The Development Of An Antiviral Therapeutic With Multiple Bioactive Molecules That Adopts A Multi-Pronged Strategy To Eradicate The SARS-CoV-2 Coronavirus And Also Address Its Effects On Various Cellular Pathways In The Human Host.

India Contact:

VEDICINALS INDIA PRIVATE LIMITED

Prakash Salunke

Mobile: 7567499944

Email :prakash160876@gmail.com /prakash@health-shield.com

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Introduction:

Since the advent of the SARS-CoV-2 coronavirus, researchers and clinical experts are being exposed to a constant onslaught of a variety of emerging clinical manifestations, research data and even autopsy studies that all indicate that the COVID-19 disease which the novel coronavirus induces, is not just a simple respiratory disease but one that is far more complex and is assaulting the human host via a variety of ways including disrupting and damaging the immune host immune system, triggering a variety of inflammation pathways, disrupting numerous human cellular pathways resulting in a variety of medical conditions arising. The long term health implications for those that have survived the disease is far more alarming.

Despite the usage of certain existing drugs under EUA approvals and antibody based treatment protocols, it has been shown that the SARS-CoV-2 coronavirus virus is fast mutating to develop drug resistant variants. Even in conditions where such treatment protocols have not been used extensively, the novel coronavirus is still evolving cum mutating and giving rise to variety of more potent and transmissible variants.

While vaccination programs are underway to hopefully control this pandemic, a group of international researchers and scientists led by German biotech entrepreneur Joachim Gerlach have developed a multiple molecular based therapeutic suspension that is not only able to effectively halt the SARS-Cov-2 replication thru a variety of targets but it is also able to address more than 71 human host cellular pathways that is directly or indirectly disrupted by the actions of the SARS-CoV-2 coronavirus.

The study team carefully selected a total of nine bioactive phytochemical molecules based on extensive silico computational docking studies, meta-analysis of thousands of past published studies on these molecules and their mechanisms along with detailed studies of conditions arising in COVID-19 patients. After going thru extensive constituent blending studies, safety studies and animal trials, the team developed a potent therapeutic compound that has performed extremely well in a WHO registered randomized clinical trial involving its use as adjuvant along with a variety of existing standard treatment protocols. Informal observation studies involving the usage of the product as a stand-alone therapeutic has also shown extremely positive results.

This white paper details the properties of the nine bioactive molecules used in the developed therapeutic that is called Vedicinals9 and also the way these molecules are able to act as an antiviral and also address more than 71 cellular pathways that are affected as a result of a SARS-CoV-2 virus infection. (Kindly note that studies are still ongoing and more pathways that are affected by the virus and is able to be treated by the Vedicinals9 therapeutic suspension is also being constantly added.)

Plans are already underway for bigger clinical trials in a variety of countries and also more detailed studies to assess the therapeutic compound as a potential prophylaxis for COVID-19 and also for treatment of a variety of Long COVID-19 conditions.

A. Background

Corona viruses (CoVs) are an etiologic agent of complex viral infections in humans as well as animals, which can lead to severe disorder in multiple organs including but not limited to the respiratory tract, digestive tract and also systemically. The new novel strain of CoV was first identified in Wuhan, China, at the end of 2019 and initially it was named as 2019-nCoV. The Emergency Committee of the World Health Organization (WHO) confirmed an outbreak in China on January 30, 2020, which still is a Public Health Emergency of International Concern. Currently, no known and proven or tested specific therapies or treatment or vaccines for COVID-19 are existing on the date of writing this based on full regulatory approvals except EUA status but worldwide a lot of resources are being diverted on finding a cure. Presently the approach remains limited to preventive and symptomatic supportive therapies, in an attempt to prevent further complications and organ failure. Several marketed drugs like Remdesivir, Liponavir, Ritonavir, Chloroquine are being used but none of them have shown promising results.

As per the warnings of global economic agency, the whole world is expected to enter into biggest economic recession and worst crisis of all time. The GDP of India is expected to reduce by over 40%. There would be tremendous unemployment which then would lead to highly unstable social environment. The COVID-19 is far more than the economic crisis. It will affect the societies and it will increase the poverty and inequalities not only in India but worldwide. Without urgent availability of COVID-19 medicine social-economics will escalate at highest degree which will jeopardize lives and livelihoods for years to come. We are already seeing the mass migration of workers in India into their respective states which is hurting the economic activity of the industries at agreat deal. Hence it is urgently required to develop an effective medicine against SARS-CoV-2 which should be a complete package and should also take care of rapid mutations of the virus along with enhancing the immune system.

As of now there is no cure for SARS-CoV-2, so far 170 plus mutations have been recorded which makes SARS-CoV-2 a very difficult virus to develop an effective vaccine. Mutations of such high degree makes SARS-CoV-2 a moving target. Researchers have never been able to develop a medically proven vaccine against any strain of CoV, work on vaccines for SARS in the early 2000s and MERS in the 2010s took so long that scientists eventually gave up as the epidemics.

B.Vedicinal Approaches and proposed Solution:

Understanding and interpreting the dynamic state of the problem we came up with broad spectrum, highly effective and synergistic nutraceutical composition which will work effectively and selectively, even on the the moving targets like constantly mutating SARS-COV-2 and includes multiple standardized Phyto components.

Our approach is scientific,systematic, structured and collaborative research performed by leading domain experts, utilising inputs from latest technology like Genetic engineering, CAD modelling, simulation andespeciallyArtificial Intelligence. Right from choosing molecules, CAD simulation, cross referencing of the results with hundreds of research papers on similar Virus strain in terms of genetic structure, way of multiplication etc. Every step has scientific backup and research. We chose each molecule for an extremely specific target / action mechanism / pathway and confirmed alternate pathways via simulations and Our simulation results are very encouraging. Once we finalized our formulation, we explored herbal plants / medicines and identified regional herbal / medicinal plants having the required molecules in abundance and could be commercially extracted. Then we worked on extraction to get purity of target compounds from 80 % minimum to 98 % maximum, and we are continuously trying to improve itsothat we have very less impurity / non-target molecules. Meanwhile we also confirmed by way of Literature Research, HPLC the remaining

molecules and checked for interaction / synergy and all looks good so far. Soon after that we sourced, secured the required molecules via herbal / plant extracts and formulated the dosages, For India, we initiated Animal acute toxicity testone week back. So far 2000 mg/kg of dosing is successfully completed, and 28 days Repeat dose study is starting in a day or two. Parallel we will explore anti-inflammatory efficacy animal testing. We are also hopeful of securing permission for Clinical Trials on Covid 19 patients and exploring In-Vitro Anti-viral testing on actual virus stain with laboratories.

We were working on HIV , MERS, SARS_Covstains for good time and immediately after we got the genetic information SARS_Cov_2 published by China mid-January our team identified similarities, differencesbetween our research so far and new Virus stain and aligned our research to SARS_Cov_2. First step was choosing compounds to attack the SARS_Cov_2 and arrest / stop replication . Our approach is UP-STREAM/ ROOT CAUSE ,which is necessity of world today due to extremely high contagious nature of the Virus. Once we had list of compounds ready, we explored Indian medicinal plants for the compounds to have a plant derived treatment similar to Ayurveda. At the same time the compounds we are using are with optimum nutrition, non-toxic, natural herbal compounds, easy to digest, have health protective and rejuvenate functions to ameliorate the symptoms and/or syndromes of COVID-19 and will promote general health and well-being of COVID-19 patients.

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Graphical representation of our approach:

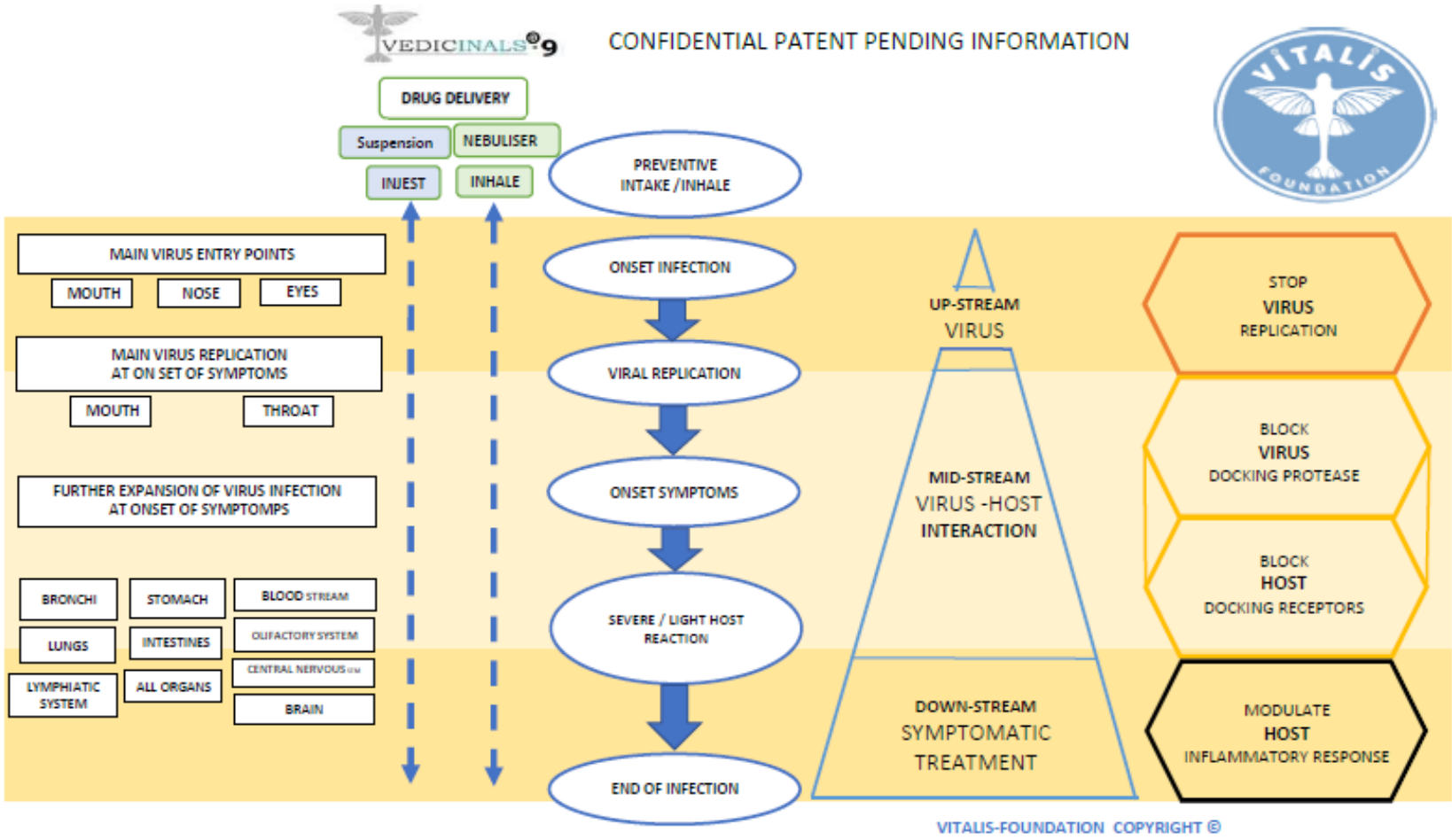

Fig1 : Graphical representation of Vedicinal approach:

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C) Phytoconstituents selected:

1) Biacalin

Baicalin is a flavonoid from the root of *Scutellariabaicalensis*gcorsi, having two pro phenolic hydroxyls with anti-inflammatory, antiallergic, antibacterial, antihypertensive effect and food condiment.[1]Baicalin has attracted increasing scientific attention because of its various pharmacological activities such as antioxidant, antitumor, anti-inflammatory and hepatoprotective effects .Baicalin also exhibited anti-viral, anti-HIV, anti-proliferative activities, inhibits cell growth and induces apoptosis [2]

2) Quercetin

Quercetin is one of the important bioflavonoids present in *Allium fistulosum, Calamus scipionum, Camellia sinensis,Centella asiatica, Hypericum hircinum, Malus domestica, Moringa olifera*, and*Morus alba.* which is known for its anti-inflammatory, antihypertensive, vasodilator effects, anti-obesity, anti-hypercholesterolemic and anti-atherosclerotic activities Some of the beneficial effects include cardiovascular protection, anticancer, antitumor, anti-ulcer, anti-allergy, anti-viral, anti-diabetic, gastroprotective effects, immunomodulatory, and anti-infective. Quercetin is known to exhibit antibacterial effects against almost all strains of bacteria, particularly affecting gastrointestinal, respiratory, urinary, and dermal system. Their anti-infective and antireplicative ability possibly contributes to the antiviral characteristics. Viruses which commonly respond to flavonoids are adenovirus, herpes simplex virus, Japanese encephalitis virus, and respiratory syncytial virus [3]

3) Luteolein

Luteolin (3′, 4′, 5′, 7′-tetrahydroxyflavone) is one of the most prevalent flavones present in variety of vegetables, fruits and herbs such as carrot, cabbage, artichoke, tea, celery and apple. Luteolin is an active compound with anti-oxidant, anti-tumor, anti-inflammatory, and anti-apoptotic activities Due to its potent anti-tumor and anti-inflammatory flavonoid, earlier studies and clinical trials on Luteolin had thus focused on cancer and inflammation. Since the 1950s to date, there has been an increase in the number of reports on the cardiovascular effects of Luteolin. Luteolin exhibits strong

cardiovascular protective activities via complex signal transduction pathways and target effectors.[4]

[1] Liu, X., Gu, J., Fan, Y., Shi, H., Jiang, M. (2013). Baicalin attenuates acute myocardial infarction of rats via mediating the mitogen-activated protein kinase pathway. *Biol. Pharm. Bull.* 36 (6), 988–994. doi: 10.1248/bpb.b13-00021

[2] Tao Y, Zhan S, Wang Y, Zhou G, Liang H, Chen X,and Shen H. (2018) Baicalin, The major component of traditional Chinese medicine *Scutellariabaicalensis* induces colon cancer cell apoptosis through inhibition of oncomic RNAs. Sci Rep 8:14477

[3] David A, Arulmoli AV, and Parasuraman, S. (2016). Overviews of biological importance of Quercetin: A bioactive flavonoid. Pharmacognosy reviews, 10(20):84–89.

[4] Luo Y, Shang P and Li D (2017) Luteolin: A flavonoid that has multiple cardio-protective effects and its molecular mechanisms. Front. Pharmacol. 8:692.

4) Rutin

Rutin (3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside) is a flavonol, abundantly found in plants, such as passion flower, buckwheat, tea, and apple. Rutin, also called as rutoside, quercetin-3-rutinoside, and sophorin is a citrus flavonoid glycoside; 'rutin' is derived from the plant *Ruta graveolens*, which also contains rutin. It has demonstrated a number of pharmacological activities, including antioxidant, cytoprotective, vasoprotective, anticarcinogenic, and cardioprotective activities[5] Rutin has demonstrated the neuroprotective effect on brain ischemia. Rutin is found to prevent oxidative damage in 'aortic endothelial cells' by lowering nitrotyrosine immunoreactivity. Rutin enhanced the in vitro serum protein binding of S- and R-warfarin. Concomitant administration of rutin possibly reduces the anticoagulant effect of racemic warfarin. Rutin in vitro caused concentration-dependent inhibition of platelet activating factor induced washed rabbit platelet aggregation, and intra-platelet free calcium concentration elevation was induced by platelet activating factor which was inhibited by rutin in a dose-dependent manner. Antiviral agents for the treatment of infections caused by retroviruses, orthomyxoviruses, herpes viruses, hepatitis B virus and hepatitis C virus are widely available.

5) Hesperedin

Hesperidin (C28H34O15) is known as a flavanone glycoside, richly found in the citrus fruits such as lemon, sweet orange (*Citrus sinensis*), and grapefruits. The presence of this compound has also been proven in unripe sour oranges, Ponderosa lemon, *Citrus unshiu*, and *C. mitis.* In addition to the Citrus species, it could be isolated from other plant genera like *Betulaceae, Fabaceae, Lamiaceae, Papilionaceae, Zanthoxylum* species, and *Acanthopanax setchuenensis*.It possesses the anti-oxidant, anti-inflammatory, and anti-carcinogenic activities (Hajialyaniet al., 2019). Hesperidin and its aglycon, hesperetin, were found to be effective on different types of cancers. Along with the anti-cancer activity of hesperidin, the effect of isoflavone on inflammation associated with cancer has been proven. It exhibited the inhibitory effect on the inflammatory-mediated cancers by regulating the level of inflammatory components like TNF-α, IL-1β, cyclooxygenase-2 (COX-2), and iNOS. Hesperidin was also found to have an anti-replicative activity against some viruses.[6]

6) Curcumin

Curcumin (1, 7- bis (4- hydroxy -3- methoxyphenyl) -1, 6-heptadiene -3,5- dione), also called diferuloylmethane, is the main natural polyphenol found in the rhizome of *Curcuma longa* (turmeric) and in others *Curcuma* spp. It has been shown to benefit inflammatory conditions, metabolic syndrome, pain, and to help in the management of inflammatory and degenerative eye conditions Inaddition, it has been shown to benefit the kidneys .While there appear to be countless therapeutic benefits to Curcumin supplementation, most of these benefits are due to its antioxidant and anti-inflammatory effects. Despite its reported benefits via inflammatory and antioxidant mechanisms, Curcumin has been shown to block NF-κB activation increased by several different inflammatory stimuli [7]

[5] Ganeshpurkar A, and Saluja AK. (2017). The Pharmacological Potential of Rutin. Saudi pharmaceutical journal : SPJ : The official publication of the Saudi Pharmaceutical Society, 25(2):149–164.

[6] Hajialyani M, HoseinFarzaei M, Echeverría J, Nabavi, SM, Uriarte E, and Sobarzo-Sánchez E. (2019). Hesperidin as a Neuroprotective Agent: A review of animal and clinical evidence. Molecules (Basel, Switzerland), 24(3):648.

[7] Hewlings SJ, and Kalman DS. (2017). Curcumin: A review of its' effects on human health. Foods (Basel, Switzerland), 6(10):92.

7) Epigallocatechin gallate

Catechins are the main flavonoids found in *Camellia sinensis*. EGCG has been found to have the highest antioxidant activity compared to others catechins. It has been reported that EGCG improves glucose tolerance and increases glucose-stimulated insulin secretion by preserving islet structure in comparison with control mice. One of beneficial effects would be a potentiation of anti-inflammatory properties induced by this flavonoid.[8] In addition, anti-inflammatory, antiaging, and antifibrosis properties of EGCG appear to involve several molecular signaling pathways and cellular machineries. several lines of evidence from various in vitro and in vivo animal studies have suggested that EGCG exerts renoprotection against Chronic Kidney Disease .[9]

8) Piperine

Piperine is a compound belonging to the alkaloids; it is found in the members of the *Piperaceae* family, been detected in several other plant species (*Rhododendron faurie*, *Vicoa indica*, *Anethum sowa*, and others). Piperine bioactivities have reported the very high spectrum of physiological effects, including antihypertensive, antiaggregant, antioxidant, antitumor, antispasmodic, antiasthmatic, antidepressant, anxiolytic, and many others. Along with an array of biological activities, piperine is known for its ability to increase the bioavailability of drugs, and thus enhance their therapeutic potential.[10]

9) Glycyrrhizin

The major bioactive constituent is glycyrrhizin of *Glycyrrhiza glabra* Linn (Family-Fabaceae), and has antitussive, demulcent, and expectorant loosening activities which may attribute due to presence of glycyrrhizin and helping to expel congestion in the upper respiratory tract as it accelerates tracheal mucus secretion. Glycyrrhizin, an already known anti-inflammatory compound, has also been found as the first plant-based inhibitor of thrombin .It prolonged the thrombin and fibrinogen clotting time and increased plasma recalcification duration. Glycyrrhizin has a prominent antiviral activity, as it does not allow the virus cell binding. Glycyrrhizin has been used for more than 60 years as treatment for chronic hepatitis under the name of SNMC (stronger neo-- minophagen-C) clinically as an antiallergic and antihepatitis agent[11]. Glycyrrhizin induced significant reduction in serum aminotransferases and improved the liver histology when compared with the placebo. An aglycone of glycyrrhizin decreases the expression of P450 E1 thereby protecting the liver. It has anti-allergic, anti-inflammatory, spasmolytic, mild laxative, antistress, antidepressive,

[8] Legeay S, Rodier M, Fillon L, Faure S, and Clere N. (2015). Epigallocatechin Gallate: A review of its beneficial properties to prevent metabolic syndrome. Nutrients, 7(7):5443–5468.

[9] Kanlaya R and Thongboonkerd V (2019) Molecular mechanisms of Epigallocatechin-3-Gallate for prevention of chronic kidney disease and renal fibrosis: Preclinical evidence, Curr Dev Nutr 3(9):1-5.

[10] Stojanovi´c-Radi´ Z, Pej˘ci´M, Dimitrijevi´ M, Aleksi´ A, Kumar A, Salehi B, Cho WC, and Sharifi-Rad J. (2019) Piperine-A Major Principle of Black Pepper: A Review of its bioactivity and studies, Applied Sciences 9, (4270):1-29.

[11] Sharma V, Katiyar A, and Agrawal RC (2018) *Glycyrrhiza glabra*: Chemistry and pharmacological activity, Sweeteners :87-100.

antiulcer,liver protective, estrogenic, emmenagogue, and antidiabetic substance, and is widely used in theIndian system of medicine.[12]

[12] Arunava G, Subrata P, Neelesh N, and Mukjerjee P. (2009). Quantification of glycyrrhizin in *Glycyrrhiza Glabra* extract by validated HPTLC densitometry. Journal of AOAC International. 93:492-495.

D) Pathways targeted:

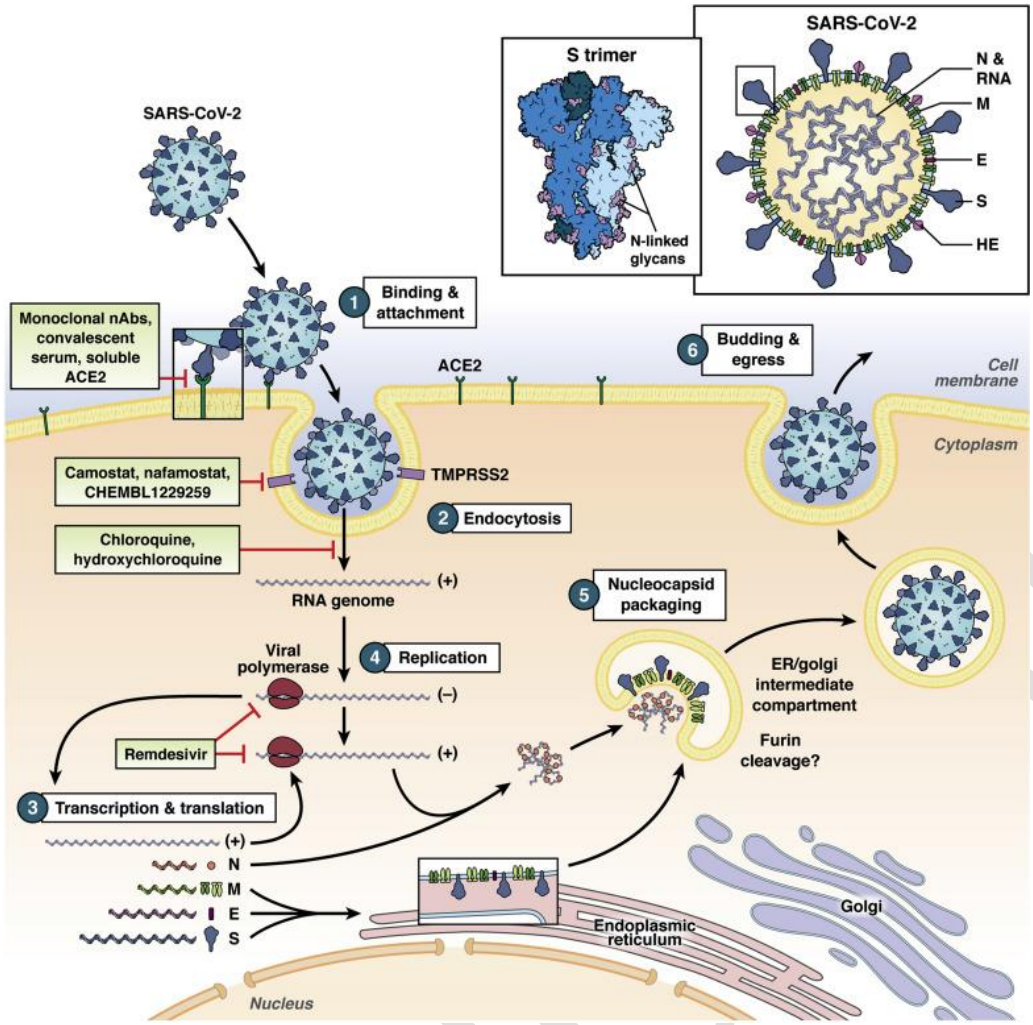

Fig : Major checkpoints for target to control Covid 19.

Search for plant-based antivirals against the SARS-CoV-2 is promising, as several plants have been shown to possess antiviral activities against betacoronaviruses (beta-CoVs). The present study aimed to evaluate bioactive compounds found in plants by using a molecular docking approach against structural and non-structural protein.

VEDICINALS®9 The Composition *for management of* **COVID-19** & *Preventing* **LONG COVID**

##	Drug Target Pathways	ACTIVE Ingredients								
		1	2	3	4	5	6	7	8	9
	STRUCTURAL PROTEINS OF SARS_CoV_2									
1	3C-LIKE PROTEASE (3CL-pro) 6LU7 INHIBITORS	✓	✓	✓	✓	✓	✓	✓	✓	✓
2	SPIKE GLYCOPROTEIN 6LYEST INHIBITORS		✓	✓		✓	✓			✓
3	ENVELOPE GYLCOPROTIEN INHIBITORS					✓				
4	NUCLEOCAPSID PROTEIN INHIBITORS					✓				
5	PAPAIN-LIKE PROTEASE (PL-pro) INHIBITORS	✓						✓		
6	RNA-DEPENDANT RNA POLYMERASE (RdRp) INHIBITORS		✓		✓				✓	✓
7	HELICASE INHIBITORS				✓	✓				
8	RBD-ACE 2 6VW1 INHIBITORS	✓	✓		✓	✓	✓	✓		✓
9	RECENT MUTATIONS & RECOMBINANT INHIBITORS	✓	✓	✓	✓	✓	✓	✓	✓	✓
	HOST RECEPTORS / CELLS / ENZYMES									
10	ZINC IONOPHORES		✓					✓		
11	DHODH INHIBITORS	✓	✓	✓			✓		✓	✓
12	ENDOCYTOSIS INHIBITORS	✓	✓		✓		✓	✓		✓
13	HEME OXYGENASE (HO-1) ACTIVATORS	✓	✓	✓	✓	✓	✓	✓	✓	✓
14	TMPRSS2 INHIBITORS	✓						✓		✓
15	FURIN INHIBITORS	✓		✓	✓	✓	✓		✓	
16	TRYPSIN INHIBITORS		✓		✓	✓		✓		✓
17	CATHEPSIN-L INHIBITORS		✓							
18	APN & CD 13 INHIBITORS						✓			
19	DPP4 INHIBITORS		✓		✓		✓	✓		
20	CALPAIN INHIBITORS		✓							
21	CASEIN KINASE-2 (CK2) INHIBITORS	✓	✓	✓				✓		✓
22	EMMPRIN CD 147 INHIBITORS	✓					✓	✓	✓	
23	GRP 78 INHIBITORS	✓	✓	✓	✓		✓	✓	✓	
24	GP 41 FUSION INHIBITORS						✓	✓		
25	ANGIOTENSIN II INHIBITORS	✓	✓	✓		✓	✓	✓		✓
26	PALS 1 INHIBITORS	✓	✓	✓		✓	✓	✓	✓	✓
27	P-SELECTIN INHIBITORS	✓	✓					✓		
28	HEPARAN SULFATE BINDING INHIBITORS							✓		
29	CCR5 INHIBITORS	✓	✓	✓				✓		
30	MYOSIN II & FILOPODIA ADHESION INHIBITORS	✓		✓				✓		
31	TYROSINE KINASE INHIBITORS	✓	✓	✓			✓	✓	✓	
32	PPAR GAMMA ACTIVATORS		✓		✓	✓	✓		✓	
33	SYNCYTIUM / SYNCYTIA FORMATION INHIBITORS	✓	✓	✓						
34	GLYCOLYSIS & GLUTAMINOLYSIS INHIBITORS		✓	✓			✓	✓	✓	
	MANAGEMENT OF COVID 19 & PREVENTION OF LONG COVID									
35	INTERLEUKIN - 6 INHIBITORS	✓	✓	✓	✓	✓	✓	✓	✓	✓
36	MACROPHAGE POLARISATION & CCL2 REGULATORS	✓	✓	✓			✓	✓		✓
37	PRO-INFLAMMATORY CYTOKINE SUPPRESSORS	✓	✓	✓	✓	✓	✓	✓	✓	✓
38	TNF ALPHA SUPPRESSORS	✓	✓	✓	✓		✓	✓	✓	✓
39	MAST CELLS STABILIZERS	✓	✓	✓	✓	✓	✓	✓	✓	✓
40	T CELLS STABILIZERS	✓	✓	✓	✓	✓	✓	✓	✓	✓
41	Nrf2 ACTIVATORS	✓	✓	✓	✓	✓	✓	✓	✓	
42	NLRP-3 & CASPASE-1 INHIBITORS	✓	✓	✓		✓	✓	✓	✓	✓
43	STAT 3 PHOSPHORYLATION SUPPRESSORS	✓	✓	✓			✓	✓	✓	✓
44	ALPHA ANTI TRYPSIN ACTIVATORS		✓				✓	✓		
45	C REACTIVE PROTEIN SUPPRESSORS	✓	✓		✓		✓	✓		✓
46	CREATININE KINEASE INHIBITORS	✓	✓			✓	✓	✓		✓
47	RAISING ANTIBODY LEVELS	✓	✓	✓	✓	✓	✓	✓	✓	✓
48	PROTECTING LUNG TISSUES	✓	✓		✓	✓	✓	✓	✓	✓
49	PROTECTING NEURONAL TISSUES	✓	✓	✓	✓	✓	✓	✓	✓	✓
50	PROTECTING KIDNEY TISSUES	✓	✓	✓	✓	✓	✓	✓	✓	✓
51	PROTECTING CARDIO-VASCULAR SYSTEM	✓	✓	✓	✓	✓	✓	✓	✓	✓
52	PROTECTING MYOCARDIAL TISSUES	✓	✓	✓	✓	✓	✓	✓	✓	
53	BDNF & REMYELINATION AGONISTS, MYELIN SHEET PROTECTORS	✓	✓	✓		✓	✓	✓	✓	✓
54	PROTECTING AGAINST THROMBOSIS	✓	✓	✓	✓	✓	✓	✓	✓	✓
55	PROTECTING PANCREATIC BETA CELLS	✓		✓		✓	✓	✓		
56	PROTECTING LIVER TISSUES	✓	✓	✓			✓	✓		
57	TREATMENT OF BACTERIAL CO-INFECTION	✓	✓	✓	✓		✓	✓	✓	✓
58	MODULATING GUT BACTERIA & GUT BRAIN AXIS	✓	✓		✓	✓	✓	✓	✓	✓
59	TREATMENT OF INTESTINAL INFLAMMATION	✓	✓	✓	✓			✓		
60	TREATMENT OF ENCEPHALOMYELITIS	✓	✓	✓				✓		✓
61	MANAGING HYPERGLYCEMIA	✓	✓	✓				✓		
62	PROTECTING AGAINST MITOCHONDRIAL DAMAGE	✓	✓	✓		✓	✓	✓		✓
63	PROTECTING & RESTORING TIGHT JUNCTIONS (INTESTINAL & BBB)	✓	✓	✓		✓	✓	✓	✓	✓
64	SENOLYTICS	✓	✓	✓		✓		✓		
65	PROTECTING & TREATMENT OF AUTO-IMMUNE CONDITIONS	✓	✓	✓	✓	✓	✓	✓	✓	✓
	TREATMENT OF CO-INFECTIONS									
66	TREATMENT OF MALARIA CO-INFECTION	✓	✓	✓	✓	✓	✓	✓	✓	✓
67	TREATMENT OF TUBERCULOSIS CO-INFECTION	✓		✓		✓	✓	✓	✓	✓
68	TREATMENT OF DENGUE CO-INFECTION	✓	✓	✓	✓			✓		✓
69	TREATMENT OF INFLUENZA CO-INFECTION	✓	✓	✓	✓	✓	✓	✓		✓
70	RETROVIRUS & REVERSE TRANSCRIPTASE INHIBITORS	✓	✓	✓	✓	✓	✓			✓
71	TREATMENT OF HIV CO-INFECTION	✓	✓	✓			✓	✓		

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Fig:Vedicinal Targated pathway (Experimental)

CHAPTER 1: STRUCTURAL VIRAL PROTEINS INVOLVED IN SARS-COV 2

Pathway *1: 3C-LIKE PROTEASE (3CL-pro) 6LU7*

The 3CLpro sequences of MERS-CoV and SARS-CoV have 51% similarity, all of the available MERS-CoV 3CLpro structures have been solved in the presence of a ligand and adopt a conformation similar to that of SARS-CoV 3CLpro, with a backbone root-mean-square deviation (RMSD) of 1.06 Å over 232 Cα atoms in the protomers[13].

3CLpro is highly conserved among the known coronavirus species, and several common features are shared among the different coronavirus 3CLpro substrates. From the N to the C terminus, the amino acids in the substrates are numbered as (-P4-P3-P2-P1↓P1′-P2′-P3'-), and the cleavage site is located between P1 and P1'. In particular, a Gln residue is almost always required in the P1 position of the substrates. Humans do not have a homologous 3CLpro, which makes 3CLpro an ideal specific antiviral target. SARS-CoV-2 and SARS-CoV are significantly different from MERS-CoV in terms of cell invasion characteristics (the S protein of MERS-CoV utilizes DPP4 as a receptor). Nevertheless, amino acid sequence alignments indicate that the similarity of the 3CLpros of SARS-CoV-2, SARS-CoV and MERS-CoV can be as high as 96.1% .[14]In CoVID-19, 3CLpro cleaves 11 sites in the polyproteins, with the recognition sequence Leu–Gln↓ (Ser, Ala, Gly), including its own N- and C-terminal autoprocessing sites, by recognising the P1′ and P1–P4 sites. 3CLpro is a cysteine protease that hydrolyses viral polyproteins (pp1a and pp1ab) to produce functional proteins, is essential for coronavirus replication and is considered an important therapeutic target for diseases caused by

[13] He J, Hu L, Huang X, Wang C, Zhang Z, Wang Y, Zhang D, and Ye W (2020). Potential of coronavirus 3C-like protease inhibitors for the development of new anti-SARS-CoV-2 drugs: Insights from structures of protease and inhibitors. International journal of antimicrobial agents, 106055.

[14] Liu PF, Han FG, Duan BB, Deng TS ,Xianglin H and Zhao MQ. (2013). Purification and antioxidant activities of baicalin isolated from the root of huangqin (Scutellariabaicalensisgcorsi). Journal of Food Science and Technology. 50(3):615–619

coronaviruses, including COVID-19.

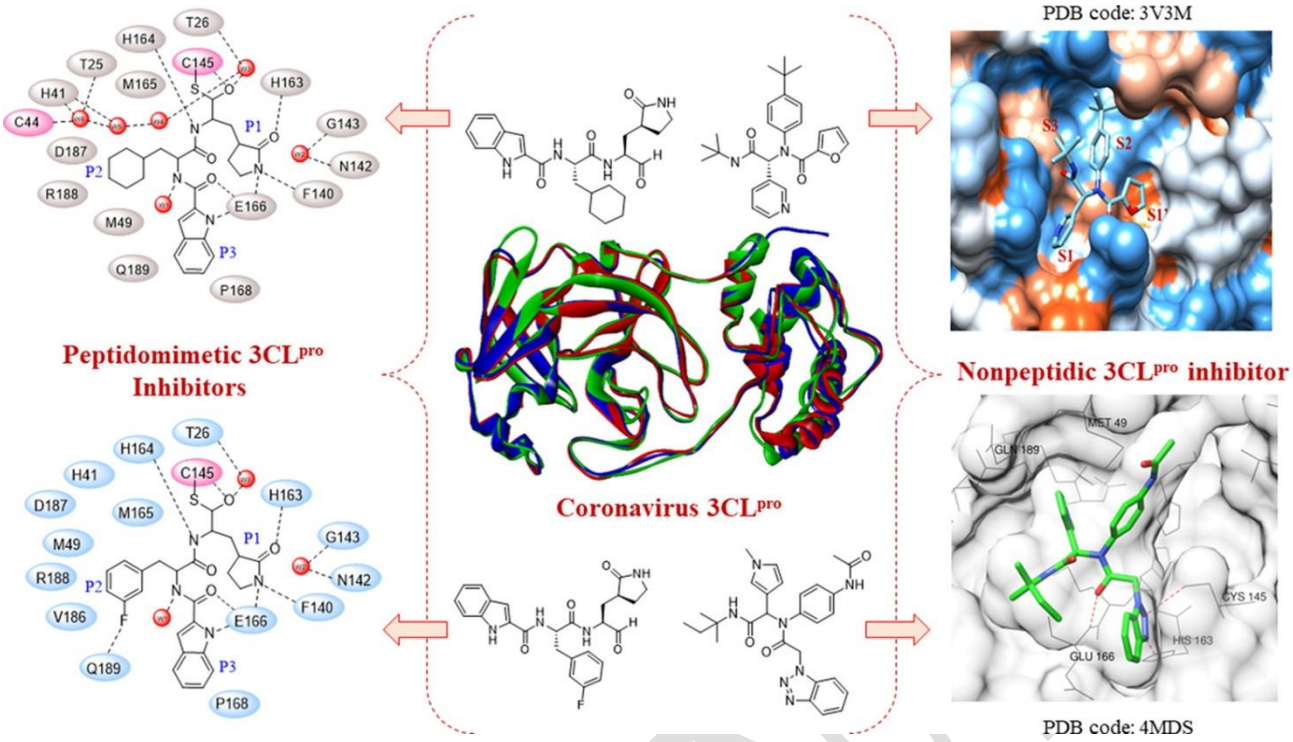

Fig4: ***3C-LIKE PROTEASE (3CL-pro) 6LU7 Inhiition Mechanism***

Studies on 3CLpro inhibitors are usually focused on substrate-binding sites (S1′−S1−S2−S3−S4), cleavage sites (P1−P4 and P1′−P4′), the catalytic dyad (His41 and Cys145) and other key residues, such as Thr190, Gln189, Glu166, Met165, Ser144, Gly143, Asn142, Leu141 and Phe140, Because of its highly conserved sequence and essential functional properties, 3CLpro has been validated as a potential target for the development of drugs to treat SARS, MERS and COVID-19.

Role of Vedicinal Constitutents: We have carefully studied and designed our formulation strategy to the highest level of confidence and it is target oriented. Specifically, curcumin, luteolin, quercetin, epigallocatechin gallate, were reported to inhibit the proteolytic activity of SARS-CoV 3CLpro.[15] Baicalin and baicalein were identified as the first non-covalent, non-peptidomimetic inhibitors of SARS-CoV-2 3CLpro and exhibited potent antiviral activities in a cell-based system. Remarkably, the binding mode of baicalein with SARS-CoV-2 3CLpro determined by X-ray protein crystallography is distinctly different from those of known inhibitors.[16],[17], [18]. Preliminary results suggested that piperine

[15] Jo S, Kim S, Shin DH, Kim MS. Inhibition of SARS-CoV 3CL protease by flavonoids. J Enzyme Inhib Med Chem. 2020 Dec;35(1):145-151. doi: 10.1080/14756366.2019.1690480. PMID: 31724441; PMCID: PMC6882434.

[16] Haixia Su, Sheng Yao, Wenfeng Zhao, Minjun Li, Jia Liu, WeiJuan Shang, Hang Xie, Changqiang Ke, Meina Gao, Kunqian Yu, Hong Liu, Jingshan Shen, Wei Tang, Leike Zhang, Jianping Zuo, Hualiang Jiang, Fang Bai, Yan Wu, Yang Ye, Yechun XubioRxiv 2020.04.13.038687; doi: https://doi.org/10.1101/2020.04.13.038687

[17] Ana-Maria Udrea, Speranta Avram, Simona Nistorescu, Mihail-Lucian Pascu, Mihaela Oana Romanitan, Laser irradiated phenothiazines: New potential treatment for COVID-19 explored by molecular docking, Journal of Photochemistry and Photobiology B: Biology,Volume 211,2020,111997,ISSN 1011-1344,

[18] Song E, Zhang C, Israelow B, Lu-Culligan A, Prado AV, Skriabine S, Lu P, Weizman OE, Liu F, Dai Y, Szigeti-Buck K, Yasumoto Y, Wang G, Castaldi C, Heltke J, Ng E, Wheeler J, Alfajaro MM, Levavasseur E, Fontes B, Ravindra NG, Van Dijk D, Mane S, Gunel M, Ring A, Kazmi SAJ,

and curcumin have the best docking scores and that they are capable of promoting structural changes in the viral protease by inducing folding of the enzyme 3CLpro.[19] EGCG exhibited the stronger molecular interactions within pockets at active sites than remdesvir and chloroquine .[20],[21]Glycyrrhizin was shown to inhibit SARS-coronavirus (SARS-CoV) replication in vitro. Amides of Glycyrrhizin and conjugates of Glycyrrhizin with two amino acid residues and a free 30-COOH function presented up to 70-fold increased activity against SARS-CoV but also increased cytotoxicity resulting in decreased selectivity index[22]. Based on above studies we can conclude that four compounds which are derivatives of Glycyrrhizin showed activities toward SARS-CoV at concentration <100 μM .[2324] Phenolic compound hesperidin dose-dependently inhibited cleavage activity of the $3CL^{pro}$, in which the IC_{50} 8.3 μM for hesperetin in the cell-based assay. Hesperidin has 38 stereoisomeric forms and several of these showed up among the top scorers, it has been reported to be a good inhibitor of the SARS-CoV 3CLpro with an IC50 of 8.3 μM in a cell-based assay .[25]The 3C-like protease 3CL-pro of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) is one of the most promising targets for discovery of drugs against SARS, because of its critical role in the viral life cycle. In this study, a natural compound called quercetin-3-beta-galactoside was identified as an inhibitor of the protease by molecular docking .Flavonoid like Quercetin, epigallocatechin gallate displayed good inhibition toward 3CL (pro) with IC (50) values of 73, and 73 μM, respectively

All selected nine compounds have 3-CL Protease inhibitory activity thereby extremely good chances of stopping SARS-COV-2 proliferation [26]. Molecular docking study showed that all nine-compounds showed very good binding affinity towards at least 5 different amino acid moieties of 3-CL Protease. We have specifically focused more towards 3-CL Protease since it is 100% conserved structure among all Corona viruses and hence even if Virus mutates itself in future, there are fare chances that 3-CL Protease will be conserved again.

Pathway 2 :SPIKE GLYCOPROTEIN 6LYEST

Coronavirus entry into host cells is mediated by the transmembrane spike (S) glycoprotein that forms homotrimers protruding from the viral surface. CoV uses its spike glycoprotein (S) for neutralization of antibody, to bind its receptor, and mediate membrane fusion and virus entry.Spike glycoprotein comprises two functional subunits responsible for binding to the host cell receptor (S1 subunit) and fusion of the viral and cellular membranes (S2 subunit). In some CoVs case, Spike glycoprotein is cleaved at the boundary between the S1 and S2 subunits, which remain non-covalently

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Zhang K, Wilen CB, Horvath TL, Plu I, Haik S, Thomas JL, Louvi A, Farhadian SF, Huttner A, Seilhean D, Renier N, Bilguvar K, Iwasaki A. Neuroinvasion of SARS-CoV-2 in human and mouse brain. J Exp Med. 2021 Mar 1;218(3):e20202135. doi: 10.1084/jem.20202135. PMID: 33433624; PMCID: PMC7808299.

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[20] Utomo, R.Y.; Ikawati, M.; Meiyanto, E. Revealing the Potency of Citrus and Galangal Constituents to Halt SARS-CoV-2 Infection. Preprints 2020, 2020030214 (doi: 10.20944/preprints202003.0214.v1).

[21] Khan S, Siddique R, Shereen MA, et al. Correction for Khan et al., "Emergence of a Novel Coronavirus, Severe Acute Respiratory Syndrome Coronavirus 2: Biology and Therapeutic Options". *J Clin Microbiol*. 2020;58(8):e01297-20. Published 2020 Jul 23. doi:10.1128/JCM.01297-20

[22] Bailly C, Vergoten G. Glycyrrhizin: An alternative drug for the treatment of COVID-19 infection and the associated respiratory syndrome? Pharmacol Ther. 2020 Oct;214:107618. doi: 10.1016/j.pharmthera.2020.107618. Epub 2020 Jun 24. PMID: 32592716; PMCID: PMC7311916.

[23] Hoever G, Baltina L, Michaelis M, Kondratenko R, Baltina L, Tolstikov GA, Doerr HW, Cinatl J Jr. Antiviral activity of glycyrrhizic acid derivatives against SARS-coronavirus. J Med Chem. 2005 Feb 24;48(4):1256-9. doi: 10.1021/jm0493008. PMID: 15715493.

[24] Cinatl J, Morgenstern B, Bauer G, Chandra P, Rabenau H, Doerr HW. Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus. Lancet. 2003 Jun 14;361(9374):2045-6. doi: 10.1016/s0140-6736(03)13615-x. PMID: 12814717; PMCID: PMC7112442.

[25] Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Xia J, Yu T, Zhang X, Zhang L. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Feb 15;395(10223):507-513. doi: 10.1016/S0140-6736(20)30211-7. Epub 2020 Jan 30. PMID: 32007143; PMCID: PMC7135076.

[26] Solnier, J., Fladerer, JP. Flavonoids: A complementary approach to conventional therapy of COVID-19?. *Phytochem Rev* (2020). https://doi.org/10.1007/s11101-020-09720-6

bound in the prefusion conformation .[27]Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein, Cell 181(2): 281-292.). For all CoVs, Spike glycoprotein is further cleaved by host proteases at the so-called S2′ site located immediately upstream of the fusion peptide. This cleavage activates the protein for membrane fusion via extensive irreversible conformational changes

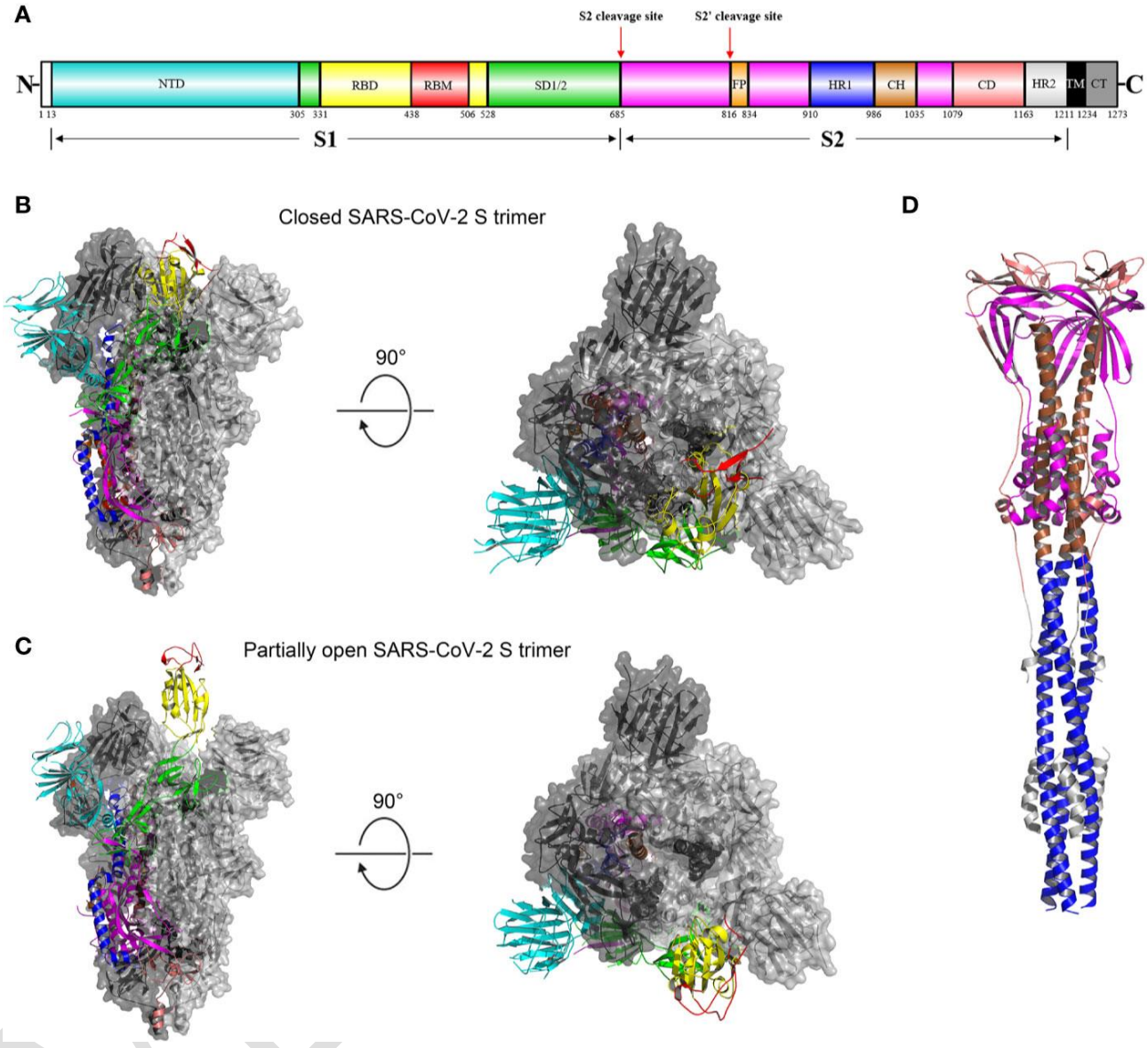

Fig5: SPIKE GLYCOPROTEIN 6LYEST Pathway

As a result, coronavirus entry into susceptible cells is a complex process that requires the concerted action of receptor-binding and proteolytic processing of the S protein to promote virus-cell fusion. The SARS-CoV-2 spike glycoprotein shares 76% amino acid sequence identity with the SARS-CoV S Urbani and 80% identity with bat SARSr-CoV ZXC21 S and ZC45 S glycoprotein .Coronavirus spike glycoproteins are densely decorated by heterogeneous N-linked glycans

[27] Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell. 2020 Apr 16;181(2):281-292.e6. doi: 10.1016/j.cell.2020.02.058. Epub 2020 Mar 9. Erratum in: Cell. 2020 Dec 10;183(6):1735. PMID: 32155444; PMCID: PMC7102599.

protruding from the trimer which participate in Spike folding, affect priming by host proteases, and might modulate antibody recognition. 20 out of 22 SARS-CoV-2 S N-linked glycosylation sequons are conserved in SARS-CoV S. Specifically, 9 out of 13 glycans in the S1 subunit and all 9 glycans in the S2 subunit are conserved among SARS-CoV-2 S and SARS-CoV S. Furthermore, S2 N-linked glycosylation sequons are mostly conserved across SARSr-CoV S glycoproteins, suggesting that accessibility of the fusion machinery to Abs will be comparable among these .

Role of Vedicinal: Compounds 2, 3, 5, 6 and 9 have strong affinity towards the SARS-CoV-2 and hence they are utilized in final composition.

(No docking results added or any other explaination)

Pathway 3: ENVELOPE GYLCOPROTIEN

The viral particle is internalized in a vesicle, whose envelope is then removed, allowing the genomic RNA to be released into the cytoplasm. The ORF1a and ORF1b RNAs are produced by the genomic RNA, and then translated into pp1a and pp1ab proteins, respectively.[28] The envelope protein is conserved across β-coronaviruses, with three variants. The multiple sequence alignment reported distinguishing feature of Sars-2-CoV envelope variants is the presence of Arg at position 69 that substitutes Glu, Gln, Asp in other homologous Sars-CoV envelope proteins. This site is followed by a deletion in position 70 corresponding to Gly or Cys in the other proteins. Sars-CoV-2 envelope sequences differ from the homologous proteins also at positions 55-56, where the dyad Ser-Phe replaces Thr-Val. Variants of the Sars-CoV-2 envelope protein differ at positions 37 and 72 where His substitutes a Leu and Leu replaces a conserved Pro, respectively [29] A homology model of the envelope protein has been built with Modeller using as a template the pentameric ion channel structure of Sars-CoV protein identified that sequence shares 91% identity to Sars-CoV-2 envelope protein and covers the segment encompassed by positions 8-65. Sars-CoV-2 Envelope protein assembled as a pentameric viroporin-like protein. This can be potential target for Drug to stop spread of covid 19 .[30]

[28] Bellavite P, Donzelli A. Hesperidin and SARS-CoV-2: New Light on the Healthy Function of Citrus Fruits. *Antioxidants*. 2020; 9(8):742. https://doi.org/10.3390/antiox9080742

29 Martina Bianchi, Domenico Benvenuto, Marta Giovanetti, Silvia Angeletti, Massimo Ciccozzi, Stefano Pascarella, "Sars-CoV-2 Envelope and Membrane Proteins: Structural Differences Linked to Virus Characteristics?", BioMed Research International, vol. 2020, Article ID 4389089, 6 pages, 2020. https://doi.org/10.1155/2020/4389089

[30] Bianchi M, Benvenuto D, Giovanetti M, Angeletti S, Ciccozzi M, Pascarella S. Sars-CoV-2 Envelope and Membrane Proteins: Structural Differences Linked to Virus Characteristics?. *Biomed Res Int.* 2020;2020:4389089. Published 2020 May 30. doi:10.1155/2020/4389089

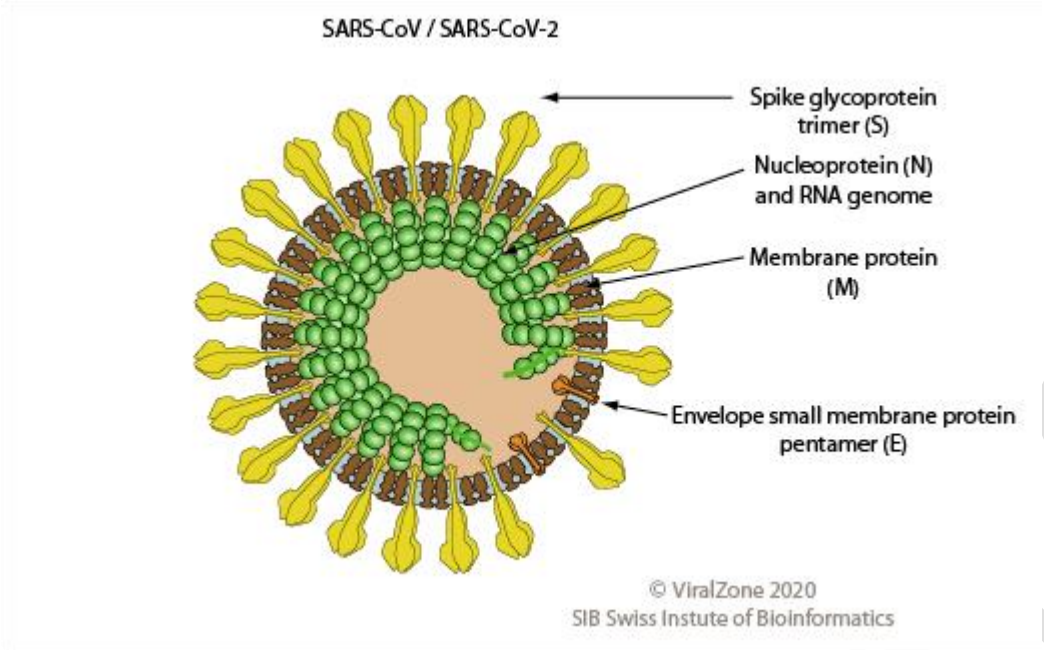

Fig6: Genome structure of SarsCov2

Role of Vedicinal: In our proposed formulation, Hesperidin showed the inhibitory activity on the molecular docking study. Selected compound inhibits the E protein thereby E Protein inhibition of formation of viral coat, an important structure for viral replication .[31]

Pathway 4: NUCLEO CAPSID PROTEIN

[31] Haggag, Y. A., El-Ashmawy, N. E., andOkasha, K. M. (2020). Is hesperidin essential for prophylaxis and treatment of COVID-19 Infection?. Medical hypotheses, 144, 109957. https://doi.org/10.1016/j.mehy.2020.109957

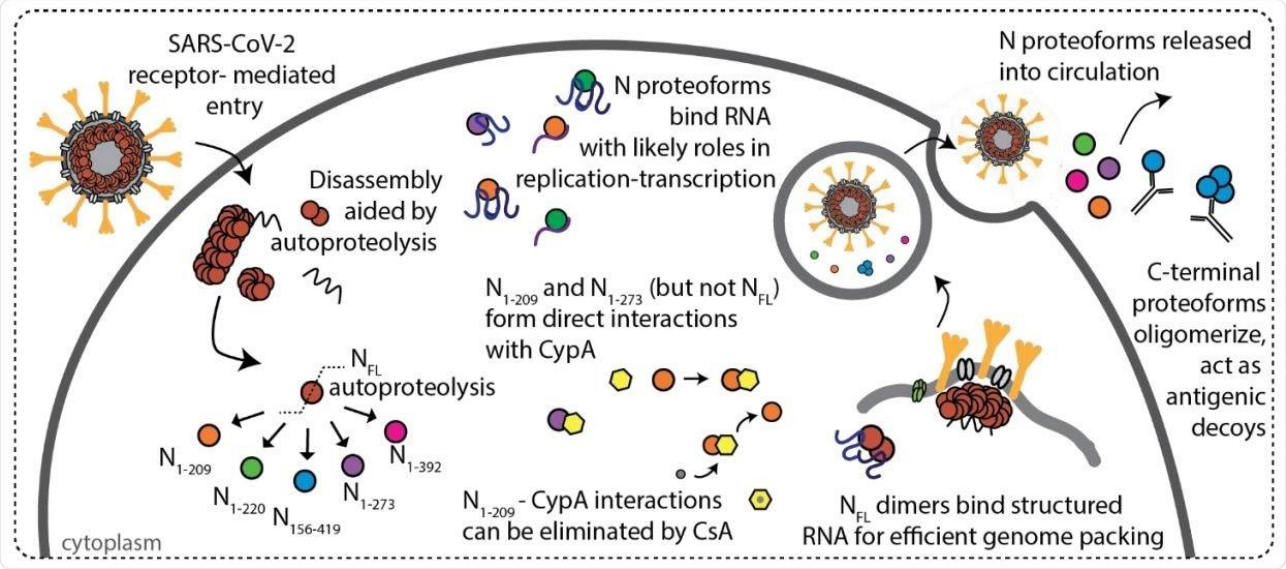

Fig7: NUCLEO CAPSID PROTEIN (Sars Cov 2Entry)

Nucleocapsid protein is similar to those of previously described, but their surface electrostatic potential characteristics are distinct. Nucleocapsid gene is more conserved and stable, with 90% amino acid homology and fewer mutations over time .[32]. Nucleocapsid proteins of many coronaviruses are highly immunogenic and are expressed abundantly during infection. Nucleocapsid protein contributes to forming helical ribonucleoproteins during the packaging of the RNA genome, regulating viral RNA synthesis during replication and transcription and modulating metabolism in infected subjects. Hence vaccine target that has some distinct advantages over other potential SARS–CoV-2 antigens

Role of Vedicinal Constituents : The pp1a and ppa1b proteins are cleaved in proteolytic process, resulting in a total of 16 non-structural proteins. Structuralproteins are incorporated into the membrane and the nucleocapsid N protein combines with the positive-sense RNA, produced through the replication process, to become a nucleoprotein complex[33]. In our proposed formulation, compound 5 showed potent N protein inhibitory activity. Our compounds inhibit the viral packaging, an important step in viral replication.

Pathway 5: **PAPAIN-LIKE PROTEASE (PL-pro)**

[32] Dutta, N. K., Mazumdar, K., & Gordy, J. T. (2020). The Nucleocapsid Protein of SARS-CoV-2: a Target for Vaccine Development. *Journal of virology*, *94*(13). https://doi.org/10.1128/JVI.00647-20

[33] (Bellavite, P., andDonzelli, A. (2020). Hesperidin and SARS-CoV-2: New Light on the Healthy Function of Citrus Fruits. Antioxidants (Basel, Switzerland), 9(8), 742. https://doi.org/10.3390/antiox9080742)

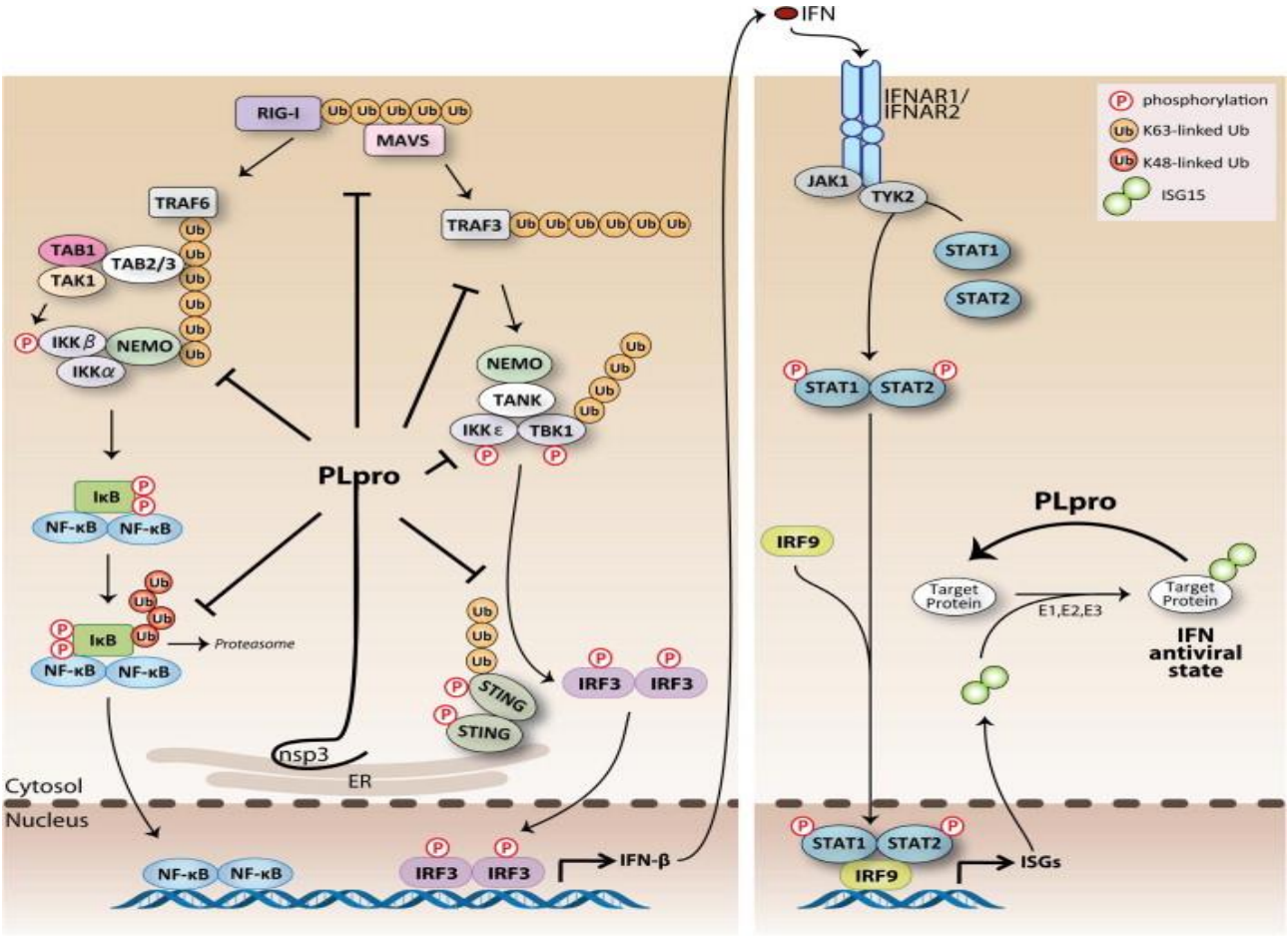

Fig8: PAPAIN-LIKE PROTEASE (PL-pro) Pathway

SARS-CoV genome encodes for two large polyproteins that are further processed by virally encoded cysteine proteases, namely, the papain-like protease (PLpro) and the 3-chymotrypsinlike protease (3CLpro, also known as the main protease-Mpro. PLpro is responsible for processing three cleavage sites of the viral polyprotein to release mature non-structural proteins 1, 2 and 3. Apart from proteolytic processing, PLpro also has a deubiquitinase and deISGylating activity. [34]

Role of Vedicinal: Molecular docking study showed that compound 1 and 7 exhibited strong binding affinity to PLpro protein, suggesting the potential utility of the compounds available in these compounds in the treatment of SARS-CoV-2.

[34] Arya, Rimanshee; Das, Amit; Prashar, Vishal; Kumar, Mukesh (2020): Potential inhibitors against papain-like protease of novel coronavirus (SARS-CoV-2) from FDA approved drugs. ChemRxiv. Preprint. https://doi.org/10.26434/chemrxiv.11860011.v2

Pathway 6:RNA-DEPENDANT RNA POLYMERASE (RdRp)

After entering into the human cell, the virus contacts this system and persuades the development of a double-membrane vesicle by developing a complex with it.[35] It generates a copy of genomic RNA. Further, it converts this Negative RNA to positive RNA which makes it mRNA. But this mRNA cannot replicate by itself and translate into a protein .The virus exploits the ribosome machinery of the human cell. The ribosome is tricked into working for the virus and translates the mRNA, creating viral proteins in thousands in each replication cycle.

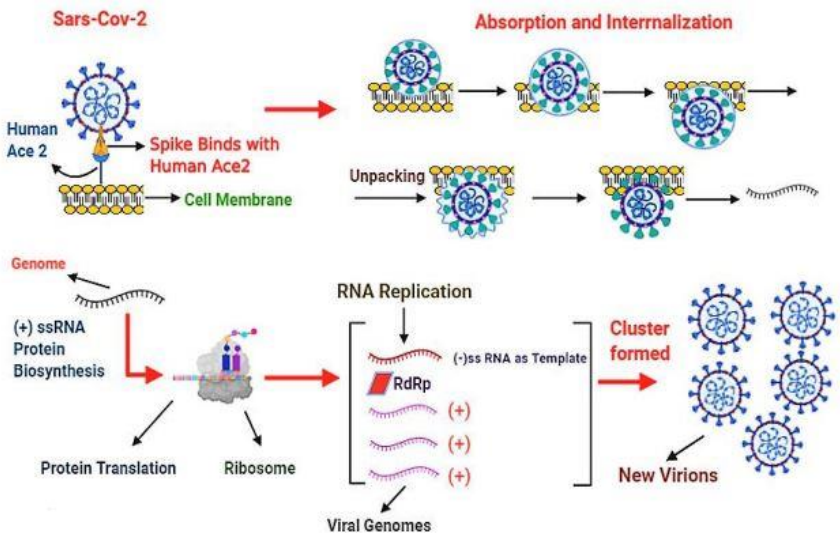

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Fig9:Working Mechanism of SARS COV 2

RNA-dependent-RNA polymerase protein (RdRp) also known as nsp12 is the engine of Sars-Cov-2 that replicates the virus using viral RNA when it gains entry into the human cell. The RdRp catalyzesthe synthesis of viral RNA and thus plays a central role in the replication and transcription cycle of COVID-19 virus, possibly with the assistance of nsp7 and nsp8 as cofactors (Gao et al., 2020) RdRp is the most significant gene in the virus genome which is encoded inside the RNA of the virus; it speeds up the process of RNA replication from the RNA template and provides safe passage to the virus that is just entered into human cells. Therefore, nsp12 is considered a primary target for nucleotide analogue antiviral inhibitors such as remdesivir, which shows potential for the treatment of COVID-19 viral infections.[36]

[35] Roomi S, Ullah W, Ahmed F, et al. Efficacy of Hydroxychloroquine and Tocilizumab in Patients With COVID-19: Single-Center Retrospective Chart Review. *J Med Internet Res*. 2020;22(9):e21758. Published 2020 Sep 1. doi:10.2196/21758

[36] QIANG GAO, LINLIN BAO, HAIYAN MAO, LIN WANG, KANGWEI XU, MINNAN YANG, YAJING LI, LING ZHU, NAN WANG, ZHE LV, HONG GAO, XIAOQIN GE, BIAO KAN, YALING HU, JIANGNING LIU, FANG CAI, DEYU JIANG, YANHUI YIN, CHENGFENG QIN, JING LI, XUEJIE GONG, XIUYU LOU, WEN SHI, DONGDONG WU, HENGMING ZHANG, LANG ZHU, WEI DENG, YURONG LI, JINXING LU, CHANGGUI LI, XIANGXI WANG, WEIDONG YIN, YANJUN ZHANG, CHUAN QIN*SCIENCE*03 JUL 2020 : 77-81

Role of Vedicinal: Our proposed formulation contains 4 compounds namely compounds 2, 4, 8 and 9 are inhibitors of RdRp[37],[38] ASP760 and ASP761 are the key amino acid residues constituting the RdRp catalytic domain. In this study, three confirmations of this enzyme have separately docked with quercetin and TYR619, CYS622, ASP623, ASP761, and SER841 were found to offer good interaction with a binding energy (Xu et al., 2003, Agrawal et al., 2020, Hecel et al., 2020, DA SILVA et al., 2021, Rahman et al., 2020, Gutierrez-Villagomez et al., 2020, Ojha et al., 2020, Bailly and Vergoten, 2020, Gomaa et al., 2021, Cinatl et al., 2003, Chanda, 2020, Shigeta et al., 2005, Maurya, 2020) RdRp is one of the most intriguing and promising drug targets for SARS-CoV-2 drug development.

Pathway 7:HELICASE INHIBITORS

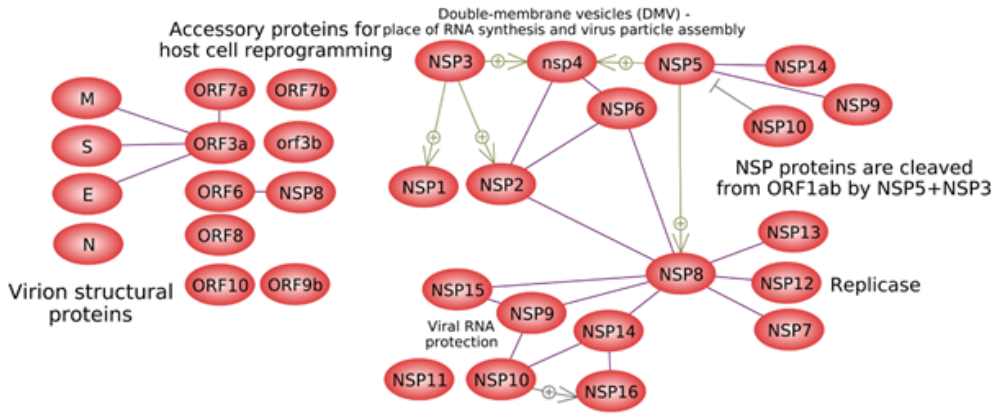

Fig 10:Helical Inhibition Pathway

Helicase also known as nsp13 in SARS-CoV-2, have shown that small molecular weight compounds with potential to inhibit the putative ATP binding site associated with the NTPase activity have promise. NTPase active site residues of SARS-CoV-2 i.e. Lys288, Ser289, Asp374, Glu375, Gln404 and Arg567 are conserved and predicted to be similar with SARS-Nsp13. SARS-nsp12 (RNA polymerase) also play a role by enhancing the helicase activity of SARS-Nsp13 through direct interactionplaying putative role of ATP hydrolysis site of the SARS-CoV. Notably differential characteristics of nsp13 in unwinding duplex RNA and duplex DNA, it is now apparent that inhibitors of the nsp13 activity offer potential therapeutic option for coronavirus including SAR-CoV-2. Among the various approaches of nsp13 activity inhibition are targeting ATP binding or direct NTPase activities, nucleic acids binding to the helicase, blocking helicase translocation, [39]

[37] Aftab, S.O., Ghouri, M.Z., Masood, M.U. *et al.* Analysis of SARS-CoV-2 RNA-dependent RNA polymerase as a potential therapeutic drug target using a computational approach. *J Transl Med* 18, 275 (2020). https://doi.org/10.1186/s12967-020-02439-0

[38] Saakre, M., Mathew, D., and Ravisankar, V. (2021). Perspectives on plant flavonoid quercetin-based drugs for novel SARS-CoV-2. Beni-Suef University journal of basic and applied sciences, 10(1), 21. https://doi.org/10.1186/s43088-021-00107-w

[39] Habtemariama S, Nabavi SF, Banach M, Neagoefgh IB, Sarkar K, PaSilPS ,Nabavi SM. (2020) Should we try SARS-CoV-2 helicase inhibitors for COVID-19 therapy, ?, Archives of Medical Research:1-5

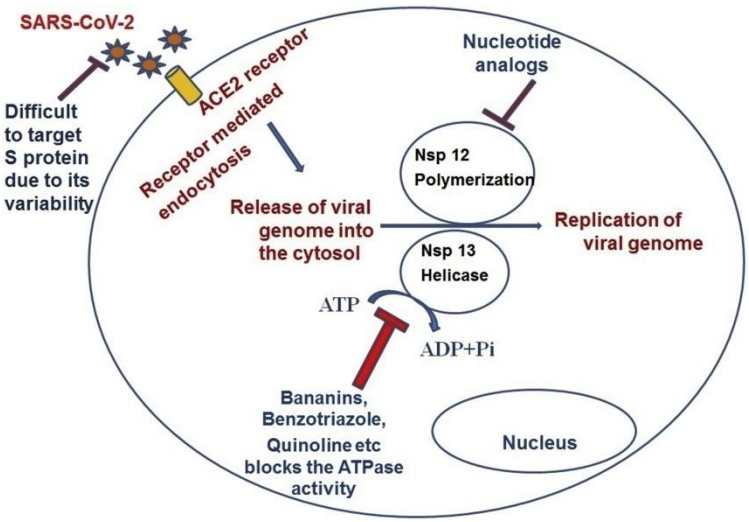

Fig 11:Receptor Mediated Endocytosis

Role of Vedicinal: Our proposed formulation contains compounds namely compound 4 and 5 are extremely good inhibitors of Helicase enzyme, an important enzyme required for viral replication.

Pathway 8:RBD-ACE-2 6VW1

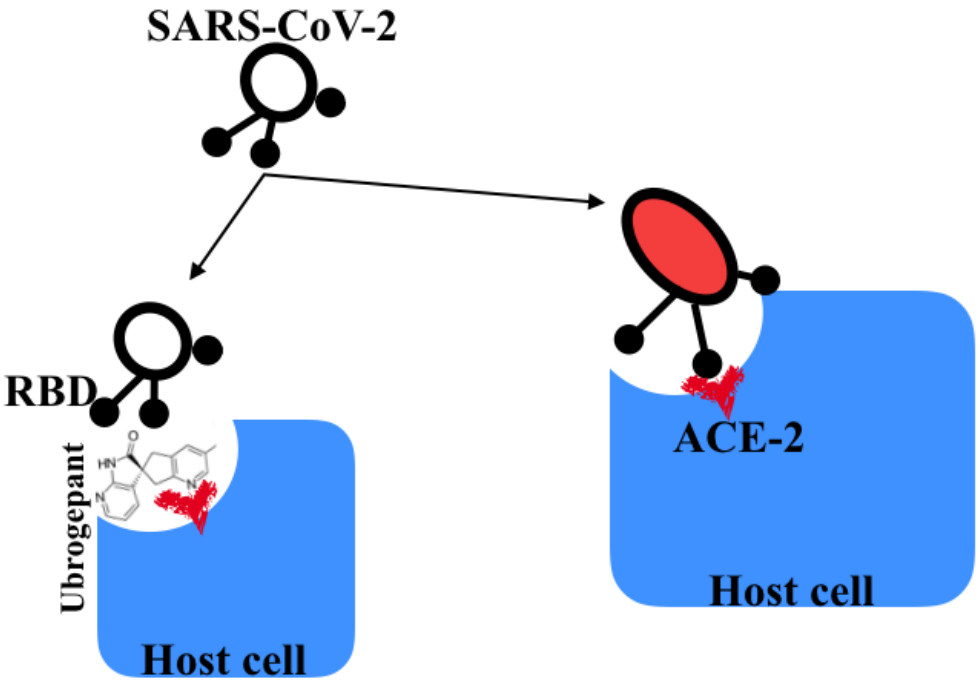

Fig12: RBD-ACE-2 6VW1 involvement

The surface glycoprotein of CoVID 19, comprises two components: S1, which contains the receptor binding domain (RBD); and S2, which contains the fusion peptide. SARS-CoV gains entry into permissive cells through interactions of the SARS-S RBD with the cell surface receptor angiotensin
converting enzyme 2 (ACE2). These interactions are followed by endocytosis, and at the low pH in endosomes, SARS-S is cleaved by a cellular protease called cathepsin L, thereby exposing the S2 domain of the spike protein for membrane fusion. Fusion of SARS-S expressing cells with ACE2 receptor-expressing cells can also take place by a pH-independent mechanism at the cell surface. SARS-S also regulates cell stress responses and apoptosis. Early studies have identified some SARS-CoV entry and replication inhibitors. Nonetheless, to date, there are no approved drugs for the treatment of SARS-CoV infection .[40]

Role of Vedicinal: Our proposed formulation contains 6 compounds namely compounds 1, 2, 4, 5, 6, 7& 9 are inhibitors of RBD (ACE2) Protein ASN121(H1), LYS114(H2), THR118(H3), ARG115(H4), GLN102(H1), ASN210(H2), ALA396(H3), VAL209, PHE390(H1), ARG393(H2), LEU100(H3), ALA99, LEU391, SER511(H1), GLY205, TYR196, GLU375(H1), ALA396, ASP206, SER128(H1), GLU145(H2)ASP350(H1), PHE390, LEU391, LYS534(H1) and LEU539(H2) are the key amino acid residues constituting the RBD (ACE2) Protein domain. In this study, three confirmations of this enzyme have separately docked with Quercetin,Luteolin, Curcumin, EGCG and Piperine and were found to offer good interaction with a binding energy and hydrogen bond interaction. RBD (ACE2) Protein is one of the most intriguing and promising drug targets for SARS-CoV-2 drug development.

[40] (Adedeji AO, Severson W, Jonsson C, Singh K, Weiss SR, and Sarafianos SG. (2013). Novel inhibitors of severe acute respiratory syndrome coronavirus entry that act by three distinct mechanisms. Journal of virology, 87(14):8017–8028.)

Pathway 9: Recent Mutations in SARS COV2

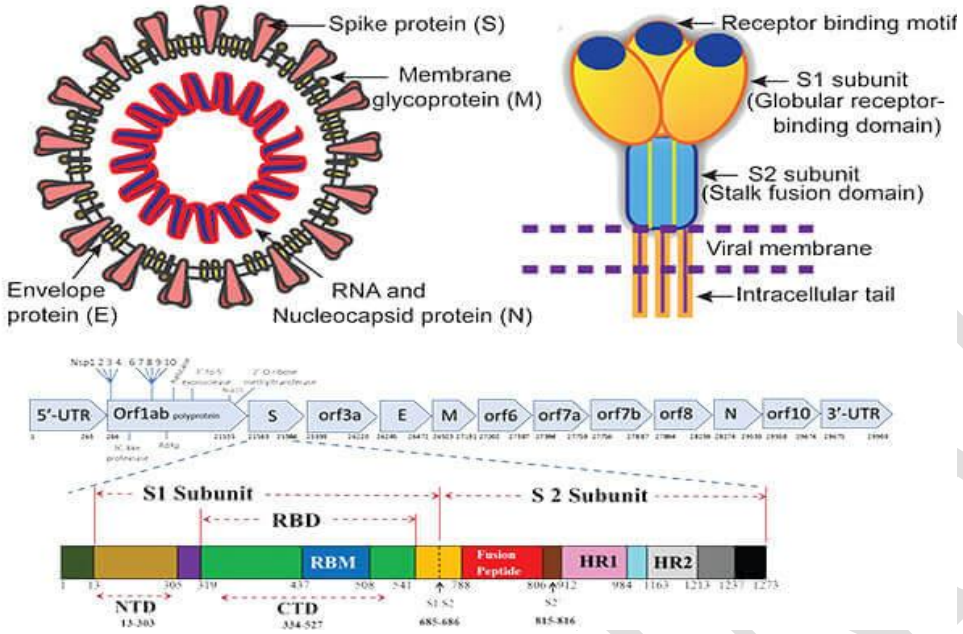

Fig: Mutation in SARS Cov2

9.1 **V483G Mutation**-

V483A is an important amino acid residue in the RBM region of the spike glycoprotein, where the Valine at position 483 has changed to Alanine, making the viral genome a unique mutant strain. V483A is one of the few mutations that have the potential to change the protein secondary structure and relative solvent accessibility in the RBM region. The RBM makes the contacts between the SARS-CoV-2 and the human ACE2 receptor acting as the core binding site. Furthermore, the RNA replication rate causes the virus to mutate at a faster rate evading host immunity, thereby posing strong drug resistance. This mutagenic capability of the virus has become the leading cause of its evolution and genomic variation. Interestingly MD simulation supports strong favorable interaction of ACE2 with RBD region containing V483A mutation. Radius of gyration analysis also showed high degree of compactness in V483A. The landscape plot and Gibbs free energy also support findings. Overall, study indicates that V483G in the RBD region can enhance its binding with the human ACE2 receptor. V483A mutation led to enhanced and broadens the virus host cell entry and transmission of the disease. . Further epitope mapping analysis revealed the mutation as antigenic determinants and thus the mutations are important while designing a therapeutic vaccine or chimeric antibody. The findings will help in further understanding the role of such arising mutations in modulating immunogenicity, viral

tropism and pathogenesis of the disease, which in lieu will help in designing vaccine more precisely to mitigate pandemic COVID-19.[41]

[42]Researchers and health officials are warning that that a mutant strain of the SARS-CoV-2 coronavirus, the V483G strain is fast making appearances in various countries including India, Brazil, The Middle-East, UK and even in the United States. According to genomic experts and immunologist, the V483 strain is resistant to the neutralizing properties of antibodies and is also deemed to be even more infectious than the D614G strains. Of the various emerging mutated strains, a mutation that is worrying and concerning that has occurred in the viral genome is the V483A mutation, which is a part of the receptor binding motif (RBM), present in the S1 domain of the spike protein. This V483A mutant virus is becoming popular in North America with 36 cases detected in random sequencing studies recently so far and considering that many sequencing studies have not been conducted, its prevalence could be far more extensive.

[41] (Ashwaq, O.; Manickavasagam, P.; Haque, S.M. **V483a – an Emerging Mutation Hotspot of Sars-Cov-2**. *Preprints* **2020**, 2020090395 (doi: 10.20944/preprints202009.0395.v1). Ashwaq, O.; Manickavasagam, P.; Haque, S.M. V483a – an Emerging Mutation Hotspot of Sars-Cov-2. Preprints 2020, 2020090395 (doi: 10.20944/preprints202009.0395.v1)

[42] Srivastava, S., Banu, S., Singh, P. *et al.* SARS-CoV-2 genomics: An Indian perspective on sequencing viral variants. *J Biosci* **46,** 22 (2021). https://doi.org/10.1007/s12038-021-00145-7

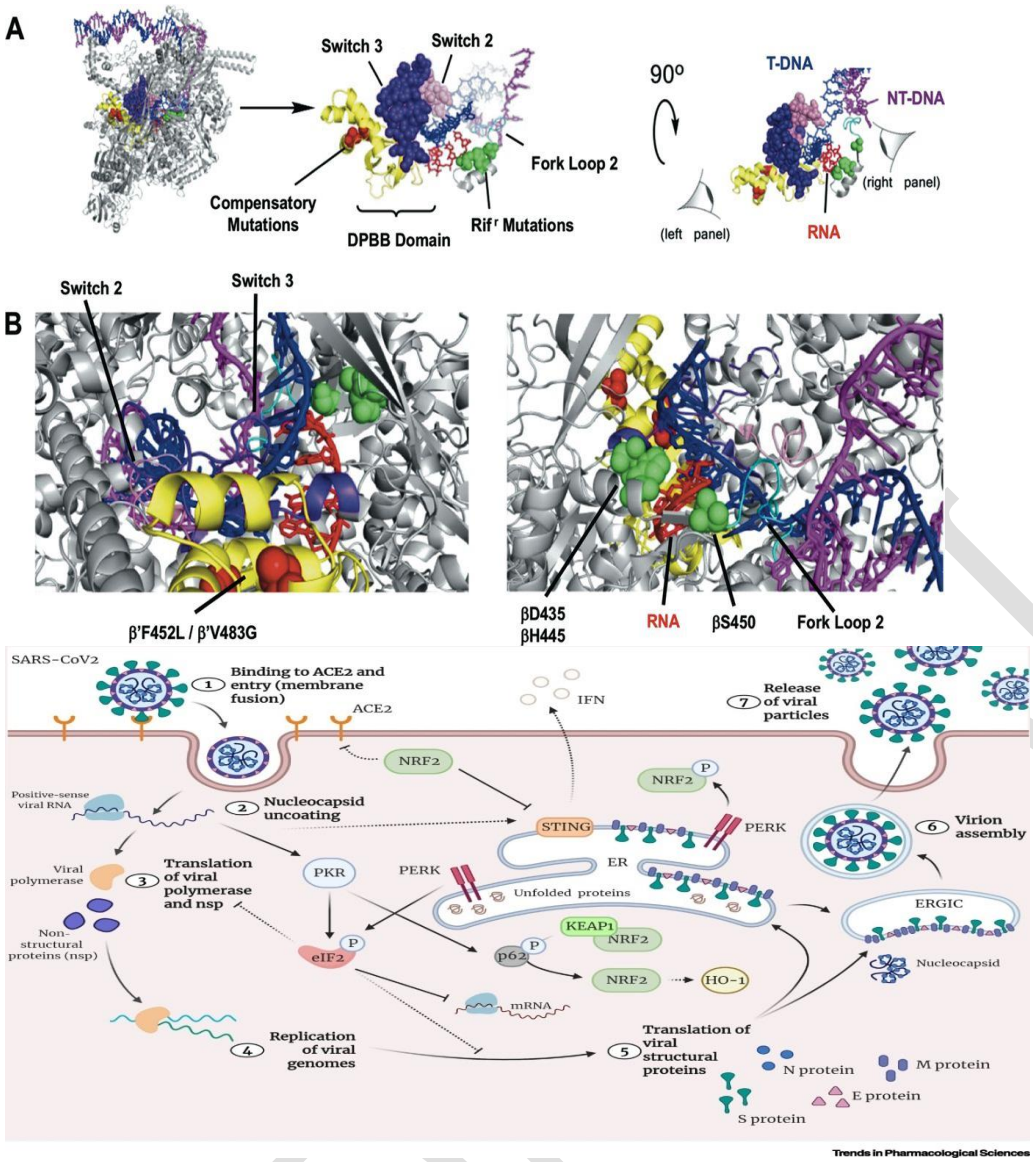

Fig: Different Mutation Domain

9.2 **E484K Mutation**-

Sometimes nicknamed Eric or Eek, E484K is a new mutation. The mutation changes the spike protein that the virus uses to enter human cells. It can make it harder for the immune system to recognize and fight the virus, if the body has been trained to fight the virus from earlier vaccines. E484K is called an "escape mutant" because it's been shown it

might be able to escape some of the antibodies produced by the vaccine. First it was the South African strain. But recently it has also been detected in the UK strain, with samples found in discovered in south-west England. [43]

The E484K mutation is present in the P.1 variant in Brazil. In the E484K mutation, a negatively charged amino acid (glutamic acid) is substituted with a positively charged amino acid (lysine). Thus, it can be expected that the mutation has a significant impact on viral sustainability and adaptive evolution. The structural analysis revealed that a new site for ACE2 binding (amino acid 75) is generated because of the E484K mutation. This appears to create a significantly stronger interaction between ACE2 and the native binding site located at the RBD and ACE2 interface (amino acid 501). The E484K mutation could potentially increase the infectivity and immune evasion potency of SARS-CoV-2. [44]

A highly diverse range of genetic mutations was observed in all Brazilian lineages with E484K mutation. On average, about 19 and 30 mutations were observed in B.1.1.33 and P.1 lineages, respectively. Further genomic analysis of the most recently emerged lineage P.2 indicated that both P.1 and P.2 lineages are evolving rapidly and have been circulating in Brazil for a more extended period. The study findings revealed that SARS-CoV-2 variants with E484K mutation are widely distributed in many regions in Brazil. The mutation was introduced in Brazil in October 2020. Because the E484K mutation is found in different viral lineages simultaneously with other mutations, the scientists suggest that this particular amino acid substitution may act as a common driving force for viral evolution in different genetic variants of SARS-CoV-2.

The N501Y mutation also occurs in the virus' RBD. This mutation is associated with increased binding specificity and faster-growing lineages. This mutation is present in the P.1 lineage but has not been detected in Brazil, except in the two cases from a distinct B.1.1.7 lineage (Claro et al. 2020 31) and a single B.1 sequence from northeast Brazil (Paiva et al. 2020 8). The frequency of N501Y in the P.1 lineage was 100% (n=7/7 genomes with information at position of interest).

[45,46]

This finding will help in further understanding the role of sucharising mutations in modulating immunogenicity, viral tropism and pathogenesis of the disease, which in lieuwill help in designing vaccine more precisely to mitigate pandemic COVID-19.

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[43] Perdiguero, B.; Esteban, M. Emerging SARS-CoV-2 Variants and Impact in Global Vaccination Programs against SARS-CoV-2/COVID-19. Vaccines 2021, 9, 243.

[44] Structural Analysis of Spike Protein Mutations in the SARS-CoV-2 P.3 Variant

Neil Andrew D. Bascos, Denise Mirano-Bascos, Cynthia P. Saloma

bioRxiv 2021.03.06.434059; doi: https://doi.org/10.1101/2021.03.06.434059

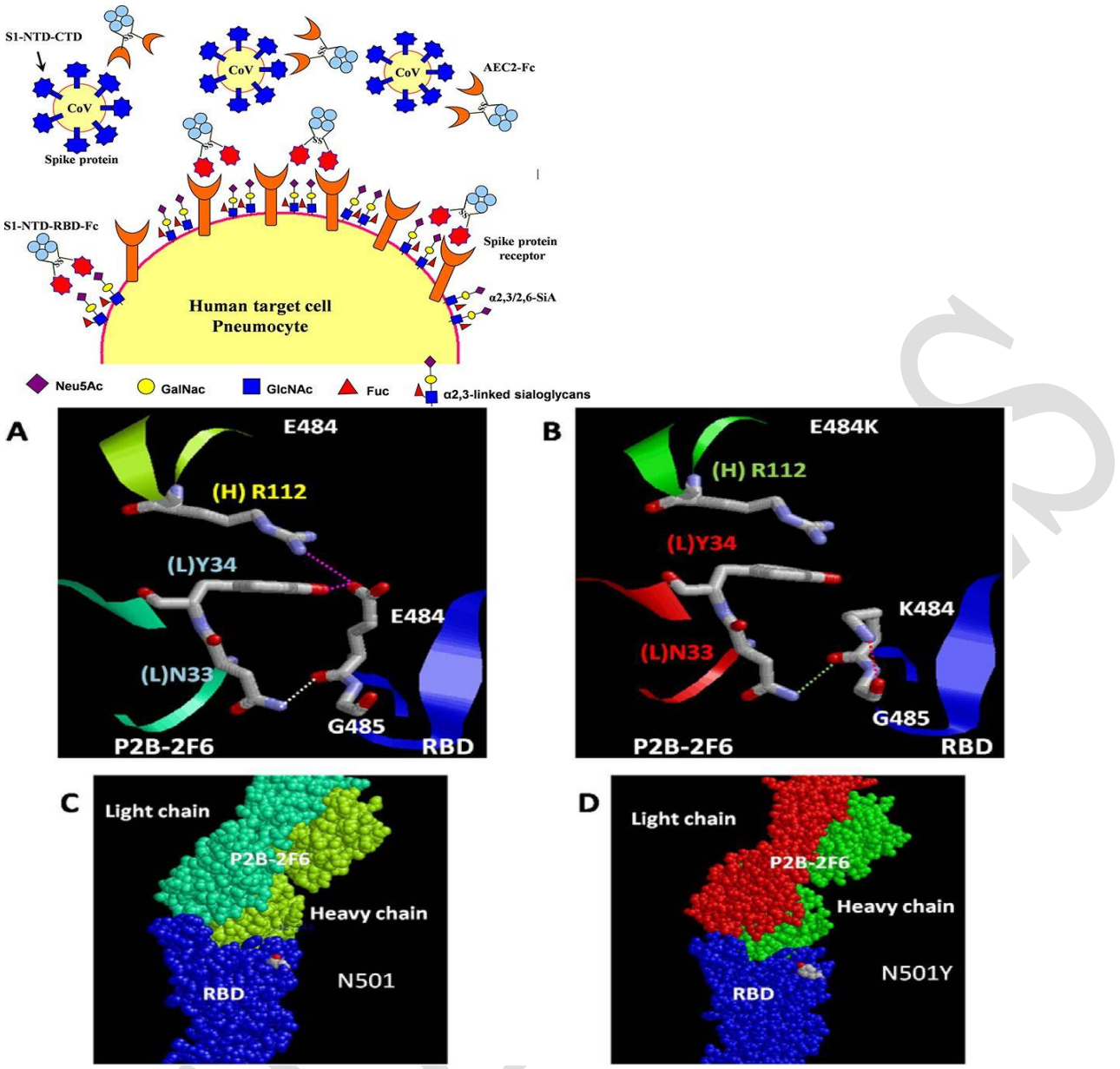
[45] Wang, P., Nair, M.S., Liu, L. *et al.* Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. *Nature* **593,** 130–135 (2021). https://doi.org/10.1038/s41586-021-03398-2

[46] Vinícius Bonetti Franceschi, Patrícia Aline Gröhs Ferrareze, Ricardo Ariel Zimerman, Gabriela Bettella Cybis, Claudia Elizabeth Thompson

medRxiv 2021.03.08.21253152; doi: https://doi.org/10.1101/2021.03.08.21253152

As per the following table comparison between wild type and mutant spike glycoproteins shows approximate same binding energy. Comp1 and Comp2 shows good binding energy as compare to wild type. As per the result and analysis except Comp4 others shows good result and useful for the inhibition of particular protein.

SN	Ligand Name	Wild type Spike glycoprotein (6VYB)	Mutant Spike Glycoprotein E484K
1	COMP 1	-6.82	-7.18
2	COMP 2	-6.06	-6.56
3	COMP 3	-6.72	-6.83
4	COMP 4	-3.09	-3.13
5	COMP 5	-4.52	-4.41
6	COMP 6	-4.90	-4.72
7	COMP 7	-5.43	-5.62
8	COMP 8	-5.23	-6.92
9	COMP 9	-9.42	-6.79



9.3 P681H Mutation-

B.1.1.7 viruses have also been shown to have a **P681H** mutation in the cleavage site of spike protein. This location is one of the residues that make up the furin cleavage site between S1 and S2 in spike. The S1/S2 furin cleavage site has been shown in animal models to promote viral entry into respiratory epithelial cells and transmission. The spike proteins of this lineage have also been shown to have a deletion at amino acids. This mutation in the receptor binding domain of spike is a recurrent deletion that has been found in various lineages associated with SARS-CoV-2 Outside of spike, a Q27 stop mutation truncates the ORF8 protein of the virus, rendering the protein inactive. An ORF8 deletion at amino acid 382 has a mild effect on virus replication in human airway cells The B.1.1.7. lineage also has five synonymous mutations in ORF1ab and one synonymous mutation in the M gene.[47],[48]

[47] Erol, Adnan. "Are the emerging SARS-COV-2 mutations friend or foe?." *Immunology letters* vol. 230 (2021): 63-64. doi:10.1016/j.imlet.2020.12.014

Hawaiian SARS-CoV-2 strains that were deposited in the GenBank in March 2020 clustered with sequences from Wuhan, China, Sweden, and the state of New York (USA). Moreover, phylogenetic tree results suggest that the virus has been brought to Hawaii from many sources. Thirteen single nucleotide polymorphisms were decoded across 13 unique SARS-CoV-2 genomes within the S gene region – with one non-synonymous mutation (P681H) detected in the two Hawaii strains.

The P681H mutation is shared in VOC-202012/01, but has emerged spontaneously several times earlier also, and there is no evidence to indicate it contributing to increased transmission of the virus in Nigeria. P681H is immediately juxtaposed to the amino [49]acid 682-685, furin cleavage site, identified at the S1/S2 linkage site, which has been predicted to enhance systemic infection based on bioinformatic analysis, and increased membrane fusion in laboratory experiments. The relevance of this to human infection is not known. This lineage has also been indirectly associated with higher virus load in samples tested by an assay using RT-qPCR and increased transmissibility. It has been hypothesized (but not proven) that this lineage may have resulted from the transmission of the virus from a chronically infected individual.

The P681H mutation is also characteristic of the new SARS-CoV-2 variants from the United Kingdom and Nigeria. SARS-CoV-2 sequences by the P681H mutation to create a ratio of sequences containing the P681H mutation to all sequences reported in the GISAID database for a given month. Inclusion criteria were for sequences providing a full month, day, and year. The D614G mutation underwent assessment in the same manner for comparison. All prevalence data converted into ratio underwent a logarithmic transformation.[50]

This finding will help in further understanding the role of sucharising mutations in modulating immunogenicity, viral tropism and pathogenesis of the disease, which in lieuwill help in designing vaccine more precisely to mitigate pandemic COVID-19.

As per the following table comparison between wild type and mutant spike glycoproteins shows approximate same binding energy. Comp1 and Comp2 shows good binding energy as compare to wild type. As per the result and analysis except Comp4 others shows good result and useful for the inhibition of particular protein. [51]

[48] Deng, X., Garcia-Knight, M. A., Khalid, M. M., Servellita, V., Wang, C., Morris, M. K., Sotomayor-González, A., Glasner, D. R., Reyes, K. R., Gliwa, A. S., Reddy, N. P., Sanchez San Martin, C., Federman, S., Cheng, J., Balcerek, J., Taylor, J., Streithorst, J. A., Miller, S., Kumar, G. R., Sreekumar, B., … Chiu, C. Y. (2021). Transmission, infectivity, and antibody neutralization of an emerging SARS-CoV-2 variant in California carrying a L452R spike protein mutation. *medRxiv : the preprint server for health sciences*, 2021.03.07.21252647. https://doi.org/10.1101/2021.03.07.21252647

[49] Stefanelli, Paola et al. "Whole genome and phylogenetic analysis of two SARS-CoV-2 strains isolated in Italy in January and February 2020: additional clues on multiple introductions and further circulation in Europe." *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* vol. 25,13 (2020): 2000305. doi:10.2807/1560-7917.ES.2020.25.13.2000305

[50] Maison DP, Ching LL, Shikuma CM, Nerurkar VR. Genetic Characteristics and Phylogeny of 969-bp S Gene Sequence of SARS-CoV-2 from Hawaii Reveals the Worldwide Emerging P681H Mutation. Preprint. *bioRxiv*. 2021;2021.01.06.425497. Published 2021 Jan 7. doi:10.1101/2021.01.06.425497

[51] Li, YD., Chi, WY., Su, JH. *et al.* Coronavirus vaccine development: from SARS and MERS to COVID-19. *J Biomed Sci* **27,** 104 (2020). https://doi.org/10.1186/s12929-020-00695-2

SN	Ligand Name	Wild type Spike glycoprotein (6VYB)	Mutant Spike GlycoproteinP681H
1	COMP 1	-6.82	-6.80
2	COMP 2	-6.06	-6.17
3	COMP 3	-6.72	-6.92
4	COMP 4	-3.09	-4.76
5	COMP 5	-4.52	-4.43
6	COMP 6	-4.90	-6.07
7	COMP 7	-5.43	-6.24
8	COMP 8	-5.23	-6.03
9	COMP 9	-9.42	-6.94

9.4 D614G Mutation-

The spike aspartic acid-614 to glycine (D614G) substitution is prevalent in global severe acute respiratory syndrome coronavirus 2 SARS-CoV-2 strains. The variant exhibits more efficient infection, replication, and competitive fitness in primary human airway epithelial cells but maintains similar morphology and in vitro neutralization properties, compared with the ancestral wild-type virus. Infection of human angiotensin-converting enzyme 2 (ACE2) transgenic mice and Syrian hamsters with both viruses resulted in similar viral titers in respiratory tissues and pulmonary disease. However, the D614G variant transmits significantly faster and displayed increased competitive fitness than the wild type virus in hamsters. The data show that the D614G substitution enhances SARS-CoV-2 infectivity, competitive fitness, and transmission in primary human cells and animal models.[52]

The D614G mutation is associated with the B.1 lineage of SARS-CoV-2 which now dominates the global pandemic, based upon global SARS-CoV-2 genome sequences shared via GISAID Retrospectively sampled viruses suggest this mutation was present in Guangzhou, Sichuan, and Shanghai Provinces, China in late January. In Europe, the 614G variant was first observed in genomes sampled on January 28 in a small outbreak in Bavaria, Germany, which was

52

Hou YJ, Chiba S, Halfmann P, Ehre C, Kuroda M, Dinnon KH 3rd, Leist SR, Schäfer A, Nakajima N, Takahashi K, Lee RE, Mascenik TM, Graham R, Edwards CE, Tse LV, Okuda K, Markmann AJ, Bartelt L, de Silva A, Margolis DM, Boucher RC, Randell SH, Suzuki T, Gralinski LE, Kawaoka Y, Baric RS. SARS-CoV-2 D614G variant exhibits efficient replication ex vivo and transmission in vivo. Science. 2020 Dec 18;370(6523):1464-1468. doi: 10.1126/science.abe8499. Epub 2020 Nov 12. PMID: 33184236; PMCID: PMC7775736.

initiated by a visitor from Shanghai and subsequently controlled through public health efforts. It is therefore likely that the D614G mutation occurred in China before being introduced on multiple occasions to European countries where it increased in frequency. This scenario is consistent with the rapid increase in February and March of European virus genomes that carry the 614G variant. In the United Kingdom, the first observation of a genome carrying the D614G mutation was in a sample collected on February 28 from a patient in Scotland who had recently traveled through Italy.[53]

The D614G mutation in SARS-CoV-2, a non-synonymous mutation resulting in a replacement of aspartic acid with glycine at position 614 of the virus's spike protein (D614G). The trimeric spike protein, composed of subunits S1 and S2, is a large glycoprotein that mediates cell entry and has been studied extensively in other corona viruses, including SARS-CoV and Midde East respiratory syndrome (MERS). SARS-CoV-2 spike protein binds to angiotensin-converting enzyme 2 (ACE2) to gain cell entry, hence mutations in this gene have the potential to alter receptor binding affinity and infectivity, as well as viral immune evasion and immunogenicity.

During the first wave of COVID-19 cases 71 percent of the SARS-CoV-2 particles identified in patients in Houston had the D614G mutation. However, in the second wave of the outbreak during the summer, this variant had 99.9 percent prevalence. The researchers say this finding mirrors a trend observed around the world; a study published in July based on more than 28,000 genome sequences found that variants carrying the D614G mutation became the globally dominant form of SARS-CoV-2 in about a month.[54]

This finding will help in further understanding the role of sucharising mutations in modulating immunogenicity, viral tropism and pathogenesis of the disease, which in lieuwill help in designing vaccine more precisely to mitigate pandemic COVID-19.

As per the following table comparison between wild type and mutant spike glycoproteins shows approximate same binding energy. Comp1 and Comp2 shows good binding energy as compare to wild type. As per the result and analysis except Comp4 others shows good result and useful for the inhibition of particular protein.

[53] Korber B, Fischer WM, Gnanakaran S, Yoon H, Theiler J, Abfalterer W, Hengartner N, Giorgi EE, Bhattacharya T, Foley B, Hastie KM, Parker MD, Partridge DG, Evans CM, Freeman TM, de Silva TI; Sheffield COVID-19 Genomics Group, McDanal C, Perez LG, Tang H, Moon-Walker A, Whelan SP, LaBranche CC, Saphire EO, Montefiori DC. Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus. Cell. 2020 Aug 20;182(4):812-827.e19. doi: 10.1016/j.cell.2020.06.043. Epub 2020 Jul 3. PMID: 32697968; PMCID: PMC7332439.

[54] Zhang L, Jackson CB, Mou H, et al. The D614G mutation in the SARS-CoV-2 spike protein reduces S1 shedding and increases infectivity. Preprint. *bioRxiv*. 2020;2020.06.12.148726. Published 2020 Jun 12. doi:10.1101/2020.06.12.148726

Table: Interaction(Binding energy) with Vedicinal 9

SN	Ligand Name	Wild type Spike glycoprotein (6VYB)	Mutant Spike Glycoprotein (7KDJ)
1	COMP 1	-6.82	-7.08
2	COMP 2	-6.06	-7.06
3	COMP 3	-6.72	-6.41
4	COMP 4	-3.09	-2.87
5	COMP 5	-4.52	-4.34
6	COMP 6	-4.90	-4.83
7	COMP 7	-5.43	-5.40
8	COMP 8	-5.23	-5.14
9	COMP 9	-9.42	-8.57

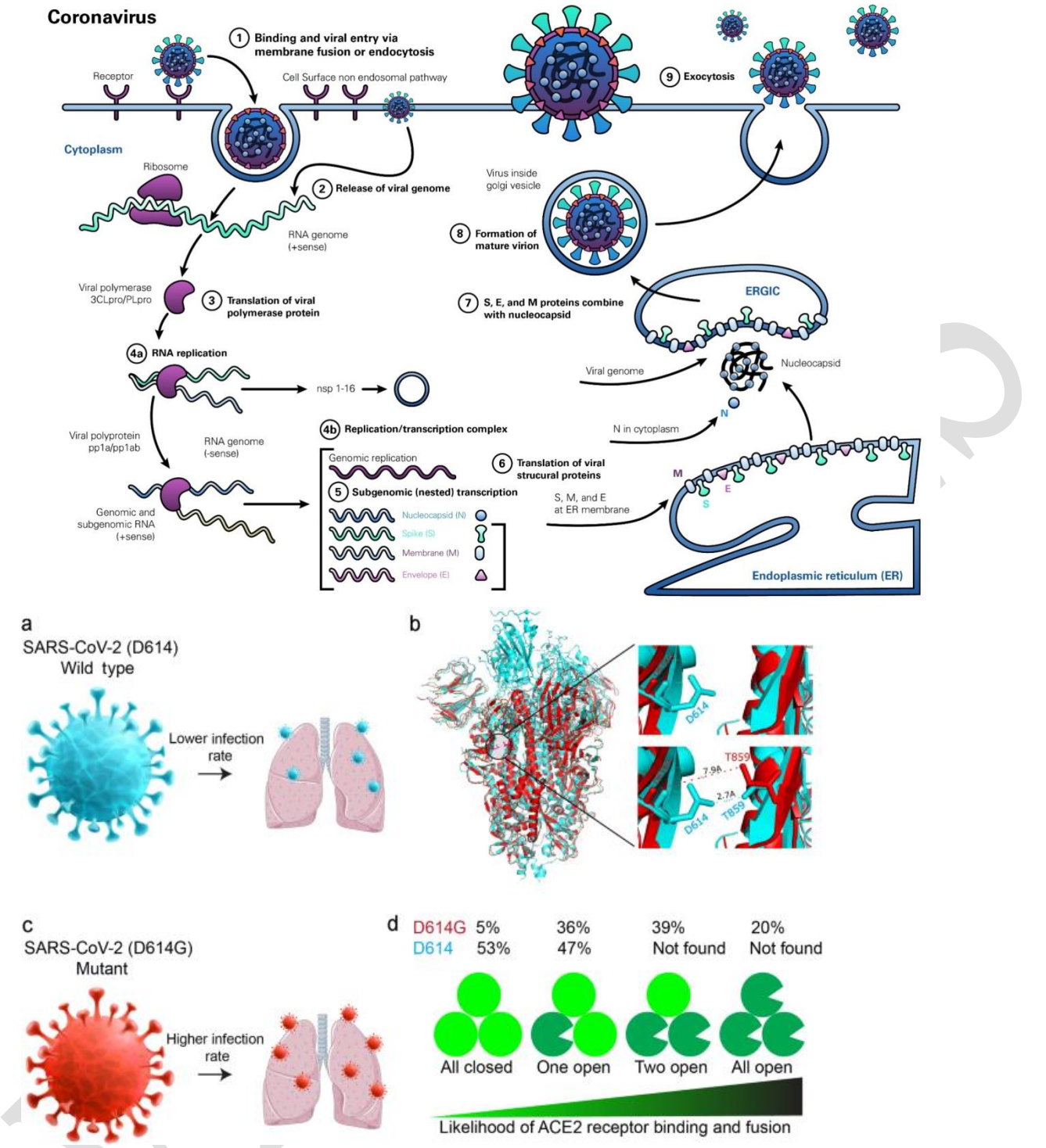
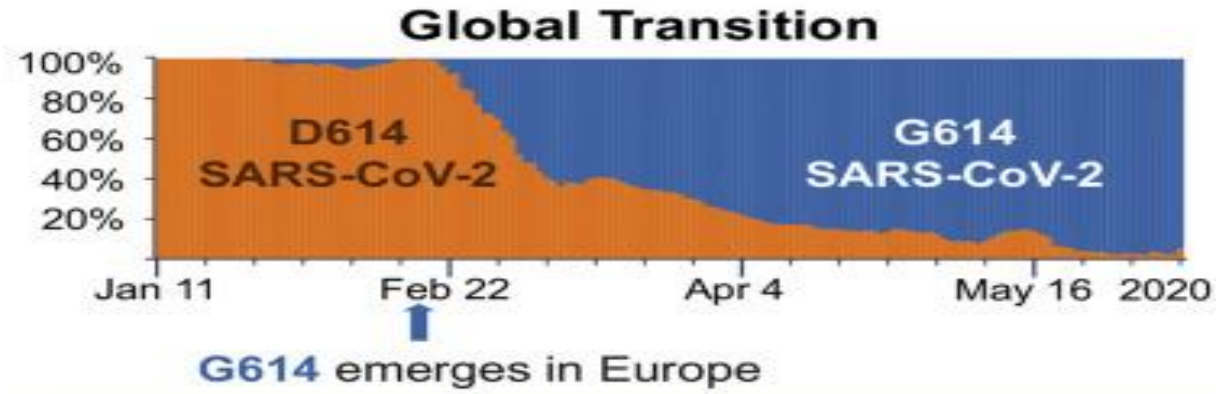
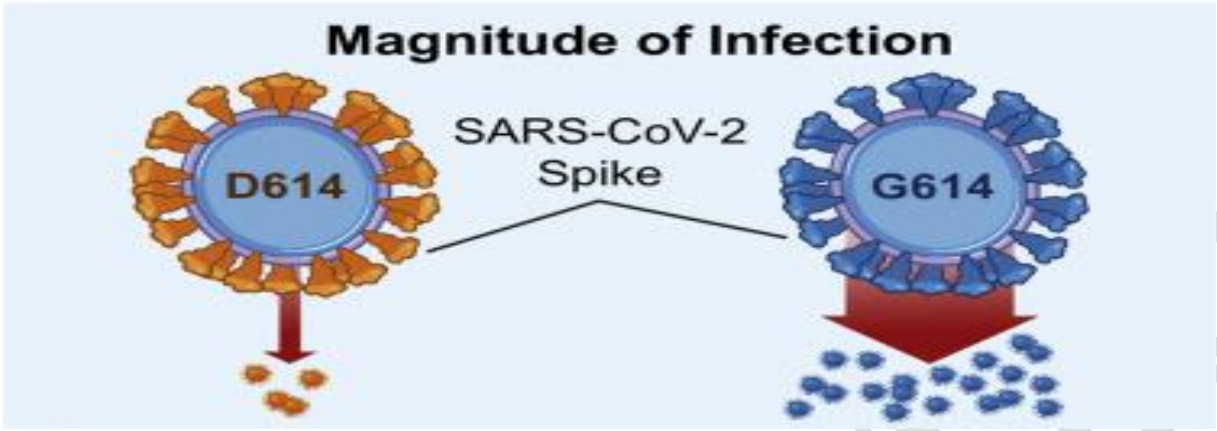


Fig 16: D614G Mutation Percentage





9.5 <u>G476S Mutation</u>-

The substitution of Gly-476 to Ser-476 in the SARS-CoV-2 Receptor-binding domain (RBD) largely affected the structural dynamics of the S-protein leading to significant influence on the interactions with ACE-2 and neutralizing antibodies. Structural properties of the S-protein such as conformation changes, residual fluctuations and residue surface area largely varied between the wild-type and G476S variant, especially in the RBD. Analyses of the interaction energies between S-protein and ACE-2 suggest that the G476S variant may have enhanced interactions with ACE-2 compared to the wild-type. The G476S variant was found to have weaker interactions with the neutralizing antibody H014 compared to the wild-type.[55]

Using integrative computational analyses investigated the structural dynamics of the G476S variant of SARS-CoV-2 and assessed the influence of this mutation on the interactions with ACE-2 and neutralizing antibodies. The substitution of Gly-476 to Ser-476 in the RBD of SARS-CoV-2 S-protein has serious implications with respect to the infectivity and antigenicity of the virus. In the case of viral infectivity, which is initiated by an interaction with the host receptor ACE-2, the G476S variant showed stronger interactions with the ACE-2 compared to the wild-type. On the neutralization of the virus by antibodies, the results suggest that the influence is dependent on the epitopes recognized by the antibody. The surveillance of the G476S mutations should be increased to track the distribution and spread of the G476S variant.[56]

Epitope mapping analysis revealed G476S mutation as antigenic determinants and thus the mutations are important while designing a therapeutic vaccine or chimeric antibody. The findings will help in further understanding the role of

[55] Kwarteng, Alexander & Asiedu, Ebenezer & Annan, Augustina. (2020). Computational analyses of the G476S variant of SARS-CoV-2: A focus on the interaction with human ACE-2 and neutralizing antibodies. 10.21203/rs.3.rs-98463/v1.

[56] Ivan Mercurio, Vincenzo Tragni, Francesco Busco, Anna De Grassi, Ciro Leonardo Pierri

bioRxiv 2020.04.17.046185; doi: https://doi.org/10.1101/2020.04.17.046185

such arising mutations in modulating immunogenicity, viral tropism and pathogenesis of the disease, which in lieu will help in designing vaccine more precisely to mitigate pandemic COVID-19.

There are 9 changes that directly affected the effective epitope:

(1) the previously likely epitopes at site of 62~75 and 459~464 were deleted.

(2) The antigenicity of epitope at site of 216~221 was decreased below the threshold which cannot be an effective epitope.

(3) The antigenicity of formerly ineffective epitope was elevated to be a effective epitope at site of 314~321.

(4) Two epitopes (372~374+384~390) fused into a new epitope (368~390), but its antigenicity was lower than the epitope before.

(5) Three alterations of epitopes improved their antigenicity with site 406~417,440~450 and 657~663.

(6) The epitope at site 486~492 was reduced antigenicity slightly. Therefore, 9 effective epitopes have predicted after G476S mutation, in which the amount of epitope-changing is the most, and the overall antigenicity was decreased.[57]

9.6 N501Y Mutation-

New severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) lineages carrying the amino acid substitution N501Y in the receptor-binding domain (RBD) of the spike protein have spread rapidly in the United Kingdom (UK) during late autumn 2020. The [58]most concerning mutation is N501Y, which co-occurs with several mutations of potential biological importance, including P681H and deletion of the amino acid at the 69th and 70th residues (Δ69/Δ70) on the spike protein. It has recently been reported that this variant is rising in frequency in the South-east of England so fast as to raise the suspicion that it has increased transmissibility.

Researchers after study investigate this aspect of the virus in terms of increased viral load. The researchers sequenced all positive samples from four Lighthouse laboratories in the UK, using their quantitative sequencing approach. This yields the number of unique mapped reads, which bears a correlation with and therefore acts as a proxy for the viral load.

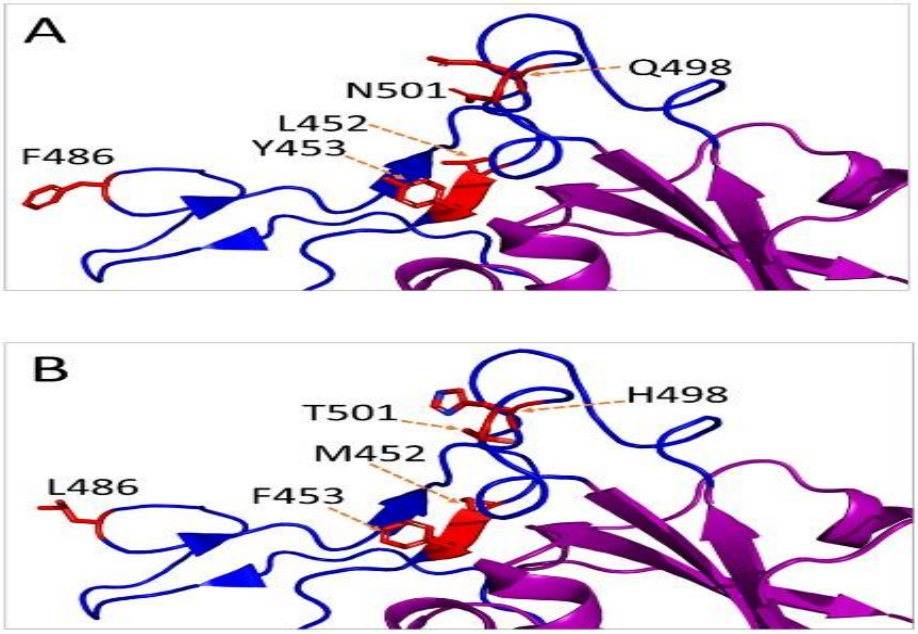
They found that the logarithm of unique mapped reads was negatively correlated with the Ct values obtained from the polymerase chain reaction (PCR) testing. They selected the presence of Y501 as a marker of the new variant. From 88 samples that showed this mutation, they considered only the samples taken in the period from October 31 to November 13, 2020. This showed that the number of unique mapped reads in the Y501 variant was more significant

[57] Jose L. Sanchez-Trincado, Marta Gomez-Perosanz, Pedro A. Reche, "Fundamentals and Methods for T- and B-Cell Epitope Prediction", *Journal of Immunology Research*, vol. 2017, Article ID 2680160, 14 pages, 2017. https://doi.org/10.1155/2017/2680160

[58] Leung K, Shum MH, Leung GM, Lam TT, Wu JT. Early transmissibility assessment of the N501Y mutant strains of SARS-CoV-2 in the United Kingdom, October to November 2020. *Euro Surveill*. 2021;26(1):2002106. doi:10.2807/1560-7917.ES.2020.26.1.2002106

than in the N501 variant, indicating that the median viral loads are increased by about three times for the Y501 variant.[59]

The mutation N501 at the receptor binding domain of the S protein results in the change of asparagine to tyrosine residue at position 501 (N501Y). This mutation is also present in the newly emerging SARS-CoV-2 variant viruses reported in the U.K. (20B/501Y.V1, B1.1.7 lineage) that is epidemiologically associated with high human to human transmission. The mutation is of particular concern because it is located in the viral receptor binding site for cell entry, increases binding to the receptor (angiotensin converting enzyme 2), and enables the virus to expand its host range to infect mice.[60,61]



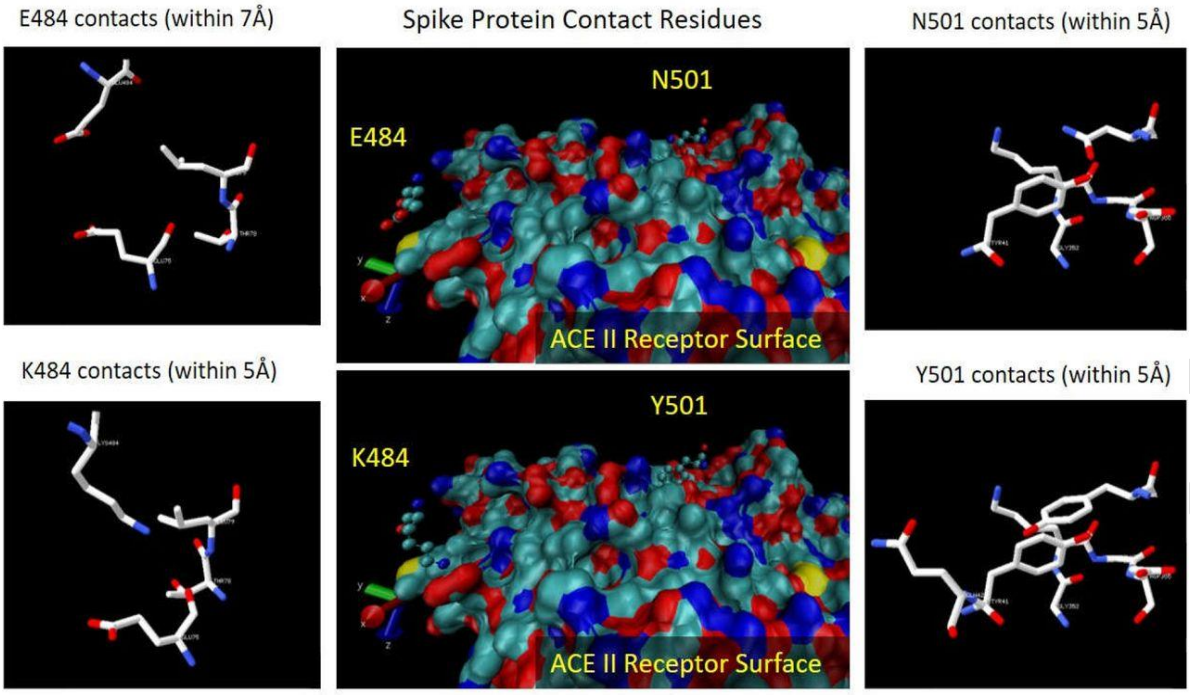
The reasons for the faster growth associated with the variant are not clear: it could be due to faster epidemic growth, demographic patterns, founder effects, or higher viral loads, among other biological mechanisms. The correlation with higher viral loads, in case, seems to suggest increased transmissibility of this virus, but further studies are required. Again, the N501Y mutation may not be the only reason for this expansion. There is more need to understand how viral levels are related to virulence since this may determine the infection's severity.[62]

[59] Borges V, Isidro J, Cortes-Martins H, et al. Massive dissemination of a SARS-CoV-2 Spike Y839 variant in Portugal. *Emerg Microbes Infect.* 2020;9(1):2488-2496. doi:10.1080/22221751.2020.1844552

[60] Tang JW, Toovey OTR, Harvey KN, Hui DDS. Introduction of the South African SARS-CoV-2 variant 501Y.V2 into the UK. *J Infect.* 2021;82(4):e8-e10. doi:10.1016/j.jinf.2021.01.007

[61] Raveen Rathnasinghe, Sonia Jangra, Anastasija Cupic, Carles Martínez-Romero, Lubbertus C.F. Mulder, Thomas Kehrer, Soner Yildiz, Angela Choi, Ignacio Mena, Jana De Vrieze, Sadaf Aslam, Daniel Stadlbauer, David A. Meekins, Chester D. McDowell, Velmurugan Balaraman, Juergen A. Richt, Bruno G. De Geest, Lisa Miorin, PVI study group, Florian Krammer, Viviana Simon, Adolfo García-Sastre, Michael SchotsaertmedRxiv 2021.01.19.21249592; doi: https://doi.org/10.1101/2021.01.19.21249592

[62] Hu, B., Guo, H., Zhou, P. *et al.* Characteristics of SARS-CoV-2 and COVID-19. *Nat Rev Microbiol* **19,** 141–154 (2021). https://doi.org/10.1038/s41579-020-00459-7



9.7 K417N Mutation-

The accompanying K417N and E484K mutations in the South African strain provide a counterpoise to the spike's increased affinity due to the N501Y mutation. They prevent the formation of two salt bridges that help to form and stabilize the RBD-ACE2 complex. This reduces ACE2 binding affinity. Thus, this strain is less infectious and less rapidly spreading than the UK strain. This is even though both share the latter substitution.[63]

The stronger binding to ACE2 caused by the substitution N501Y, the South African 501.V2 variant that has undergone two additional mutations (K417N and E484K) is unlikely to exhibit an increased infectivity and likely transmissibility."

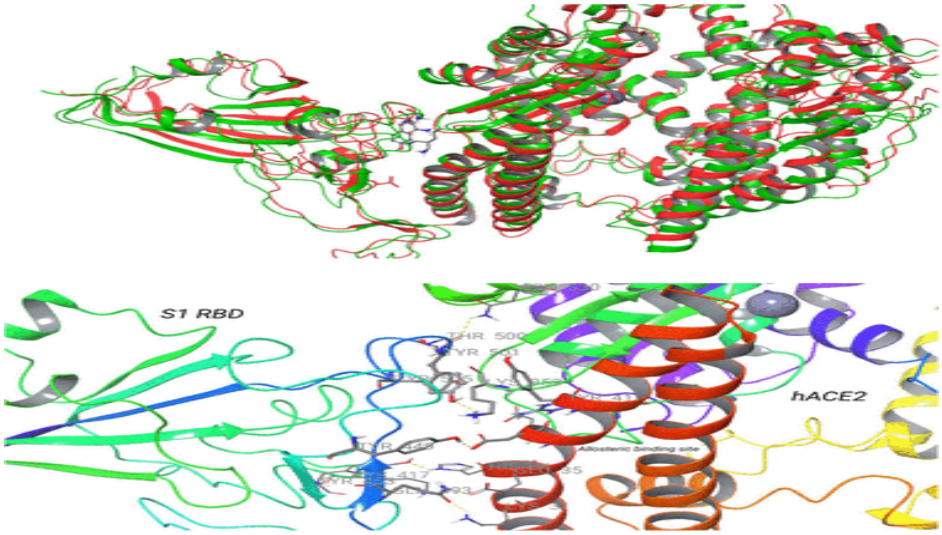
Deep mutational scanning suggests that the K417N mutation has minimal impact on binding affinity to hACE235. The spike RBD is the main target of neutralizing antibodies (NAbs) elicited during SARSCoV-2 infection. NAbs to the RBD can be broadly divided into four main classes. Of these, class 1 and class 2 antibodies appear to be most frequently elicited during SARS-CoV2 infection, and their epitopes directly overlap the hACE2 binding site. Class 1 antibodies have

63
Houriiyah Tegally, Eduan Wilkinson, Marta Giovanetti, Arash Iranzadeh, Vagner Fonseca, Jennifer Giandhari, Deelan Doolabh, Sureshnee Pillay, Emmanuel James San, Nokukhanya Msomi, Koleka Mlisana, Anne von Gottberg, Sibongile Walaza, Mushal Allam, Arshad Ismail, Thabo Mohale, Allison J Glass, Susan Engelbrecht, Gert Van Zyl, Wolfgang Preiser, Francesco Petruccione, Alex Sigal, Diana Hardie, Gert Marais, Marvin Hsiao, Stephen Korsman, Mary-Ann Davies, Lynn Tyers, Innocent Mudau, Denis York, Caroline Maslo, Dominique Goedhals, Shareef Abrahams, Oluwakemi Laguda-Akingba, Arghavan Alisoltani-Dehkordi, Adam Godzik, Constantinos Kurt Wibmer, Bryan Trevor Sewell, José Lourenço, Luiz Carlos Junior Alcantara, Sergei L Kosakovsky Pond, Steven Weaver, Darren Martin, Richard J Lessells, Jinal N Bhiman, Carolyn Williamson, Tulio de OliveiramedRxiv 2020.12.21.20248640; doi: https://doi.org/10.1101/2020.12.21.20248640

a VH3-53 restricted mode of recognition centred on [64]spike residue K417. The K417N mutation would abolish key interactions with class 1 NAbs, and likely contributes toward immune evasion at this site.

Single Spike RBD-RBM amino acid substitutions, which were 310 found in other new SARS-CoV-2 Spike variants, namely the 501.V2 variant from South Africa 311 (K417N).

Results predicted that the combined effect of the three amino acid 322 substitutions N501Y, K417N and E484K was less than that of the single N501Y (Fig. 5A) in terms 323 of increased computed affinity for ACE2 (from -50.26 to -56.37 Kcal/mol as compared to -67.49 324 Kcal/mol of N510Y). According to research, the addition of K417N mutation led to a dramatic decrease of the STE90-C11 antibody binding to virus's S1 RBD. The default FEP sampling protocol calculated a ΔΔG value of 5.74 kcal/mol. In the case when both N501Y and K417N mutations are present at the same time this value further increased to 8.61 kcal/mol but this simulation was not well converged and after its extension to 15 ns we obtained a value of 5.83 kcal/mol. Thus, it seems that the effect of these mutations is not additive and only K417N mutation can abolish the interaction with STE90-C11 antibody. These results also suggest that even well tolerated to mutations, antibodies eventually would be resisted to this variant of SARS-CoV-2. 4). The K417N mutation also increases the S1 RBD binding to ACE2, however by only -0.39 kcal/mol.



[64] : Villoutreix, B.O.; Calvez, V.; Marcelin, A.-G.; Khatib, A.-M. In Silico Investigation of the New UK (B.1.1.7) and South African (501Y.V2) SARS-CoV-2 Variants with a Focus at the ACE2–Spike RBD Interface. Int. J. Mol. Sci. 2021, 22, 1695. https:// doi.org/10.3390/ijms22041695

9.8 N440K Mutation-

The N440K mutation is associated with greater binding affinity with human host receptors and is associated with greater infectivity ad transmission capability.
The genetically tweaked variant with a mutation named N440K has been found in nearly 34% of the 272 SARS-CoV-2 genomes analyzed from Andhra Pradesh in December 2020. The variant has also been seen in Karnataka, Maharashtra and Telangana.

For India, N440K high frequency variant was observed out of 19 immune escape variants. This mutation is located at C135 interaction interface. In wild type strain, N440 forms a strong H-bond network with D54 and weak H-bond networks with P52 and R55 of C135 antibody. Interestingly, it is observed 100% co-occurrence on N440K mutation along C64F mutation in membrane glycoprotein of SARS-CoV-2 apart from globally dominant D614G (S protein) and P323L (ORF1ab).

The calculated binding-free energy of mutant RBDs of spike protein complexed with human ACE2 revealed only four RBD mutant types (D364Y, N440K, N450K, S477R) displaying a much lower binding-free energy (ΔG), indicating a significantly higher affinity for the ACE2, which could influence the pathogenicity of SARS-CoV-2.

Ligands / Mutation	Baicalin	Quercetin	Luteolin	Rutin	Hesperidin	Curcumin	EGCG	Piperine	Glycyrrhizic
E484K	-7.18	-6.56	-6.83	-3.13	-4.41	-4.72	-5.62	-6.92	-6.79
V483G	-7.70	-7.29	-7.54	-4.35	-5.59	-6.97	-6.36	-7.72	-8.00
P681H	-6.80	-6.17	-6.92	-4.76	-4.43	-6.07	-6.24	-6.03	-6.94
K417N	-7.26	-7.35	-7.54	3.81	-6.65	-6.89	-6.89	-7.38	-7.28
D614G	-7.08	-7.06	-6.41	-2.87	-4.34	-4.83	-5.40	-5.14	-8.57
G476S	-7.43	-7.40	-7.01	-4.18	-5.06	-6.48	-6.80	-6.60	-7.76
N501Y	-7.39	-7.35	-7.06	-4.75	-5.72	-6.43	-7.25	-6.86	-7.88
N440K	-7.55	-7.96	-7.21	-4.91	-6.31	-6.98	-7.07	-7.40	-7.69

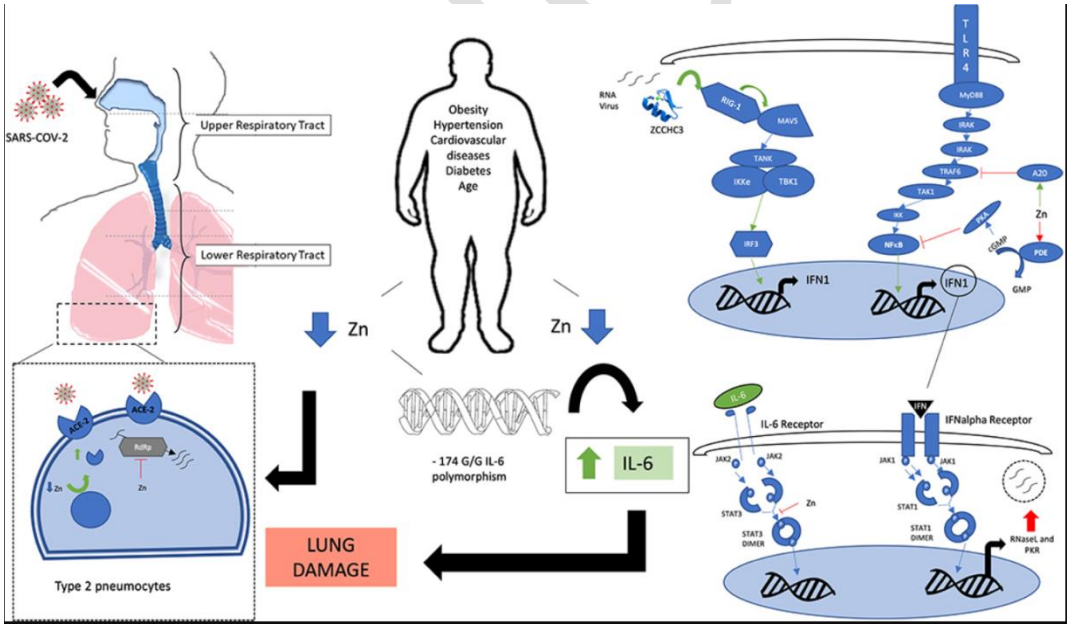
Table: Summarized Vedical 9 Interaction information against all Mutation

CHAPTER 2:Pathway Involved in Host cells/Receptors /Enzymes

Pathway 10th : Zinc Ionophores

Zinc deficiency may be present in up to 17% of the population worldwide. In mild SARS-CoV-2, with C-reactive protein (CRP) levels of 15 mg/L, a 10% decrease in zinc is observed. In severe infectious diseases, CRP levels can reach 100–200 mg/L, with a much greater decrease in zinc levels (40–60%).

Zinc also has a role in viral recognition. The zinc-finger protein ZCCHC3 binds RNA and facilitates the detection of intracellular RNA viruses by activating retinoic acid-inducible gene-I (RIG-1)-like receptors (RLRs), including RIG-I and MDA5. This action triggers the activation of the anti-viral response mediated by downstream activation of antiviral genes. In this process, kinases such as TBK1 and IκK further phosphorylate the interferon regulatory transcription factor 3 (IRF3) and IκB-alpha, the NK-κB inhibitor, leading to activation of IRF3 and NF-κB, which results in interferon type 1 upregulation. Interferon alpha-induced signaling results in upregulation of antiviral proteins (RNase L and PKR), known to degrade viral RNA and inhibit its translation. Zinc also exerts an inhibitory effect on the activation of NF-κB, through the expression of the A20 protein. A20 is a zinc-finger protein that negatively regulates tumor necrosis factor receptor (TNFR) and toll-like receptor (TLR)-initiated NF-κB pathways. Furthermore, zinc acts as an inhibitor of cyclic nucleotide phosphodiesterase (PDE). When PDE is inhibited, cyclic nucleotide cGMP (cyclic guanosine monophosphate) is elevated, leading to the activation of PKA (protein kinase A), and subsequent inhibition of NF-κB. Additionally, zinc supplementation has been shown to downregulate inflammatory cytokines by decreasing gene expression of IL-1β, TNF-alpha, and by inhibiting NF-κB activation. [65]



[65] Mayor-Ibarguren, A., & Robles-Marhuenda, Á. (2020). A hypothesis for the possible role of zinc in the immunological pathways related to COVID-19 infection. *Frontiers in immunology*, *11*, 1736.

Fig: **Zinc Ionophores**

IL-6 appears to be important in triggering severe lung damage during SARS-CoV-2 infection. Sustained elevation of IL-6 is postulated as being responsible for severe immune-mediated lung damage as well as for macrophage activation syndrome (MAS) that might overlap in patients with severe COVID-19.

As a virus, SARS-CoV2 is highly dependent on the metabolism of the host cell. It was suggested that zinc can prevent fusion with the host membrane, decreases the viral polymerase function, impairs protein translation and processing, blocks viral particle release, and destabilizes the viral envelope. Low-dose zinc supplementation together with small concentrations of the zinc ionophores pyrithione or hinokitol decreased RNA synthesis in influenza, poliovirus, picornavirus, the equine arteritis virus, and SARS-CoV by directly inhibiting the RNA-dependent RNA polymerase of the virus. There is evidence that zinc can enhance the effect of chloroquine, another known zinc ionophore, while zinc ionophores like epigallocatechin-gallate or quercetin. [66]

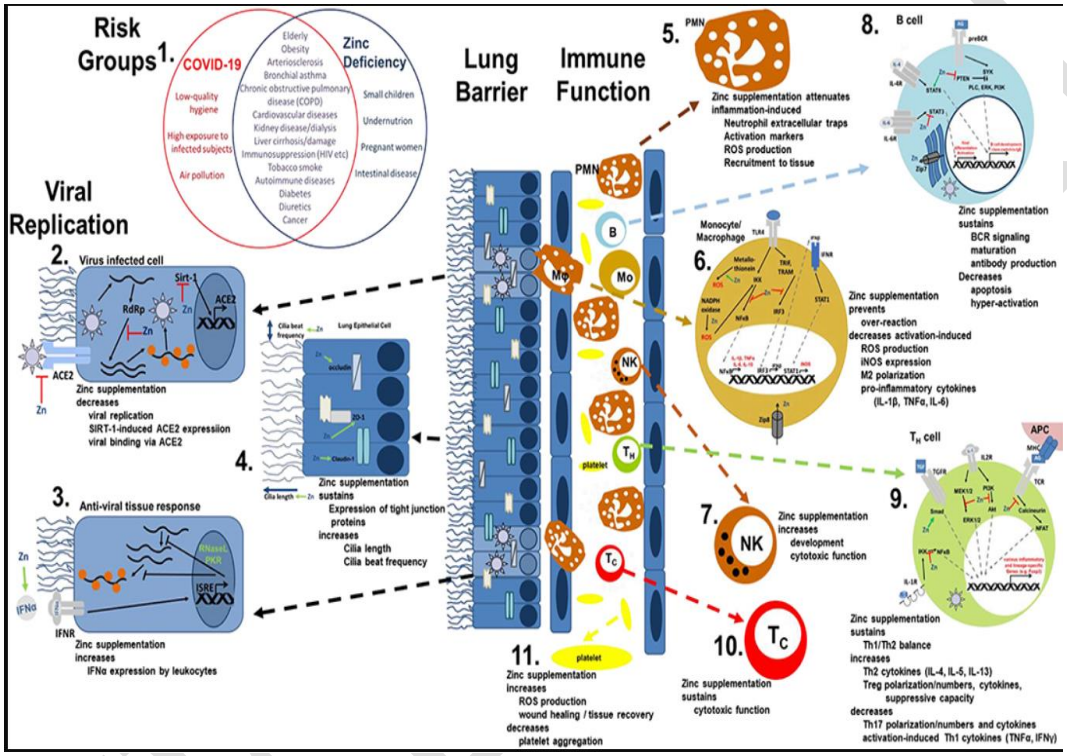


Fig:Mechanism via JAK/STAT1 signaling Pathway

A lack of adequate secretion of type I and type II interferons was reported for COVID-19 patients. For human interferon alpha (IFN-α) it was shown that zinc supplementation can reconstitute its expression by leukocytes and potentiates its anti-viral effect via JAK/STAT1 signaling as observed for rhinovirus-infected cells. However, as it was suggested that SARS-CoV2 might take advantage of the interferon-dependent expression of ACE2.

Zn^{2+} cations especially in combination with Zn ionophore pyrithione were shown to inhibit SARS-coronavirus RNA polymerase (RNA dependent RNA polymerase, RdRp) activity by decreasing its replication. Findings demonstrate that

[66] Wessels, I., Rolles, B., & Rink, L. (2020). The potential impact of zinc supplementation on COVID-19 pathogenesis. *Frontiers in immunology*, *11*, 1712.

chloroquine is a zinc ionophore increasing Zn^{2+} flux into the cell. Moreover, it is also proposed that chloroquine-mediate zinc influx may underlie anticancer activity of the compound. Similarly, it was hypothesized that increasing intracellular Zn^{2+} concentration by chloroquine may also mediate its antiviral effect against SARS-CoV-2. In this view zinc supplementation without chloroquine might have similar positive effects without adverse side-effects of chloroquine treatment. Hypothetically, such an effect may be also observed using other zinc ionophores like quercetin and epigallocatechin-gallate with substantially lower toxicity, although clinical trials supported by experimental *in vitro* studies are required to support this hypothesis. [67]

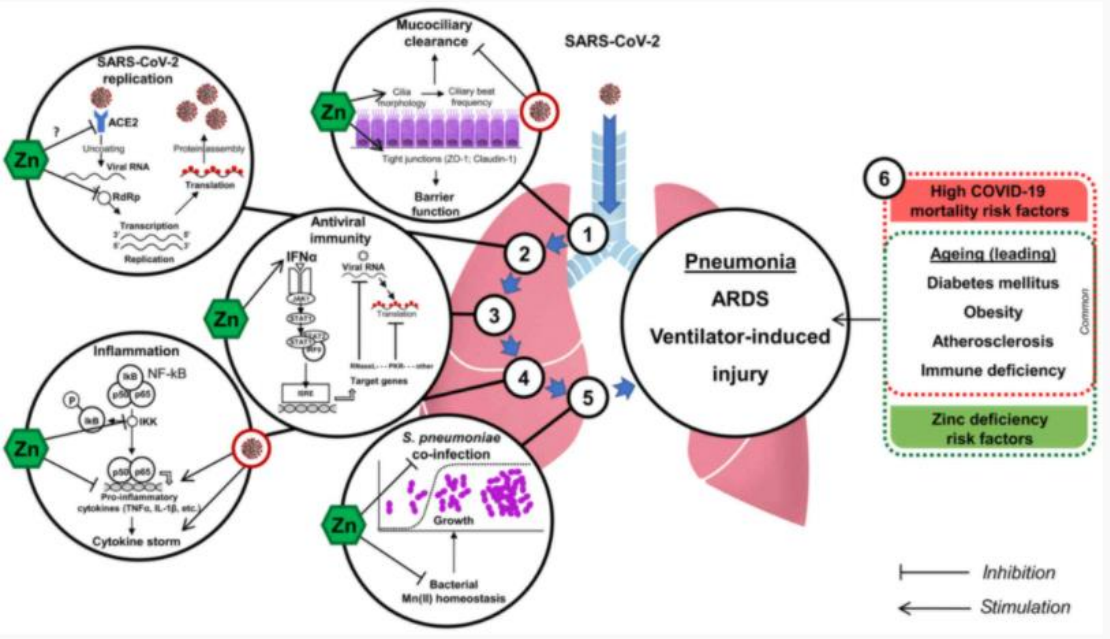


Fig: ARDS mediated Pathway

[67] Skalny, A. V., Rink, L., Ajsuvakova, O. P., Aschner, M., Gritsenko, V. A., Alekseenko, S. I., ... & Tinkov, A. A. (2020). Zinc and respiratory tract infections: Perspectives for COVID-19. *International journal of molecular medicine*, *46*(1), 17-26.

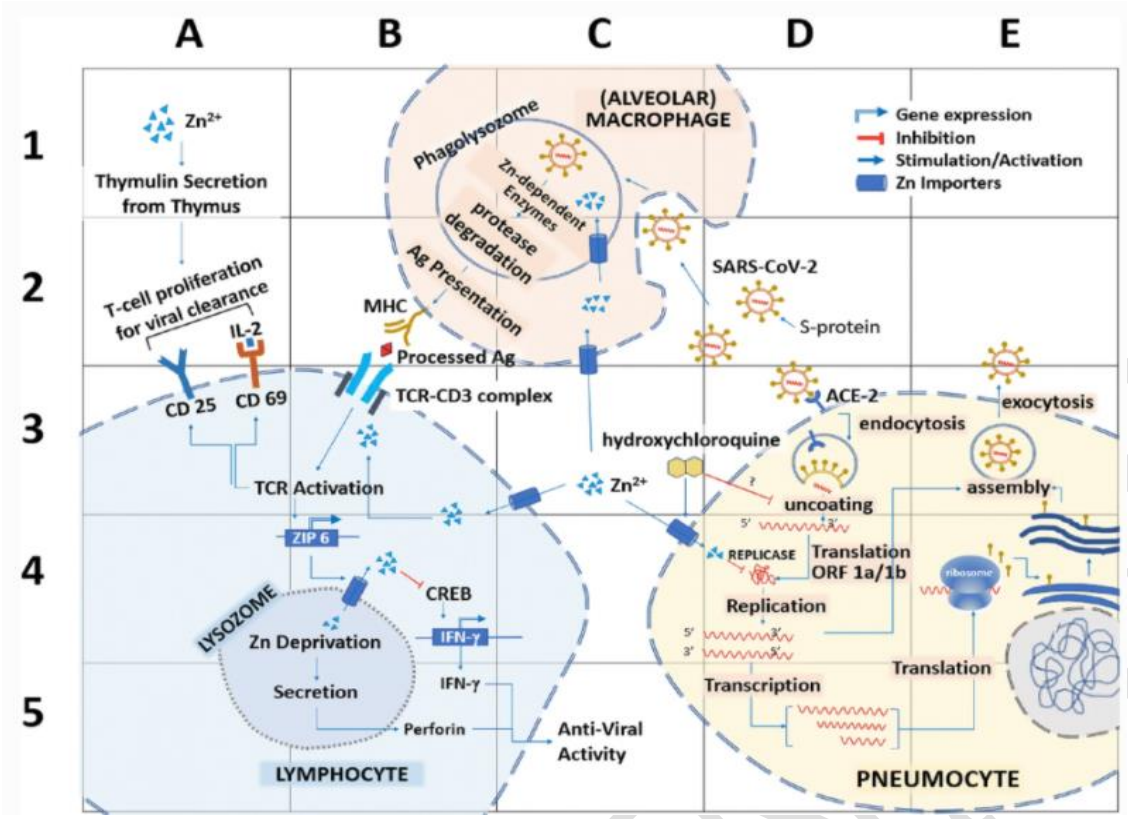


Fig: Potential sites of action of Zn

Above figure represents potential sites of action of Zn to counter SARS-CoV-2 in pneumocytes. SARS-CoV uses spike (S) proteins to bind to angiotensin-converting enzyme 2 (ACE2) on pneumocytes (D3). Virus enters the host cell through endocytosis and releases therein the viral RNA (D3). The replicase enzyme complex is translated from the viral genome that mediates both replication and transcription (D4). Virions are shed from the infected cell through exocytosis (E3). The primary site of Zn^{2+} could be the inactivation of the viral replicase (D4). At the same time, the additional Zn supplement might initiate interferon-© (a common anti-viral agent) production by T lymphocytes (B5). However, Zn deprivation in the lysosome of the lymphocytes triggers to secrete perforin, which also exert anti-viral activity (B5). A pool of Zn importing inside the T lymphocytes activates T cell receptors as well as CD25 and CD69 to aid T cell proliferation and stabilization (A2–3). Added Zn also contributes to the production of thymulin from the thymus and triggers T lymphocyte production (A1–2). In alveolar macrophages, Zn can help to degrade the phagocytosed viral particle by the enzymes of the phagolysosome (B-C2). That in turn will help to present the processed Antigen by the major histocompatibility complex (MHC) (B2). [68]

Role of Vedicinal: Our proposed formulation contains 6 compounds namely compounds 2 and 7 are inhibitors of Zinc Ionophores Proteindomain. In this study, three confirmations of this enzyme have separately docked with Quercetin and EGCG were found to offer good interaction with a binding energy and hydrogen bond interaction. Zinc Ionophores Protein is one of the most intriguing and promising drug targets for SARS-CoV-2 drug development.

[68] Rahman, M. T., & Idid, S. Z. (2020). Can Zn be a critical element in COVID-19 treatment?. *Biological trace element research*, 1-9.

Pathway 11: Dihydroorotate dehydrogenase

Pyrimidine de novo biosynthesis is an important biosynthetic pathway. The de novo pathway consists of six enzymes, which are encoded by single genes or are parts of larger multifunctional proteins. Dihydroorotate dehydrogenase (DHODH; EC 1.3.99.11) is the fourth enzyme of this pathway and catalyzes the conversion of dihydroorotate (DHO) to orotate. In most eukaryotes, DHODH is located at the inner mitochondrial membrane, facing the intermembrane space. For its activity, mitochondrial DHODH depends on a functional respiratory chain and requires ubiquinone as a direct electron acceptor. [69]

Pyrimidines serve as crucial building blocks for the biosynthesis of DNA, RNA, phospholipids, and glycoproteins, which is essential for the cell survival as well as proliferation. Human DHODH belongs to the class 2 DHODH family and is a flavin-dependent mitochondrial enzyme catalyzing the oxidation of dihydroorotate to orotate, the fourth step also a rate limiting step in the de novo biosynthesis of pyrimidine-based nucleotides. By consequence, DHODH is an attractive therapeutic target for multiple diseases including cancer and autoimmune diseases.[70]

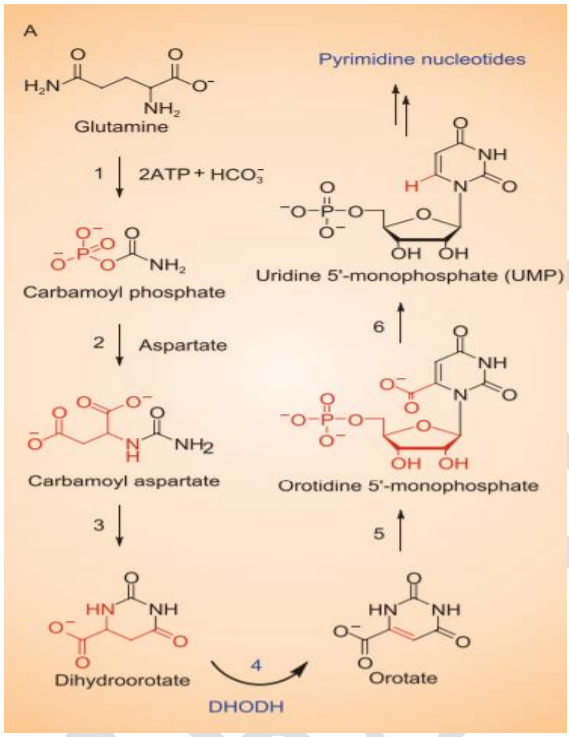


Fig. DHODH catalyzes the fourth step in the de novo pyrimidine biosynthesis pathway.

[69]Zameitat, E., Freymark, G., Dietz, C. D., Löffler, M., &Bölker, M. (2007). Functional expression of human dihydroorotate dehydrogenase (DHODH) in pyr4 mutants of Ustilago maydis allows target validation of DHODH inhibitors in vivo. *Applied and environmental microbiology*, *73*(10), 3371-3379.

[70]Xu, Y., & Jiang, H. (2020). Potential treatment of COVID-19 by inhibitors of human dihydroorotate dehydrogenase. *Protein & Cell*, *11*(10), 699-702.

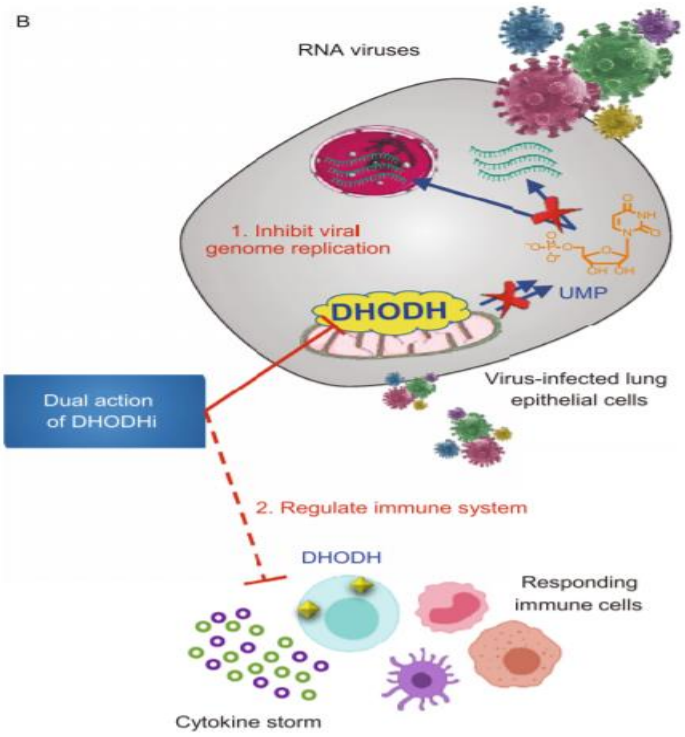


Fig. DHODH inhibitors (DHODHi) are broad-spectrum antivirals against RNA viruses with the dual action of inhibiting viral genome replication and regulating the immune system.

DHODH inhibitors prevent viral replication. Simplified depiction of de novo pyrimidine synthesis pathway and its role in viral replication depicted in Fig.3. A series of enzymes catalyse the 6-step pathway conversion of glutamine, bicarbonate (HCO3) and ATP into the final product, Uridine monophosphate (UMP), necessary for the production of pyrimidines. De novo pyrimidine synthesis is catalysed by the trifunctional CAD (carbamoyl phosphate synthase, aspartate carbamoyl transferase and dihydroorotase), DHODH and the bifunctional UMPS (orotate phosphoribosyltransferase and orotidine monophosphate decarboxylase). DHODH catalyses the fourth step, the oxidation of dihydroorotate to orotate, and is linked to the mitochondrial electron transport chain (ETC) via ubiquinone (UbQ) redox-cycling. Inhibition of DHODH leads to pyrimidine nucleosides depletion that primarily affects RNA virus replication. The decrease of pyrimidine pools further triggers host antiviral genes expression and promotes innate immune responses. Both responses are a consequence of the reduction of pyrimidines and cause inhibition of virus replication and infection.71

[71]Coelho, A. R., & Oliveira, P. J. (2020). Dihydroorotate dehydrogenase inhibitors in SARS-CoV-2 infection.

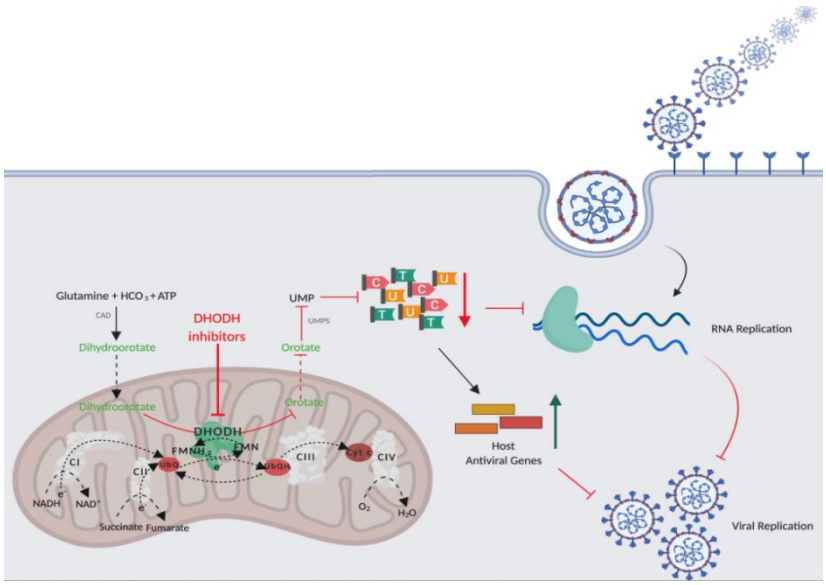


Fig.Dde novo pyrimidine synthesis pathway

Two of the most well-known DHODH inhibitors are brequinar and leflunomide. Brequinar competitively inhibits DHODH at the UbQ-binding site, while leflunomide, which is metabolized to the active agent teriflunomide (A77 1726), promotes noncompetitively inhibition. None of the drugs interferes with the FMN-binding site. Targeting de novo pathway is an important approach, because a large nucleotide pool is more critical for pathogens replication, being less prone to cause toxicity. DHODH inhibitors were already demonstrated to have antiviral activity against positive and negative -sense RNA and DNA viruses, namely foot-and-mouth disease virus, Junín virus,rotavirus, and particularly against Ebola.[72][73]

SARS-CoV-2 also impairs innate immune responses. Thus, DHODH inhibitors could have antiviral capacity by inducing two different mechanism of action. On one hand DHODH inhibition causes pyrimidine pool depletion and consequently interferes with replication of the viral genome, on the other hand it induces antiviral genes expression, namely interferon-simulated genes, which stimulate innate immune responses.[74][75]

Role of Vedicinal: Our proposed formulation contains 6 compounds namely compounds 1, 2, 3, 6, 8 & 9 are inhibitors of DHODH inhibitors, LYS184(H1), GLU229(H2), THR63(H1), LEU67(H2), LEU359(H3) SER120(H1), TYR356(H2), SER305(H3), THR357(H4) TYR147(H1), LEU359, PRO364, PHE98 GLN47(H1) PHE73(H1), ASP174(H2) and LYS110 are the key amino acid residues constituting the DHODH inhibitors domain. In this study, three confirmations of this enzyme have separately docked with Baicalin, Luteolin and Piperine were found to offer good interaction with a binding energy and hydrogen bond interaction. DHODH inhibitors is one of the most intriguing and promising drug targets for SARS-CoV-2 drug development.In this study, compounds are separately docked with

[72]Liu, Z., Hu, Q., Wang, W., Lu, S., Wu, D., Ze, S., ... & Huang, J. (2020). Natural product piperine alleviates experimental allergic encephalomyelitis in mice by targeting dihydroorotate dehydrogenase. *Biochemical pharmacology*, *177*, 114000.

[73]Zameitat, E., Freymark, G., Dietz, C. D., Löffler, M., &Bölker, M. (2007). Functional expression of human dihydroorotate dehydrogenase (DHODH) in pyr4 mutants of ustilago maydis allows target validation of DHODH inhibitors in vivo. *Applied and environmental microbiology*, *73*(10), 3371–3379.

[74]Zeng, Z., &Konopleva, M. (2018). Targeting dihydroorotate dehydrogenase in acute myeloid leukemia. *Haematologica*, *103*(9), 1415–1417.

[75]Wu, D., Wang, W., Chen, W., Lian, F., Lang, L., Huang, Y., Xu, Y., Zhang, N., Chen, Y., Liu, M., Nussinov, R., Cheng, F., Lu, W., & Huang, J. (2018). Pharmacological inhibition of dihydroorotate dehydrogenase induces apoptosis and differentiation in acute myeloid leukemia cells. *Haematologica*, *103*(9), 1472–1483.

DHODH were found to offer good interaction with a binding energy. Binding energies of Compound 1 showed -6.60 kcal/mol, compound 2 showed -7.20 kcal/mol, compound 3 -7.42 kcal/mol, compound 6 -6.69 kcal/mol, compound 8 showed -8.22 kcal/mol, and compound 9 -6.38 kcal/mol respectively. DHODH is one of the most intriguing and promising drug targets for SARS-CoV-2 drug development. On consideration of all the above properties, DHODH can be a potential inhibitor and can emerge as an important drug in the treatment of SARS-CoV in future ahead.

Pathway 12: ENDOCYTOSIS

Endocytic pathways in mammalian cells involve budding of clathrin coated vesicles by action of a large GTPase dynamin, in alternative noncanonical pathways such as caveolae, flotillin-1associated endocytosis, Clathrinn independent carrier / glycosylphosphatidylinositol anchored protein enriched endosomal compartment endocytosis and macropinocytosis. Viruses use this entire pathway to infect host cell, Mechanistic details of these pathways may vary considerably between cell types, and diversity of endocytosis in airway epithelium is currently poorly understood. Understanding endocytic viral entry in the respiratory tract may therefore offer a promising therapeutic strategy to treat viral infections.[76]

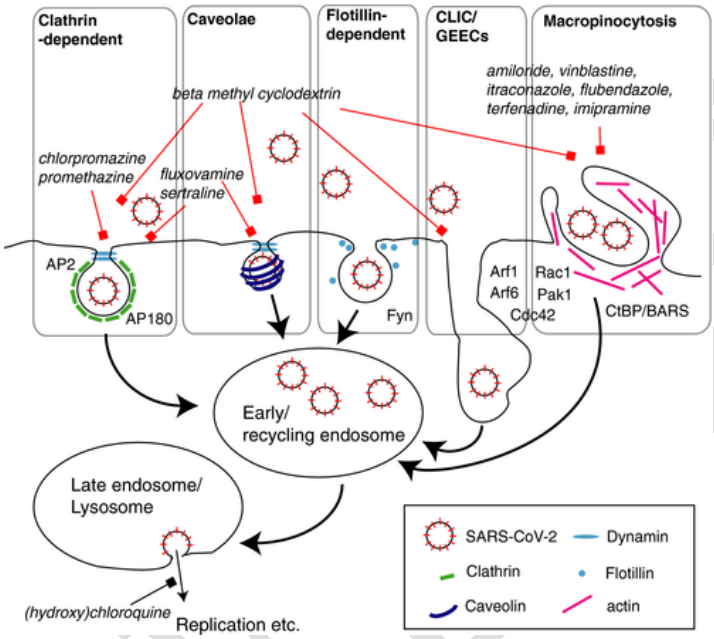


Fig :Endocytosis

Role of Vedicinal: Our proposed formulation contains 6 compounds namely compounds 1, 2, 4, 6, 7& 9 are inhibitors of Endocytosis Inhibitors domain. In this study, three confirmations of this enzyme have separately docked with Baicalin, Quercetin, Rutin, Curcumin, EGCG and Glycyrrhizin were found to offer good interaction with a binding energy and hydrogen bond interaction. Endocytosis Inhibitors is one of the most intriguing and promising drug targets for SARS-CoV-2 drug development.

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[76] Glebov O. (2020) Understanding SARS-CoV-2 endocytosis for COVID-19 drug repurposing, the FEBS journal 287(17):3664-3671.).

Pathway 14: TMPRSS2 INHIBITORS

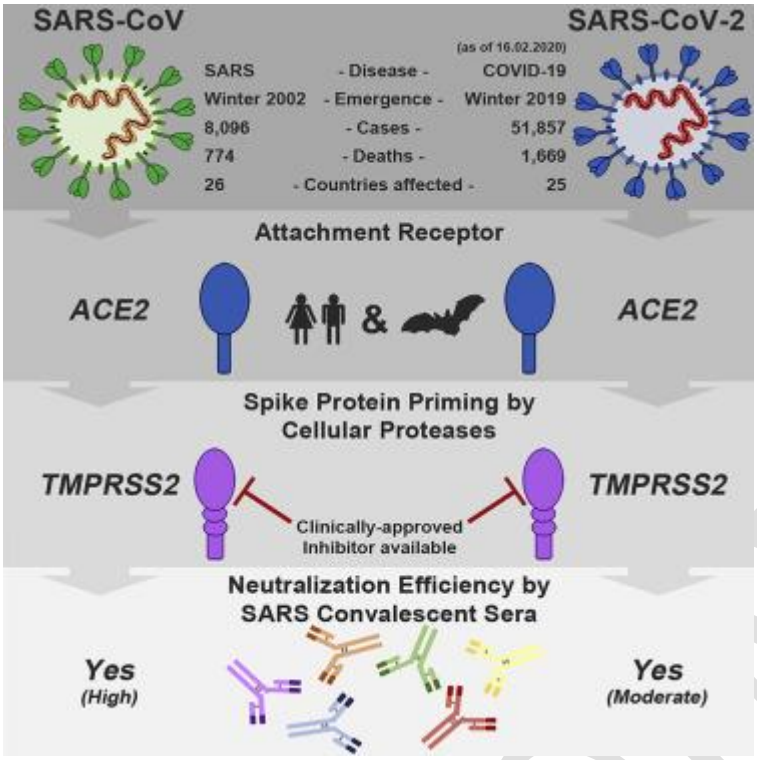

Fig: TMPRSS2 INHIBITION Pathway

Cleavage and activation of the spike protein (S protein) of SARS-CoV is required for membrane fusion and host cell entry is mediated by transmembrane protease/serine subfamily member 2 (TMPRSS2), a serine protease present in airway and alveolar cell[77]. Recent studies demonstrated SARS-CoV-2 employs TMPRSS2 for SARS-CoV-2 S protein priming and S protein-driven cell entry, it was shown that inhibition of TMPRSS2 in human lung Calu-3 cells by camostatmesilate significantly reduced infection with SARS-CoV-2

Role of Vedicinal: TMPRSS serine proteases facilitate virus infection by inducing S cleavage and exposing the fusion peptide for efficient viral entry. SARS-CoV-2 are activated by TMPRSS2 and thus can be inhibited by TMPRSS2 Inhibitors. Compound 7 in our formulation is TMPRSS2 inhibitor .[78]

[77] (McKee DL, Sternberg A, Stange U, Laufer S, and Naujokat C. (2020). Candidate drugs against SARS-CoV-2 and COVID-19. Pharmacological research, 104859:1-157.)

[78] .(Wu, Canrongand Liu, Yang and Yang, Yueyingand Zhang, Peng and Zhong, Wu and Wang, Yaliand Wang, Qiqiand Xu, Yang and Li, Mingxueand Li, Xingzhouand Zheng, Mengzhuand Chen, Lixiaand Li, Hua. (2020). Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. Acta Pharmaceutica Sinica B. 10. 10.1016/j.apsb.2020.02.008.).

Pathway 13: Heme oxygenase-1 (HO-1)

Heme oxygenase-1 (HO-1) is a metabolic enzyme that catalyzes the degradation of heme into carbon monoxide, biliverdin, and free iron. This enzyme has anti-inflammatory and antioxidant properties, which modulate host innate and adaptive immune responses. Heme is the functional group of a variety of heme–proteins, including cytochromes and hemoglobin (Hb), and is therefore crucial for many different cellular processes. Excess of free heme has been shown to exacerbate and contribute to the pathogenesis of a wide variety of inflammatory diseases. The immunomodulatory capacity of HO-1 has been demonstrated in several models, such as the LPS-induced acute lung inflammation in which HO-1 activation decreased the migration of polymorphonuclear leukocytes to the lung, reducing oxidative tissue damage. Furthermore, the pharmacological induction of HO-1 inhibits dendritic cell (DC) activation and immunogenicity, suppressing cytokine secretion and the capacity to prime T cells.[79][80]

Heme oxygenase-1 (HO-1), whichistranscribedbyactivatednuclearfactorerythroid2-relatedfactor2(Nrf2),abasicleucinezippertranscriptionfactor,istheratelimitingenzymeintheconversionofhemetobiliverdin,releasingfreeironandcarbonmonoxide(CO).Itiscriticalinpreventingoxidativestress,inflammationandmetabolicdysregulation.HO1inducerssuchasheminorsubstancesthatproducecarbonmonoxidesuchascobaltprotoporphyrin,reduceinflammatorycytokineexpressioninob/obmiceandZuckerratsandimproveinsulinsensitivityindiabeticanimals.Moreover,inductionofHO1islikelytoprotectagainstmacrophagemediatedinflammatoryresponsesbypreferentiallypromotingtheM2phenotype.[81]

Following hemolysis, Hb is released that can be scavenged by serum haptoglobin (Hp). Free Hb outside the erythrocyte will turn into methemoglobin, which readily liberates its heme group. Normally, this free heme gets scavenged by hemopexin (hpx) to prevent its injurious actions. In case of severe hemolysis, or in blood clots, the hemoglobin and heme scavengers may be overwhelmed, exhausted, or physically not able to interact and neutralize free Hb and heme. Alternative protective mechanisms against free heme are then pivotal for cellular survival. When heme enters the cell, it can modify proteins, DNA, and lipids. Heme can also be transported out of the cell by Breast Cancer Resistance Protein (BCRP) that increases its chance of survival. Finally, heme can be intracellularly degraded by heme oxygenase (HO) into biliverdin, iron, and carbon monoxide (CO). Biliverdin is then directly converted into the antioxidant bilirubin by biliverdin reductase (BVR), whilst iron gets scavenged by co-induced ferritin. Heme oxygenase activity causes resolution of inflammation. The HO-effector molecules biliverdin/bilirubin, CO, and ferritin have each shown to be beneficial. Bilirubin signaling mediates protection against a variety of inflammatory diseases. Increasing bilirubin has also been shown to increase the antioxidant capacity of the serum and may protect against the oxidative properties of heme in a similar fashion as ascorbic acid possibly improves the condition of some COVID-19 patients.

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[79]Espinoza, J. A., León, M. A., Céspedes, P. F., Gómez, R. S., Canedo-Marroquín, G., Riquelme, S. A., ... &Kalergis, A. M. (2017). Heme oxygenase-1 modulates human respiratory syncytial virus replication and lung pathogenesis during infection. *The Journal of Immunology*, *199*(1), 212-223.

[80]Wagener, F. A., Pickkers, P., Peterson, S. J., Immenschuh, S., & Abraham, N. G. (2020). Targeting the heme-heme oxygenase system to prevent severe complications following COVID-19 infections. *Antioxidants*, *9*(6), 540.

[81]Kim, C. S., Choi, H. S., Joe, Y., Chung, H. T., & Yu, R. (2016). Induction of heme oxygenase-1 with dietary quercetin reduces obesity-induced hepatic inflammation through macrophage phenotype switching. *Nutrition research and practice*, *10*(6), 623.

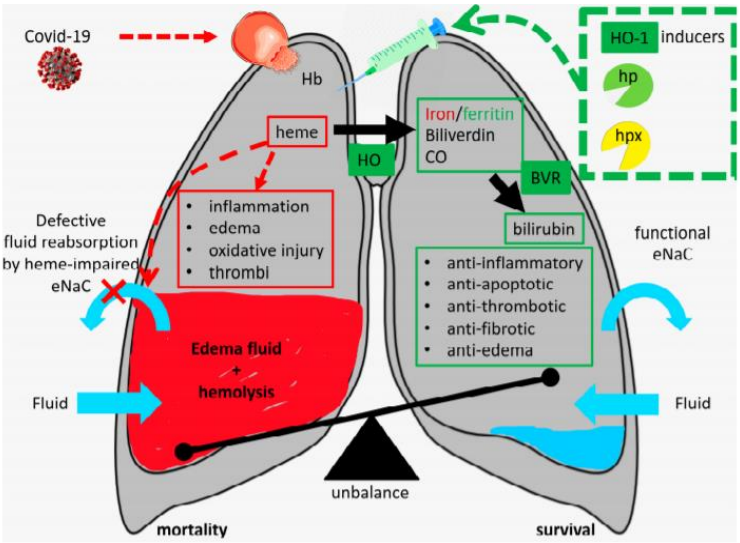


Fig: **Heme oxygenase-1 (HO-1)**

Knocking out HO-1 genes in animal models increases lung damage caused by sepsis. Conversely, raising HO-1 and other stress reduces inflammation and improves insulin resistance and in animal models lengthens life span. The cytoprotective properties of HO-1 are largely the result of HO-1 enzymatic cleaving of heme into biliverdin, ferrous iron, and carbon monoxide. These products limit inflammation and oxidative stress, protecting tissues.[82]

Heme oxygenase catalyzes the oxygen- and NADPH-dependent oxidation of hemoproteins' heme moieties at the alpha-meso carbon bridge, yielding equimolar amounts of ferrous iron, carbon monoxide (CO), and biliverdin (BV), the latter being further reduced into bilirubin (BR) by biliverdin reductase. Heme oxygenase exists as two main isoforms, named HO-1 and HO-2. Although these isozymes share the same mechanism of action, their regulation and distribution are quite different. Heme oxygenase-1 is the inducible isoform and both its gene transcription and protein levels increase in response to free radicals, e.g., reactive oxygen species and reactive nitrogen species (ROS and RNS, respectively). Furthermore, HO-1 is the major isoform detected in both the liver and spleen, even if it is expressed, at lower levels, in some brain areas, such as the hippocampus and hypothalamus. Conversely, the constitutive isoform HO-2 is involved in the physiological turnover of heme and is mainly detectable in neurons and testes.[83]

After a viral infection, type 1 IFNs are among the first cytokines produced by the host cells to inhibit viral replication. However, like other viruses, SARS-CoV-2 suppresses type 1 IFN induction as well as translation and also suppresses IFN stimulated genes in order to survive and replicate. IFNs act by inhibiting viral replication and produce immunomodulatory effects by increasing natural killer cell cytotoxicity and proliferation, and expression of major

[82]Hooper, P. L. (2020). COVID-19 and heme oxygenase: novel insight into the disease and potential therapies. *Cell Stress and Chaperones*, *25*, 707-710.

[83]Mhillaj, E., Tarozzi, A., Pruccoli, L., Cuomo, V., Trabace, L., & Mancuso, C. (2019). Curcumin and heme oxygenase: neuroprotection and beyond. *International journal of molecular sciences*, *20*(10), 2419.

histocompatibility complex-1. Furthermore, IFNs have been reported to possess broad-spectrum antiviral activity including against SARS-CoV.

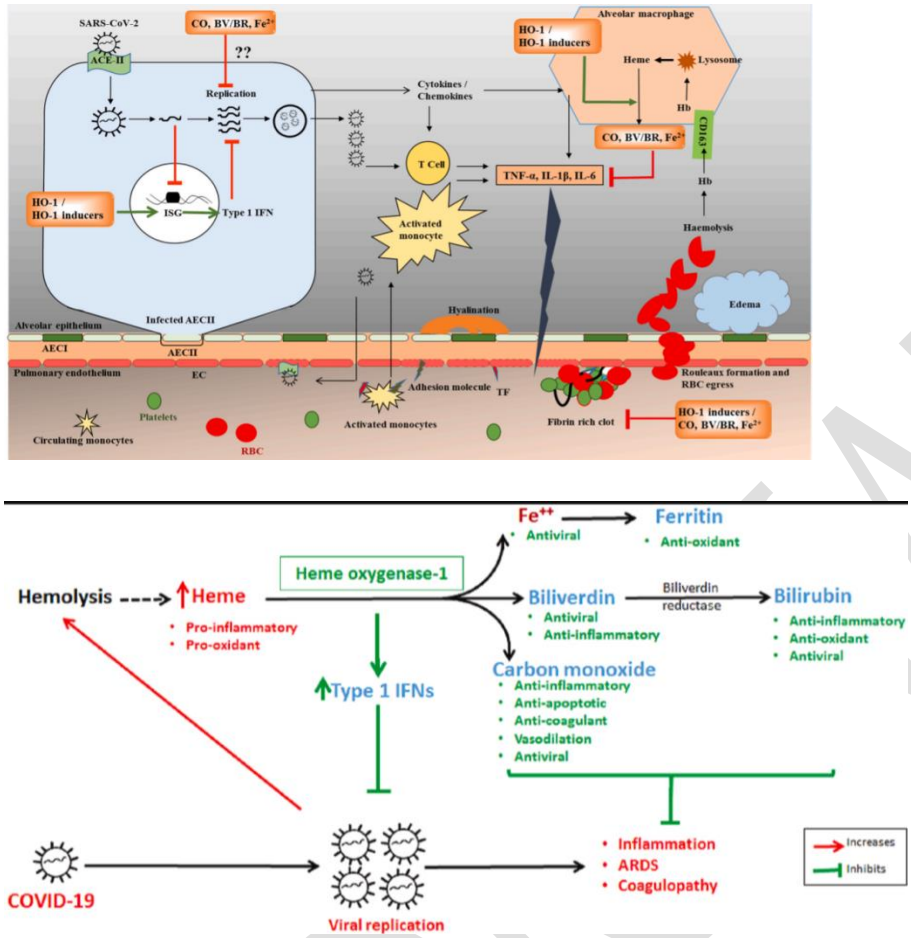


Fig. 2 Hypothetical mechanism of HO-1 induction and heme degradation end-products such as CO, BV, and BR in COVID-19. SARS-CoV-2 virus infects nasal, bronchial epithelial cells and alveolar epithelial cell type II (AECII) via angiotensin converting enzyme-2 (ACE-2) receptors.

In AECII, SARS-CoV-2 replicates and interferes with interferon induction and signaling, thereby stimulating infected cells to release inflammatory signaling molecules like cytokines and chemokines. These molecules in turn stimulate resident alveolar macrophages, and T-cells to release inflammatory mediators like TNF-α, IL-1β and IL-6. With further disease progression, these inflammatory mediators activate endothelial cells (EC) in pulmonary capillaries thereby inducing the expression of adhesion molecules. Adhesion molecules, in turn cause activation and recruitment of activated monocytes to alveoli where they release various inflammatory mediators, thus altering the balance between pro-inflammatory and anti-inflammatory cytokines. All these events ultimately affect epithelial-endothelial integrity, thereby further increasing the egress of monocytes and red blood cells. Pro-inflammatory cytokines also induce expression of tissue factor (TF) on ECs which when comes in contact with platelets; activate coagulation cascade forming a fibrin rich clot. In later stages, virus affects EC directly causing apoptosis, loss of barrier integrity thereby

activating further inflammation and coagulation. Increased heme released after hemolysis as observed in the COVID-19 patients with ARDS, further increases pro-inflammatory cytokines.[84]

Role of Vedicinal: Our proposed formulation contains 6 compounds namely compounds 1 to compound 9 are inhibitors of Heme oxygenase-1 (HO-1) domain. In this study, three confirmations of this enzyme have separately docked with all were found to offer good interaction with a binding energy and hydrogen bond interaction. Heme oxygenase-1 (HO-1) is one of the most intriguing and promising drug targets for SARS-CoV-2 drug development.

Pathway 14th:TMPRSS2

TMPRSS2 gene is located on human chromosome 21: 41, 464, 551-41, 531, 116 (Fig. 1). A significant feature of the TMPRSS2 gene is that several androgen receptor elements (AREs) are located upstream of the transcription start site and the first intron. As shown in Fig. 2, the TMPRSS2 gene encodes a predicted protein of 492 amino acids which anchors to the plasma membrane. It converts to its form through autocatalytic cleavage between Arg255 and Ile256. After cleavage, the mature proteases are mostly membrane-bound, yet a noticeable portion of them can be liberated into the extracellular milieu. The protease catalytic domain contains a catalytic triad consisting of the amino acid residues His296, Asp345 and Ser441, corresponding to His57, Asp102 and Ser195 of chymotrypsinogen.[85]

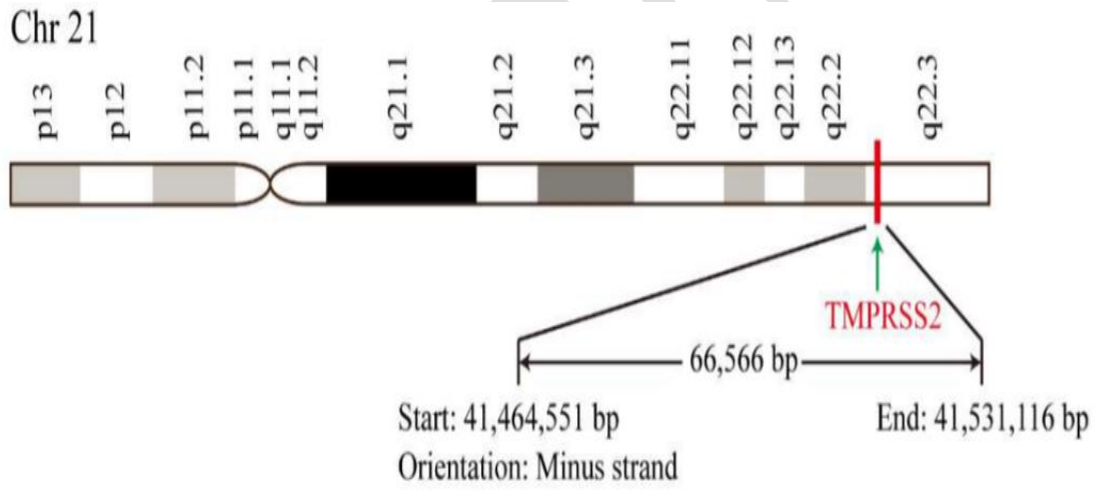


Fig: A schematic diagram of TMPRSS2 genomic location.

[84]Singh, D., Wasan, H., &Reeta, K. H. (2020). Heme oxygenase-1 modulation: A potential therapeutic target for COVID-19 and associated complications. *Free Radical Biology and Medicine, 161*, 263-271.

[85]Shen, L. W., Mao, H. J., Wu, Y. L., Tanaka, Y., & Zhang, W. (2017). TMPRSS2: A potential target for treatment of influenza virus and coronavirus infections. *Biochimie, 142*, 1-10.

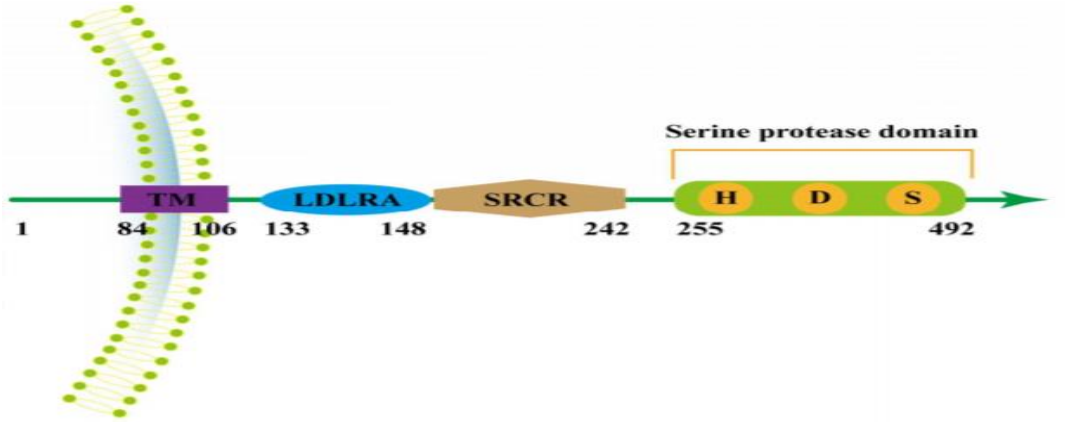


Fig.:The location and structure of TMPRSS2 protein. TM: transmembrane domain; LDLRA: low-density lipoprotein receptor domain class A; SRCR: Scavenger receptor cysteine-rich domain; Letters H: histidine; Letters D: aspartate; Letters S: serine.

After SARS-CoV-2 binds to the cell receptor ACE2 (angiotensin-converting enzyme 2), a lysosomal endopeptidase enzyme known as cathepsin L can cleave its S protein, activating membrane fusion for subsequent cell entry. Likewise, the plasma membrane-associated protease TMPRSS2 can also cleave the aforementioned S-protein and activate viral entry at the cell surface. Furthermore, human cells that express both ACE2 and TMPRSS2 are present in multiple tissues, which include lungs, nasal mucosa, buccal mucosa, ileum, colon, and myocardial epithelial cells.

SARS-CoV-2 fusion can be activated by either or both of two pathways. The coronaviruses bind the cellular receptor ACE2 and must be activated by proteolysis with either a surface-expressed protease like TMPRSS2 or by cathepsin L in the endosome. Only cathepsin L-mediated proteolysis requires endosomal acidification. Camostat mesylate inhibits TMPRSS2 activity, whereas hydroxychloroquine, like ammonium chloride, inhibits endosomal acidification.[86]

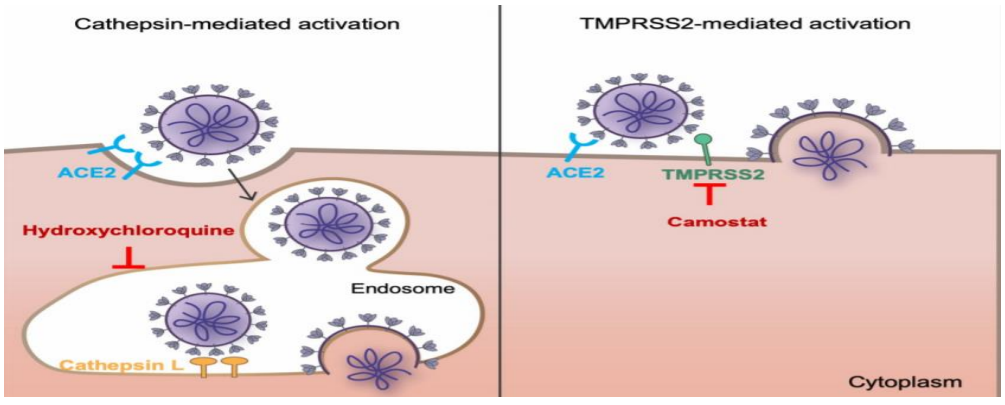


Fig. ACE2 cytoplasmic tail cleavage mediated by TMPRSS2

[86]Zipeto, D., da Fonseca Palmeira, J., Argañaraz, G. A., &Argañaraz, E. R. (2020). ACE2/ADAM17/TMPRSS2 interplay may be the main risk factor for COVID-19. *Frontiers in immunology*, *11*.

Another important mechanism, in this complex pathophysiological equation, is the ACE2 cytoplasmic tail cleavage mediated by TMPRSS2. The cleavage of the ACE2 tail by TMPRSS2 increases viral uptake in target cells and, therefore, TMPRSS2 could promote SARS-CoV-2 entry by two mechanisms: i) by SARS-S cleavage, which activates the S protein for membrane fusion, and ii) by ACE2 cleavage, which might promote viral uptake through the cathepsin L-dependent pathway, which would then infect the cell by fusing with the endosomal membrane.[87]

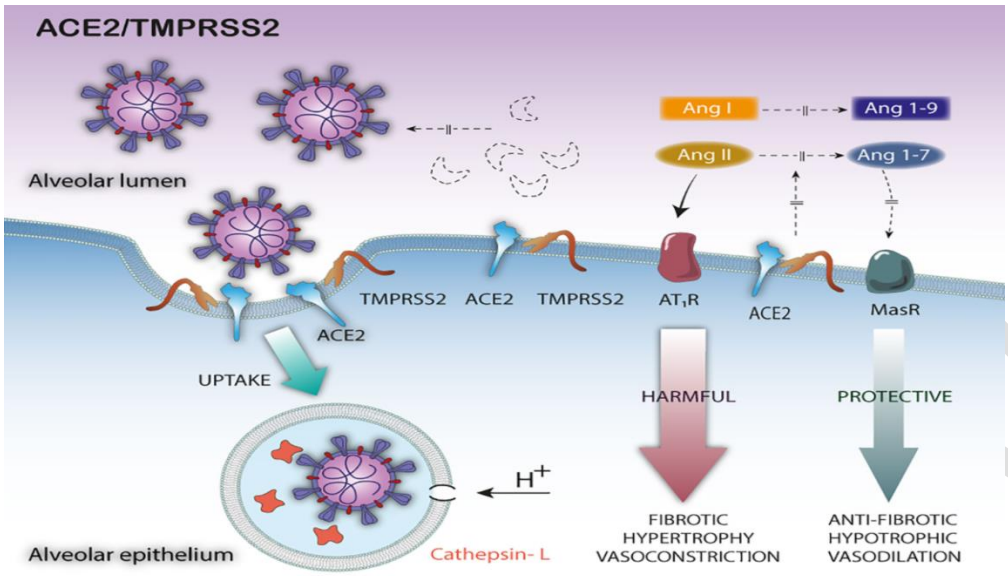


Fig:ACE2/TMPRSS2

Role of Vedicinal: Our proposed formulation contains 6 compounds namely compounds 1, 7 & 9 are inhibitors of trans-membrane protease serine 2 (TMPRSS2) domain. In this study, three confirmations of this enzyme have separately docked with Baicalin, EGCG and Glycyrrhizin were found to offer good interaction with a binding energy and hydrogen bond interaction. trans-membrane protease serine 2 (TMPRSS2) is one of the most intriguing and promising drug targets for SARS-CoV-2 drug development.

[87] Ou, T., Mou, H., Zhang, L., Ojha, A., Choe, H., & Farzan, M. (2021). Hydroxychloroquine-mediated inhibition of SARS-CoV-2 entry is attenuated by TMPRSS2. *PLoS pathogens*, *17*(1), e1009212.

Pathway15: FURIN INHIBITORS

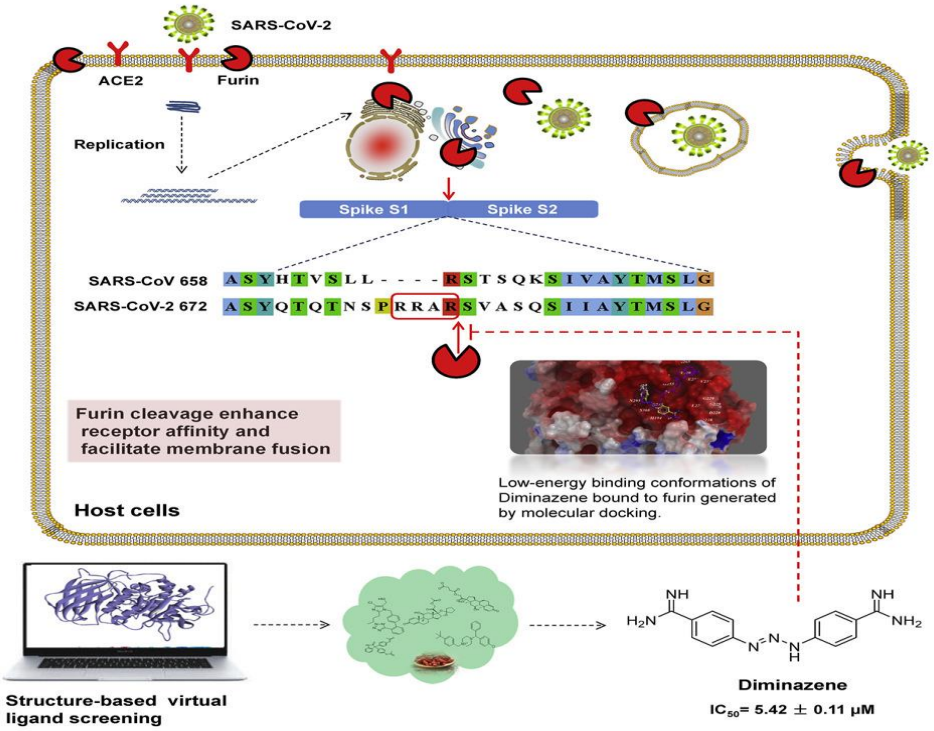


Fig:Furin Inhibition pathway

Several proteases process coronavirus S proteins, like Proprotein convertases, extracellular proteases Proteases can process the protein depending on whether spikes are cleaved during virus packaging (e.g. furin), after virus release (e.g. elastase), after virus attachment to the host cell (e.g. TMPRSS2), or during virus entry by endocytosis (e.g. cathepsin L), respectively.[88]This generates a novel, putative, furin cleavage signal not seen in other clade members. It was subsequently shown that prior furin cleavage enhanced entry of a pseudovirus containing the SARS-CoV-2 S protein into different cell lines expressing hACE2. The furin cleavage site is also required for SARS-CoV-2 infection of human lung cells

Role of Vedicinal: Our proposed formulation contains 6 compounds that can inhibit the Furin activity among which compound 1 is the most potent Furin inhibitor. Furin inhibition will stop the entry of corona viruses into the host cells. Our proposed formulation contains 6 compounds namely compounds 1, 3, 4, 5, 6&8areLYS449(H1), TYR313(H2), TYR571(H3), SER311(H1), ALA532(H2), ASN310(H3), GLY265(H4), GLY307(H1), ASP530(H2), ASN529(H3), TRP531, GLY229(H1), ASP154(H2), ASP191(H3), GLY255(H4), PRO256, TYR308, GLU236, ARG490(H1), ASN529(H2), GLY527(H3), SER311, ILE312, TYR313,GLY307,ARG483(H1), THR442, and SER575 inhibitors of trans-membrane Furin Inhibitors domain. In this study, three confirmations of this enzyme have separately docked with Baicalin, Luteolin, Piperine and Glycyrrhizinwere found to offer good interaction with a binding energy and hydrogen bond interaction. trans-membrane Furin Inhibitors is one of the most intriguing and promising drug targets for SARS-CoV-2 drug development.

[88] Klimstra WB, Tilston-Lunel NL, Nambulli S, Boslett J, McMillen CM, Gilliland T, Dunn MD, Sun C, Wheeler SE, Wells A, Hartman AL, McElroy AK, Reed DS, Rennick LJ and Duprex PW. (2020) SARS-CoV-2 growth, furin-cleavage-site adaptation and neutralization using serum from acutely infected, hospitalized COVID-19 patients, bioRxiv preprint: 1-30.)

Pathway 16: TRYPSIN INHIBITOR

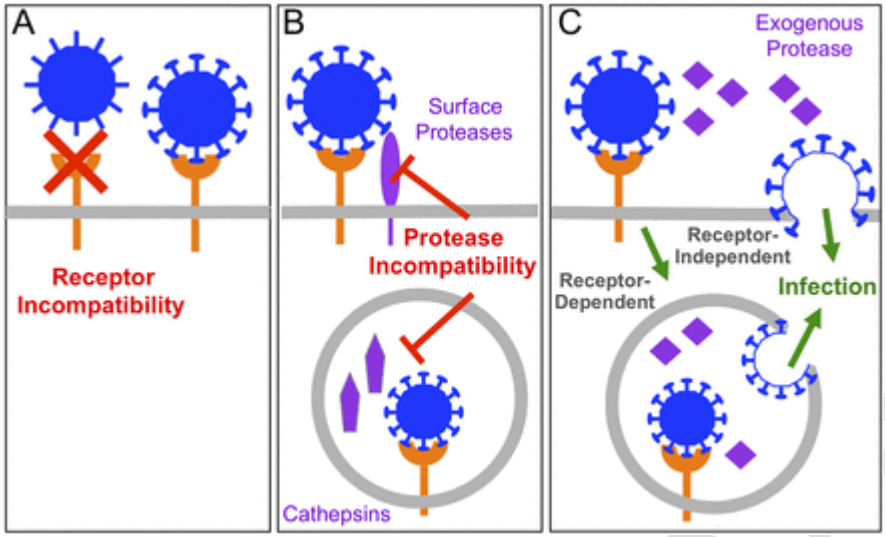


Fig: TRYPSIN Inhibition pathway

Aminopeptidase N (APN, CD13) belongs to the M1 family of zinc metallopeptidases3, also termed gluzincins, which comprises enzymes of medical interest for infectious and autoimmune diseases, cancer and hypertension.[89] The proteases TMPRSS2 and TMPRSS11a, which exist in the respiratory tract richly and become experiment on cell surfaces, promote the entry of SARS-CoV-1-virus. Protease TMPRSS11d similar to trypsin has confessed of the human respiratory tract - a proteolytic activation of the spike protein was proved by SARS-CoV

Role of Vedicinal: 5 of our compounds have strong Trypsin inhibition activity which may alter the attachment pathway by inducing the lack of receptor compatibility. Our proposed formulation contains 6 compounds namely compounds 2, 5, 5, 7 & 9 are inhibitors of Trypsin Inhibitors domain. In this study, three confirmations of this enzyme have separately docked with Quercetin, Rutin, Hesperidin, EGCG and Glycyrrhizin were found to offer good interaction with a binding energy and hydrogen bond interaction. Trypsin Inhibitors is one of the most intriguing and promising drug targets for SARS-CoV-2 drug development.

Pathway 17: CATHEPSIN L INHIBITORS

[89] (Bittmann S, Luchter E, Weissenstein A, Villalon G, and MoschÃring-Alieva E (2020). TMPRSS2-Inhibitors play a role in cell entry mechanism of COVID-19: An insight into Camostat and Nafamostat. J Regen Biol Med 2(2):1-3.)

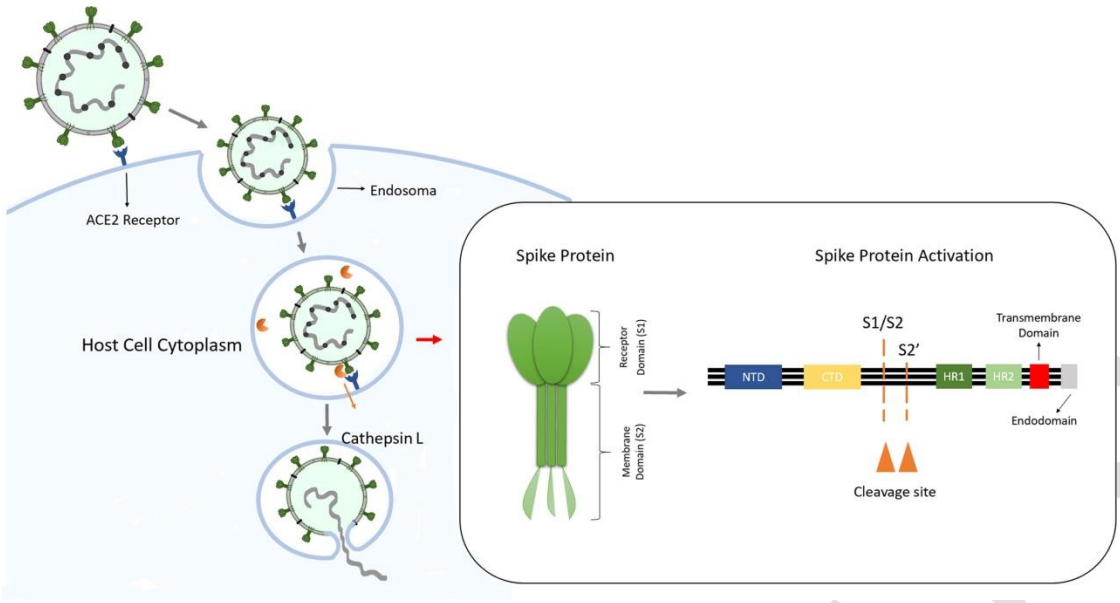


Fig: **CATHEPSIN L Inhibition Pathway**

Interactions are followed by endocytosis, and at the low pH in endosomes, SARS-S is cleaved by a cellular protease called cathepsin L, thereby exposing the S2 domain of the spike protein for membrane fusion[90]. Novel inhibitors of severe acute respiratory syndrome coronavirus entry that act by three distinct mechanisms. Journal of virology, 87(14):8017–8028.). The process of viral entry involves interactions between the receptor binding domain of SARS-S and the receptor ACE2. SARS-S is hydrolyzed by cathepsin L to S1 and S2 domains. The viral and cellular membranes eventually fuse through a type I fusion mechanism. After endocytosis of the virus, cathepsin L cleaves SARS-S to S1 and S2, allowing subsequent fusion of the viral membrane with the endosomal membrane . Novel inhibitors of severe acute respiratory syndrome coronavirus entry that act by three distinct mechanisms. Journal of virology, 87(14):8017–8028.). Prevention of viral entry by blocking the interaction of SARS-S with the ACE2 receptor by impeding viral entry by inhibiting cathepsin L processing of the SARS-S envelope in the endosome, and inhibit viral entry by preventing fusion of the viral membrane with the host cellular membrane.

Role of Vedicinal: Inhibition of Cathepsin will inhibit entry of SARS-COV-2 into the cell and ultimately stop the viral replication. In our formulation compound 2 shows the Cathepsin L inhibition activity. Our proposed formulation contains 6 compounds namely compounds 2 are inhibitors of Cathepsins Inhibitors domain. In this study, three confirmations of this enzyme have separately docked with Quercetin were found to offer good interaction with a binding energy and hydrogen bond interaction. CathepsinsInhibitors is one of the most intriguing and promising drug targets for SARS-CoV-2 drug development.

[90] Adedeji AO, Severson W, Jonsson C, Singh K, Weiss SR, and Sarafianos SG. (2013)

Pathway 18: APN / CD 13 Inhibitors

Aminopeptidase N (APN/CD13) is a transmembrane protease present in a widely variety of human tissues and cell types (endothelial, epithelial, fibroblast and leukocyte) and its expression is dysregulated in inflammatory diseases as well as in cancers. CD13 is known to serve as receptor for human Coronavirus. It was recently shown that CD13 deficient pigs are resistant to Coronavirus infection. Coronavirus recognition of APN is species-specific, and specificity is associated with N-linked glycosylations in the APN protein. Hence blockage of CD13 could serve as prophylaxis for COVID-19.

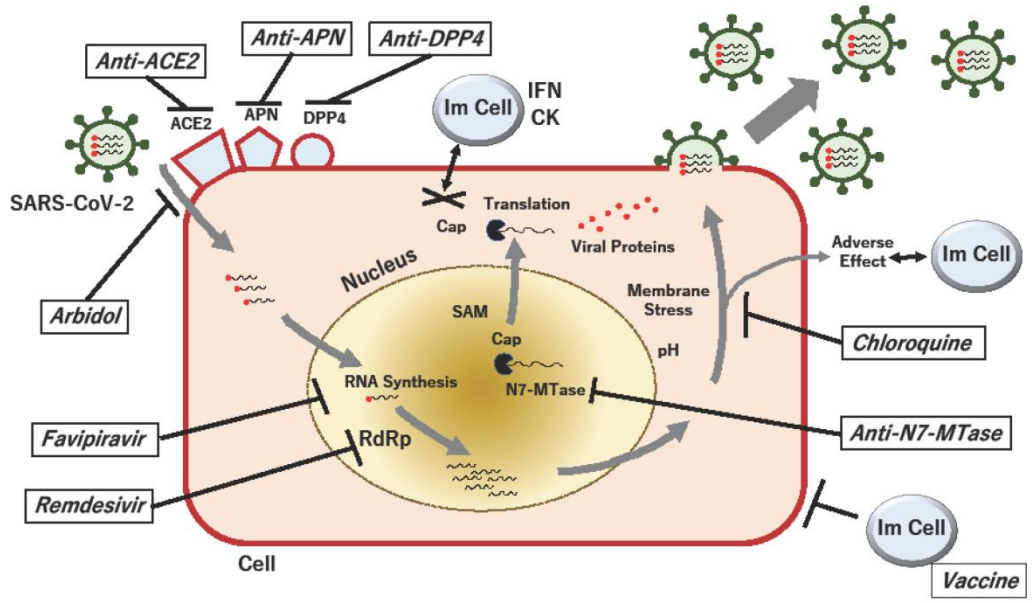


Fig: Pathway 18: APN / CD 13 Inhibiton pathway

Role of Vedicinal: Compound 6 in our formulation binds to CD13 and inhibits APN/CD13 irreversibly which indicates that inhibition of APN/CD13 could indeed be effective in preventing the Coronavirus infection. Our proposed formulation contains 6 compounds namely compounds 6 inhibitors of trans-membrane APN & CD 13 Inhibitors domain. In this study, three confirmations of this enzyme have separately docked with Curcumin were found to offer good interaction with a binding energy and hydrogen bond interaction. trans-membrane APN & CD 13 Inhibitors is one of the most intriguing and promising drug targets for SARS-CoV-2 drug development.

Pathway 19 : DDP4 INHIBITORS

DDP4 family/system was implicated in various physiological processes and diseases of the immune system, and DDP4/CD26 is variously expressed on epithelia and endothelia of the systemic vasculature, lung, kidney, small intestine and heart[91]. In particular, DDP4 distribution in the human respiratory tract facilitates the entrance of the virus into the airway tract itself and could contribute to the development of cytokine storm and immunopathology in causing fatal COVID-19 pneumonia .[92] DDP4 inhibitors affect glucose control through several mechanisms, including enhancement of glucose-dependent insulin secretion, slowed gastric emptying, and reduction of postprandial glucagon and of food intake

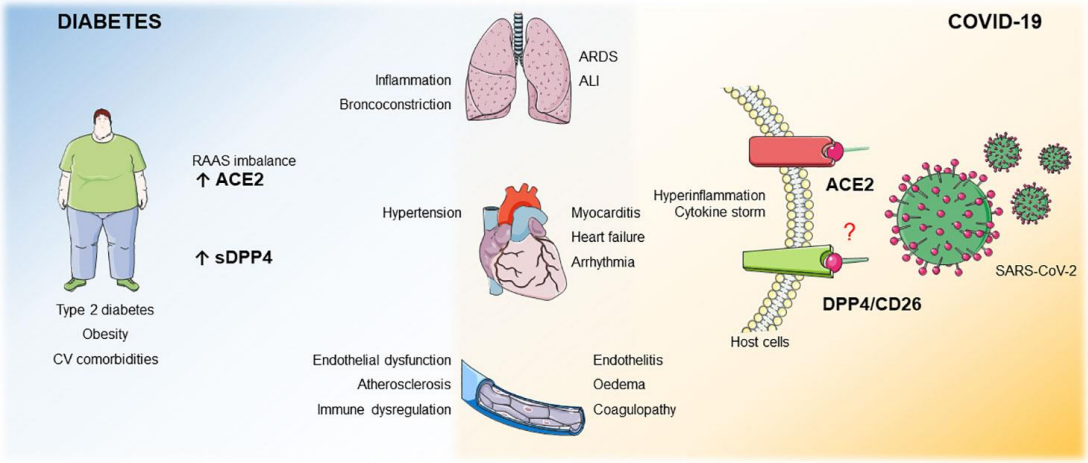


Fig:DPP4 Pathway

DPP4 inhibitor is known to modulate inflammation and is known to suppress T cell proliferation and production of pro-inflammatory cytokine. In COVID-19 infection, SARS Co-V has been shown to infect T cells through S protein-mediated membrane fusion although it's not clear whether the virus replicates inside the T cells or it leads to apoptosis .[93]Decrease in the counts of CD3 + T, CD4 + T, CD8 + T, NK cells, as well as increases in the CD4/CD8 ratio in COVID-19 patients compared to recovered patients have been reported. Lower levels have been reported to correlate with severity of infection. Is DPP4 inhibition a comrade or adversary in COVID-19 infection. Diabetes research and clinical practice, 164:108216.). Regulatory T cells (Tregs) which have a very important role in autoimmune conditions did not have a significant role in COVID-19. Baseline suppressed T cell immunity secondary to DPP4i may be a disadvantage in COVID-19 infection and lead to a more severe disease

Role of Vedicinal: Compound 2, 4, 6 and 7 are DPP4 inhibitors and may offer a simple way to reduce the virus entry and replication into the airways and to hamper the sustained cytokine storm and inflammation within the lung in patients diagnosed with COVID-19 infection. Our proposed formulation contains 6 compounds namely compounds 2, 4,

[91] (Solerte SB, Di Sabatino A, Galli M, and Fiorina P. (2020). Dipeptidyl peptidase-4 (DPP4) inhibition in COVID-19. Acta diabetologica, 57(7):779–783.)

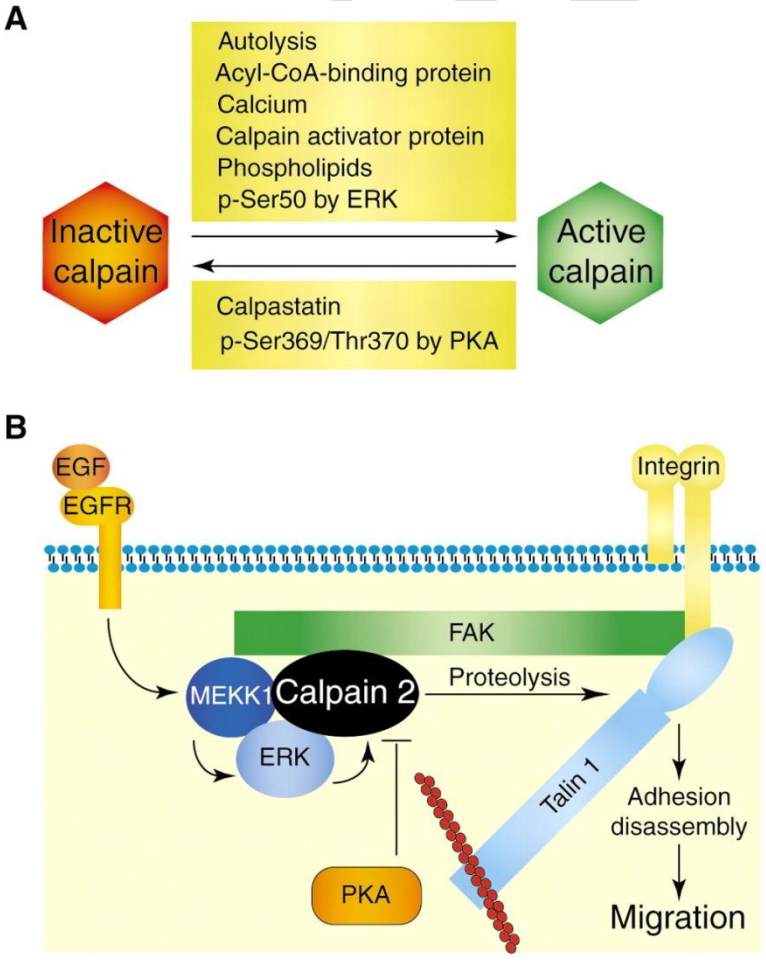
[92] (Solerte SB, Di Sabatino A, Galli M, and Fiorina P. (2020). Dipeptidyl peptidase-4 (DPP4) inhibition in COVID-19. Acta diabetologica, 57(7):779–783.).

[93] (Dalan R. (2020). Is DPP4 inhibition a comrade or adversary in COVID-19 infection. Diabetes research and clinical practice, 164:108216.).

6 & 7 are inhibitors of trans-membrane DPP4 Inhibitors domain. In this study, three confirmations of this enzyme have separately docked with Quercetin, Rutin, Curcumin and EGCG were found to offer good interaction with a binding energy and hydrogen bond interaction. trans-membrane DPP4 Inhibitors is one of the most intriguing and promising drug targets for SARS-CoV-2 drug development.

Pathway 20: CALPAINS INHIBITORS

Calpains are intracellular calcium-dependent cysteine proteases that are activated by intracellular calcium and inhibited by the endogenous molecule calpastatin. It is believed that calpains are part of the cellular proteolytic system and are involved in different processes of cytoskeleton remodeling and apoptosis. Several studies have shown that calpains play an important role in viral replication and in activation of virus-induced apoptosis [94] [95]. In line with this, a research group has shown that Z-Val-Phe-Ala-CHO (calpain inhibitor III) and Val-Leu-CHO (calpain inhibitor VI) effectively inhibited in vitro SARS-CoV replication



[94] (De Clercq E. (2006). Potential antivirals and antiviral strategies against SARS coronavirus infections. Expert review of anti-infective therapy, 4(2), 291–302. https://doi.org/10.1586/14787210.4.2.291)

[95] Elshabrawy HA. (2020) SARS-CoV-2: An update on potential antivirals in light of SARS-CoV antiviral drug discoveries, Vaccines 8 (335):1-30.

Fig: **CALPAINS INHIBITION Pathway**

Role of Vedicinal: Compound 2 from our formulation is Calpain inhibiators, it fit perfectly into the binding sites of Mpro. Binding sites are specific sites on the structure of an enzyme where the substrate (the compound on which an enzyme acts) binds. once bound to the site, calpain inhibitor changes its structure so that it fits snugly into the active site. When the active site is taken, the enzyme cannot work. Our proposed formulation contains 6 compounds namely compounds 2 is inhibitors of Calpain Inhibitors domain. In this study, three confirmations of this enzyme have separately docked with Quercetin was found to offer good interaction with a binding energy and hydrogen bond interaction. trans-membrane Calpain Inhibitors is one of the most intriguing and promising drug targets for SARS-CoV-2 drug development

Pathway 21: CASEIN KINASE-2 (CK2) INHIBITION

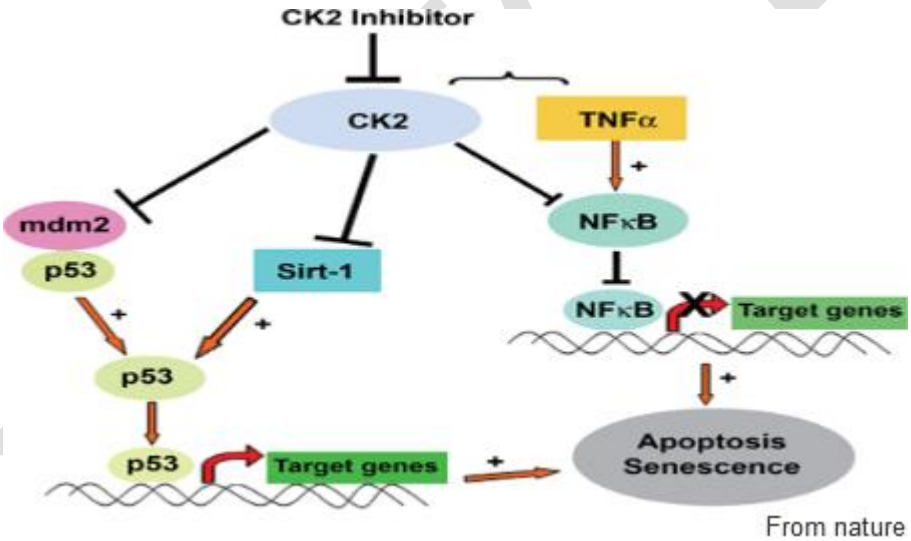


Fig: CASEIN KINASE-2 (CK2) INHIBITION Pathway

Casein kinase 2 (CK2) is a ubiquitous eukaryotic serine/threonine protein kinase that plays an important role in cell cycle progression. Over-expression of CK2 in cells or colorectal carcinoma cells that have truncated mutant APC

proteins down-regulates cell proliferation .[96] The complex formation between CK2 and full-length APC regulates CK2 activity that, in turn, regulates cell cycle progression, whereas truncated APC in colorectal carcinomas are unable to regulate the cell cycle. In the process to look for the downstream target for CK2, researchers found eukaryotic translation initiation factor 5 (eIF5) is phosphorylated by CK2 in vivo as well as in vitro These results suggest an important role of CK2 on promotion of cell growth through eIF5

Role of Vedicinal: CK2 has been found to be stimulated in other viral infections , and has

various roles in the infection cycles of different viruses,Compound 2, 3, 7 and 9 shows inhibitory potential towards CK2 catalytic subunits. Our proposed formulation contains 6 compounds namely compounds 1, 2, 3, 7 & 9 are inhibitors of casein kinase-2 (CK2) Inhibitorsdomain. In this study, three confirmations of this enzyme have separately docked with Baicalin, Quercetin, Luteolin, EGCG and Glycyrrhizinwere found to offer good interaction with a binding energy and hydrogen bond interaction. trans-membrane casein kinase-2 (CK2) Inhibitorsis one of the most intriguing and promising drug targets for SARS-CoV-2 drug development.

Pathway 22 : EMMPRIN/CD147

SARS-CoV-2 virus can invade host cells not via a receptor called CD147,This receptor is also called "extracellular matrix metalloproteinase inducer" (EMMPRIN) or Basigin. EMMPRIN/CD147 induces the production of matrix metalloproteinases (MMP) such as MMP-9 and MMP-2. Upregulation of EMMPRIN was reported in oral premalignant cells and primary and metastatic cell lines of OSCC leading to oral carcinogenesis. COVID-19 in OSCC patients might exhaust the EMMPRIN due to excessive binding with the 'S' receptor and carcinogenesis. In absence of free ACE-2, ACE2-Ang-1-7-MAS pathway is inhibited. It is conceivable that in the ACE-2 depleted situation in OSCC, EMMPRIN receptor might get high jacked by SARS-CoV-2 for the entry into the host cells. This further increases the complexity of COVID-19 infectivity prediction and disease progression in OSCC patients, which needs serious consideration in the future .[97]

[96] (Homma MK, and Homma Y. (2005) Regulatory role of CK2 during the progression of cell cycle. Mol Cell Biochem 274:47–52.).

[97] (Varadarajana S, Balaji TM, Sarode SC, SarodeGS,Sharmad NK, Gondivkare S, Gadbail A, Patil S. (2020) EMMPRIN/BASIGIN as a biological modulator of oral cancer and COVID-19 interaction: Novel propositions, Medical hypotheses 143:1-4.)

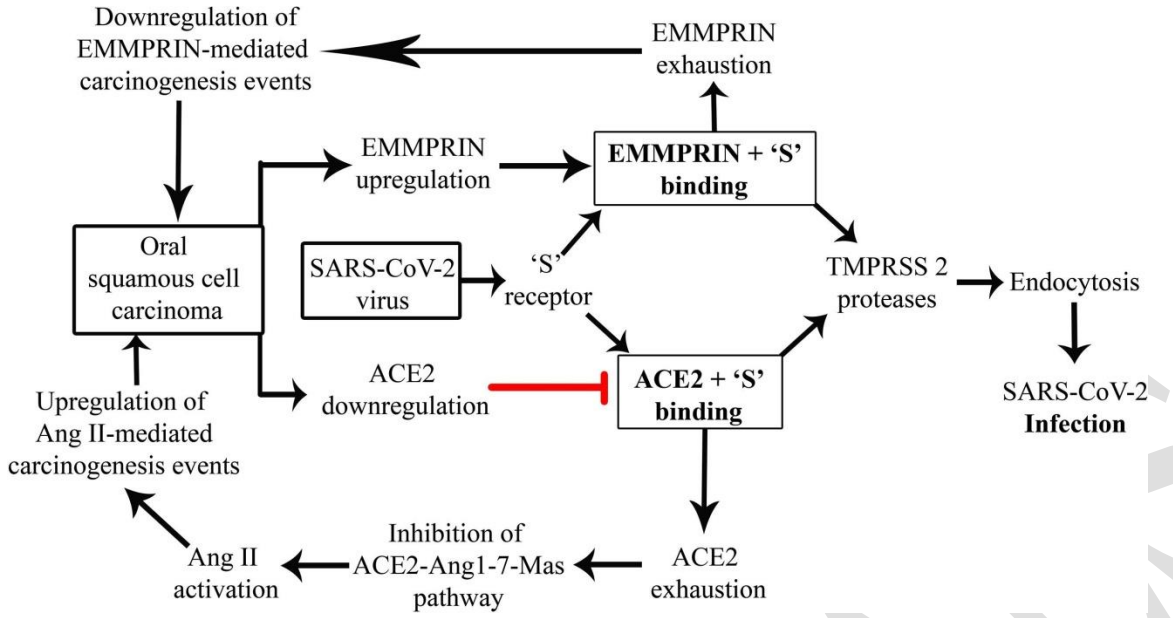


Fig: EMMPRIN/CD147

Role of Vedicinal: Broadly expressed transmembrane glycoprotein of the immunoglobulin superfamily CD147 (also known as basigin or EMMPRIN) facilitates SARS-CoV invasion for host cells, compounds 1, 6, 7 and 8 from Vedicinal block EMMPRIN and prevent from CoVs infection. Our proposed formulation contains 6 compounds namely compounds 1, 6, 7& 8 are inhibitors of trans-membrane Emmprin CD 147 Inhibitors domain. In this study, three confirmations of this enzyme have separately docked with Baicalin, Curcumin, EGCG and Piperine were found to offer good interaction with a binding energy and hydrogen bond interaction. trans-membrane Emmprin CD 147 Inhibitors is one of the most intriguing and promising drug targets for SARS-CoV-2 drug development.

Pathway 23 :GRP78

CoV spike glycoprotein recognizes a 78-kDa glucose–regulated protein (GRP78) or heat shock 70 kDa protein 5 (HSPA5), known as binding immunoglobulin protein (BiP) or Byun1, encoded by the HSPA5 gene. HSP5A is a ER-resident unfolded protein response (UPR) protein. GRP78 modulates CoV entry in the presence of the DPP4 as a host cell receptor. Stress-overexpressed GRP78 can avoid ER retention and is translocated to the membrane. GRP78 translocated to the cell PM can recognize viruses by its substrate-binding domain (SBD) for virus entry into the cell. RBD of the CoV spike protein recognizes the GRP78 SBDβ as the host cell receptor. This process is the mechanism underlying the cell surface HSPA5 (GRP78) exposure and this is exploited to be used for pathogen entry. GRP78 or BiP is a chaperone protein located in the ER lumen. Unfolded protein response are upregulated, Overexpressed GRP78 can avoid ER retention and is translocated to the membrane. This process is the mechanism underlying the cell surface HSPA5 (GRP78) exposure and this is exploited to be used for pathogen entry. Therefore, natural products can inhibit

cell-surface.[98] .

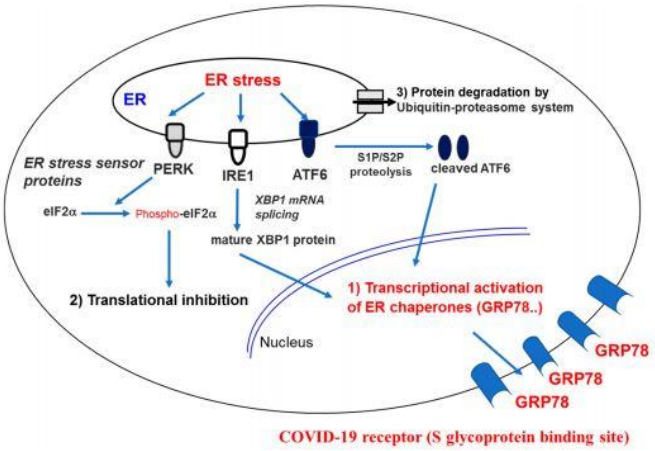


Fig: GRP78Pathway

Role of Vedicinal:GRP78, which is a stress-inducible, multi-faceted chaperone protein serving critical functions in the endoplasmic reticulum (ER) and other cellular compartments, impacting both health and disease,GRP78 has the potential to disrupt multiple stages of the viral life cycle including entry, production and subsequent cellular infection and phytocompound 1, 2, 3, 4, 6, 7 and 8 induce GRP78. Our proposed formulation contains 6 compounds namely compounds 1, 2, 3, 4,6, 7&9 are inhibitors of GRP 78 Inhibitors domain. In this study, three confirmations of this enzyme have separately docked with Baicalin, Quercetin, Luteolin, Rutin, Curcumin, EGCG and Glycyrrhizinwere found to offer good interaction with a binding energy and hydrogen bond interaction. trans-membrane Furin Inhibitors is one of the most intriguing and promising drug targets for SARS-CoV-2 drug development.

Pathway 24: GP41

HIV-1 gp41 and SARS-CoV S2 protein showed some similar structural motifs such as N-terminal leucine/isoleucine heptad repeat sequence on residues 913–1000; C-terminal leucine/isoleucine heptad repeat motif on residues 1151–1185. SARS-CoV S2 and HIV gp41 share very similar helix structure on residues 879–942, these discoveries suggest a similar membrane fusion mechanism for the two viruses .[99]

[98] (Kim CH. (2020) SARS-CoV-2 Evolutionary Adaptation toward Host Entry and Recognition of Receptor O-Acetyl Sialylation in Virus–Host Interaction, Molecular sciences 21(4549):1-34)

[99] (Zhang W, and Leng YY. (2004) Structural similarity between HIV-1 gp41 and SARS-CoV S2 proteins suggests an analogous membrane fusion mechanism. Theochem, 677 1:73–76. https://doi.org/10.1016/j.theochem.2004.02.018).

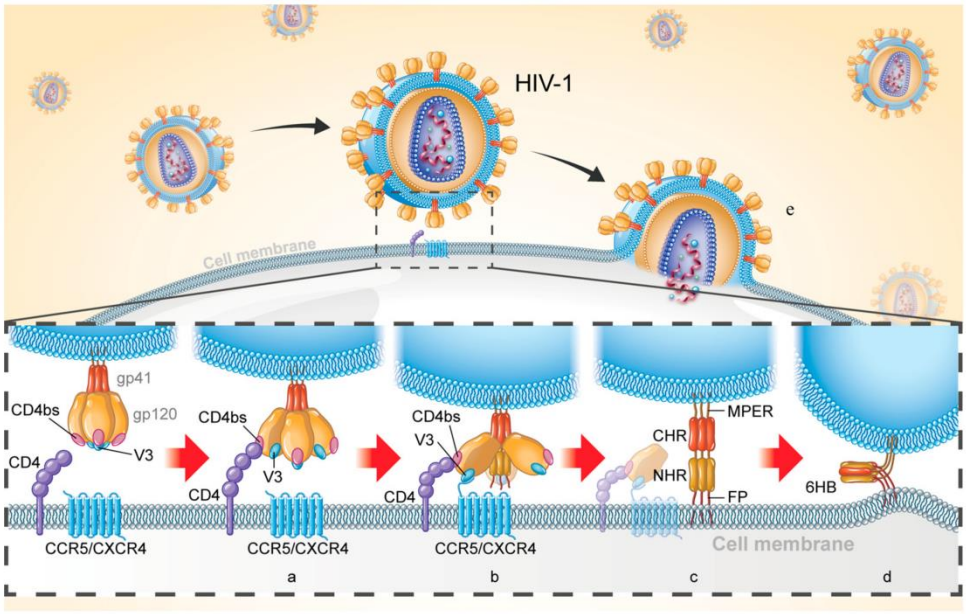


Fig: HIV gp41 **Pathway**

Role of Vedicinal: Phytocompound 6 and 7targets the gp41 amino-terminal region exposed in the transient extended state, blocking the ultimate collapse into the trimer-of hairpins and inhibiting membrane fusion. This vulnerability of this transient extended state has stimulated the development of new agents, ranging from small molecules to large proteins, that bind to gp41 and inhibit its structural transformations. Our proposed formulation contains 6 compounds namely compounds 6 &7 areinhibitors of trans-membrane GP 41 Fusion Inhibitorsdomain. In this study, three confirmations of this enzyme have separately docked with Curcumin and EGCG were found to offer good interaction with a binding energy and hydrogen bond interaction. trans-membrane GP 41 Fusion Inhibitorsis one of the most intriguing and promising drug targets for SARS-CoV-2 drug development.

Pathway 25: ANGIOTENSIN II

ACE2 receptor has important implications for understanding SARS-CoV-2 transmissibility and pathogenesis. SARS-CoV-2 lead to downregulation of the ACE2 receptor, through binding of the spike protein with ACE2 [100]

[100] (Zhang H, Penninger JM, Yimin Li, Nanshan Zhong and Arthur S. Slutsky 2020 Angiotensin-converting enzyme 2 ACE2 as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target, Intensive Care Medicine 46:586–590.)

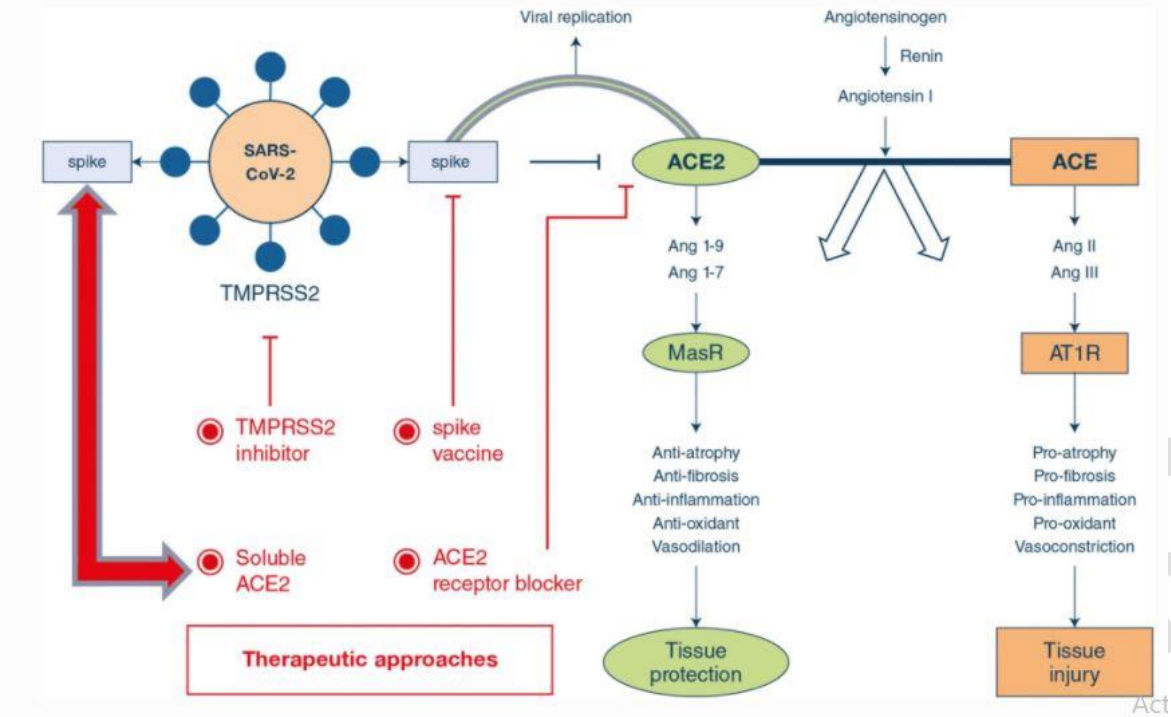


Fig: ANGIOTENSIN II involvement

Role of Vedicinal: ACE2 downregulation and the imbalance between the RAS and ACE2/angiotensin-(1–7)/MAS after infection may also contribute to multiple organ injury in COVID-19. Phytocompound 1, 2, 3,5, 6, 7 and 9 block SARS-CoV-2 spike glycoprotein, which binds to ACE2. Restoring the balance between the RAS and ACE2/angiotensin-(1–7)/MAS may help attenuate organ injuries. Our proposed formulation contains 6 compounds namely compounds 1, 2, 3, 5, 6, 7&9 inhibitors of Angiotensin II Inhibitorsdomain. In this study, three confirmations of this enzyme have separately docked with Baicalin, Quercetin, Luteolin, Hesperidin, Curcumin, EGCG and Glycyrrhizin were found to offer good interaction with a binding energy and hydrogen bond interaction. trans-membrane Angiotensin II Inhibitorsis one of the most intriguing and promising drug targets for SARS-CoV-2 drug development.

Pathway 26th: PALS 1 INHIBITORS

PALS1 (Protein Associated with Lin Seven 1), a tight junction-associated protein, is a member of the CRUMBS3-PALS1-PATJ (PALS1-associated tight junction) polarity complex, which is crucial for the establishment and maintenance of epithelialcell polarity[101]. PALS1 is required for the normal polarized localization of the vesicular markers sec8 and syntaxin4, and for the distribution of E-cadherin and myelin proteins PMP22 and MAG at the plasma membrane.The polarity protein PALS1 plays an essential role in the radial and longitudinal extension of the myelin sheath, likely involving a functional role in membrane protein trafficking.

In vitro, mild silencing of PALS1 in mammalian epithelial cells leads only to a delay in the formation of tight junctions, while a stronger silencing disturbs the formation of tight and adherens junctions.

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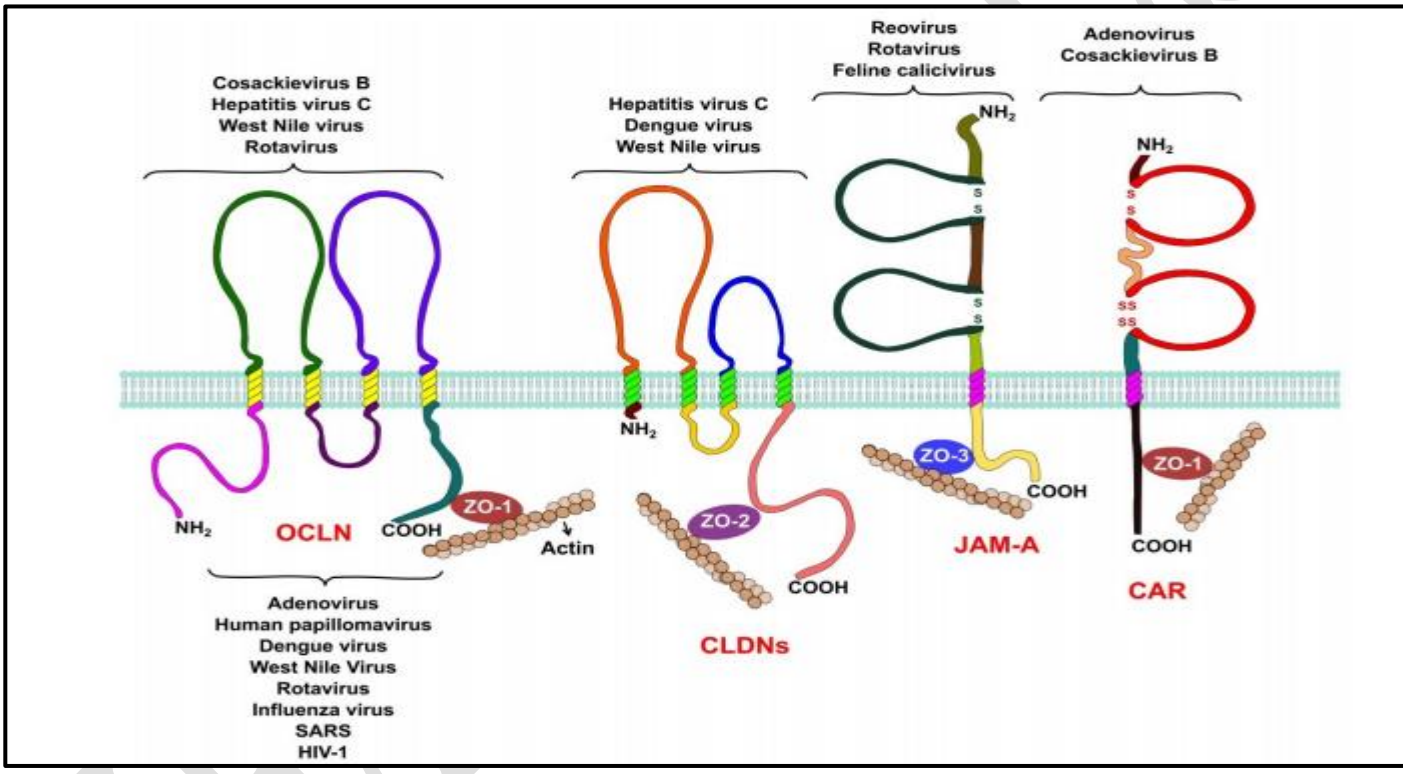


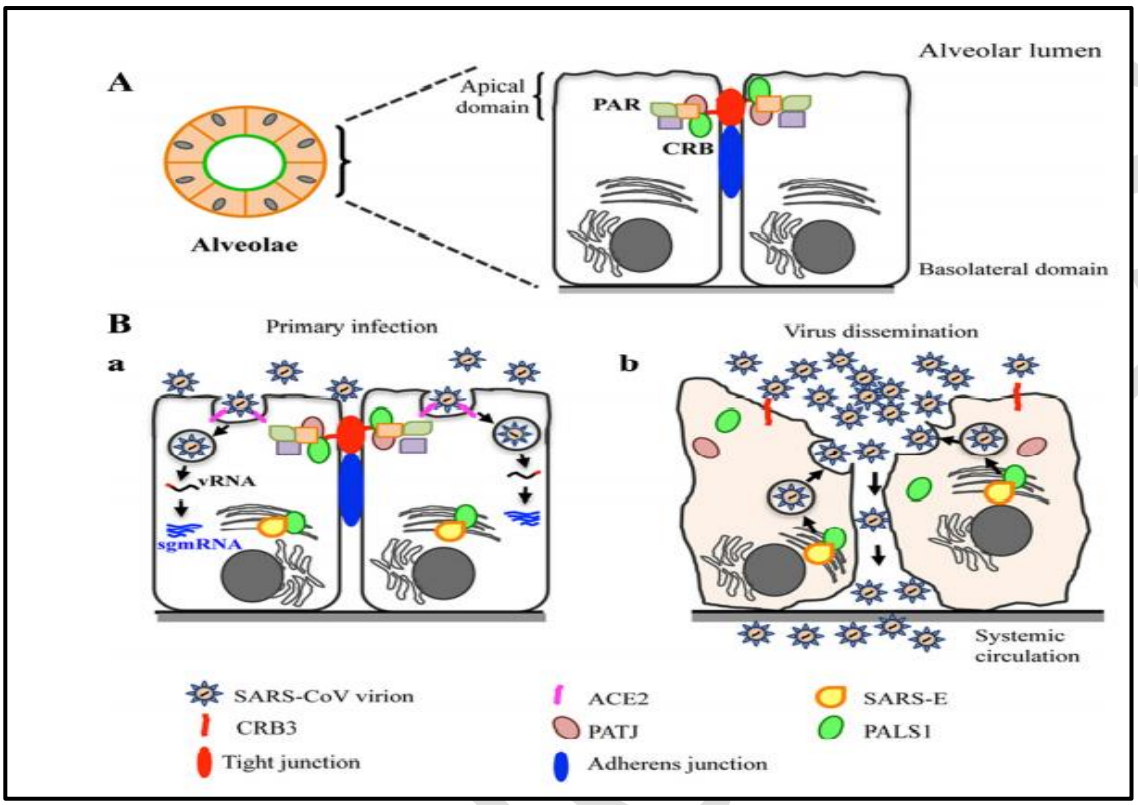
[Figure 1. Tight junction proteins and virus replication.]

In epithelial cells, PALS1is involved in cell polarization and so far this is the only function that has been shown for this molecule. Pals1 is not required for the distribution of particular myelin proteins, but rather for the myelin sheath genesis itself. While PALS1 mutations or silencing have been shown to affect adherens and tightjunctionformation in epithelial cells .An impairment of this mechanism in SCs(Schwann cells) silenced for PALS1 is thus likely to reduce myelination[102].

[101]Teoh, Kim Tat et al. 2010. "The SARS Coronavirus E Protein Interacts with PALS1 and Alters Tight Junction Formation and Epithelial Morphogenesis." *Molecular Biology of the Cell* 21(22): 3838–52.

[102]Özçelik, Murat et al. 2010. "Pals1 Is a Major Regulator of the Epithelial-like Polarization and the Extension of the Myelin Sheath in Peripheral Nerves." Journal of Neuroscience 30(11): 4120–31.

PALS1, a membrane-associated guanylate kinase protein. PALS1 is positioned as a crucial adaptor at the tight junction that forms the core of the epithelial polarity complex[103].PALS1 is a cellular protein involved in maintaining tight junctions between epithelial cells. Therefore, interactions between the SARS E protein and PALS1 induced relocation of PALS1 to the virus assembly site and disrupted tight junctions promoting virus spread[104].In-silico modelling analyses of E proteins conformation and docking provide evidences of a strengthened binding of SARS-CoV-2 E protein with the tight junction-associated PALS1 protein[105].



[Figure . Model of the potential consequences of SARS-CoV infection on polarity and intercellular junctions formed by alveolar epithelial cells.]

PALS1 is a cellular protein involved in maintaining tight junctions between epithelial cells also via the interaction with PALS1-associated tight junction (PATJ) protein. The PALS1-PATJ complex is fundamental for the development and maintenance of apical-basal polarity of epithelial cells. Therefore, interactions between the SARS E protein and PALS1 induced relocation of PALS1 to the virus assembly site and disrupted tight junctions promoting virus spread. The hypothesis that characteristic virulence of SARS-CoV-2 virus could depend on the strengthened interaction between

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[103] Helfand, Brian T et al. 2003. "Maintaining the Shape of Nerve Cells □." Molecular Biology of the Cell 14(December): 5069–81.

[104] Maio, Flavio De et al. 2020. "Enhanced Binding of SARS-CoV-2 Envelope Protein to Tight Junction-Associated PALS1 Could Play a Key Role in COVID-19 Pathogenesis." : 1–14.

[105] De Maio, Flavio et al. 2020. "Improved Binding of SARS-CoV-2 Envelope Protein to Tight Junction-Associated PALS1 Could Play a Key Role in COVID-19 Pathogenesis." Microbes and Infection 22(10): 592–97. https://doi.org/10.1016/j.micinf.2020.08.006.

SARS-CoV-2 E protein and PALS1 prompting a strong alteration of the tight junctions. The enhanced binding to PALS1 represents only the first step of the immune pathogenic process associated to SARS-CoV-2 infection. PALS1 E binding alters E-cadherin intracellular traffic with change in cell polarizatio.Several studies show that E protein has a role in pathway signalling, for example, one study of E protein showed that the E protein in SARS-CoV-2 in the CT region had changed in several amino acids, which could affect the PALS1, which plays a key role in tight junction, causing the virus to become more pathogenic than other coronaviruses[106].

Role of Vedicinal: Our proposed formulation contains 6 compounds namely compounds 1, 2, 3, 5, 6, 7, 8&9LYS410(H1), ASN635(H2), THR579 (H1), GLN580(H2), TYR363(H3), ARG578, TYR638(H1), GLN633(H2), ASN388(H3), PHE639(H1), HIS637(H2), MET469(H3), PRO670, TYR466, GLU467, ASN388(H1), LYS583(H2), TYR363, PHE354, TYR356, PRO408, ASN388(H1), GLN580(H2), GLY409(H3), GLY409(H1), GLN633(H2),LYS795(H1), LYS814(H2), LEU806 and PRO807 inhibitors of trans-membrane PALS 1 Inhibitorsdomain. In this study, three confirmations of this enzyme have separately docked with Baicalin, QuercetinLuteolin, Curcumin, EGCG, Piperine and Glycyrrhizin were found to offer good interaction with a binding energy and hydrogen bond interaction. Trans-membrane PALS 1 Inhibitors is one of the most intriguing and promising drug targets for SARS-CoV-2 drug development.

[106]Asghari, Arghavan et al. 2020. "The Novel Insight of SARS-CoV-2 Molecular Biology and Pathogenesis and Therapeutic Options." *DNA and Cell Biology* 39(10): 1741–53.

Pathway :27thP-SELECTIN INHIBITORS

P-selectin is a type-1 transmembrane protein that in humans.P-selectin functions as a cell adhesion molecule (CAM) on the surfaces of activated endothelial cells, which line the inner surface of blood vessels, and activated platelets[107]. In inactivated endothelial cells, it is stored in granules called Weibel-Palade bodies. In inactivated platelets P-selectin is stored in granules. Other names for P-selectin include CD62P, Granule Membrane Protein 140 (GMP-140), and Platelet Activation-Dependent Granule to External Membrane Protein (PADGEM)[108].

P-selectin is constitutively expressed in megakaryocytes (the precursor of platelets) and endothelial cells.P-selectin expression is induced by two distinct mechanisms. First, P-selectin is synthesized by megakaryocytes and endothelial cells, where it is sorted into the membranes of secretory granules[109].When megakaryocytes and endothelial cells are activated by agonists such as thrombin,P-selectin is rapidly translocated to the plasma membrane from granules. P-selectin may play an important role in the delivery of protein to the cell surface. In ischemic stroke patients, plasma P-selectin concentration was reported to be highly correlated to plasminogen activator inhibitor-1 activity and tissue plasminogen activator activity[110].

The extracellular region of P-selectin is composed of three different domains like other selectin types; a C-type lectin-like domain in the N-terminus, an EGF-like domain and a complement-binding protein-like domains (same as complement regulatory proteins: CRP) having short consensus repeats (~60 amino acids). P-selectin plays an essential role in the initial recruitment of leukocytes (white blood cells) to the site of injury during inflammation. When endothelial cells are activated by molecules such as histamine or thrombin during inflammation, P-selectin moves from an internal cell location to the endothelial cell surface[111].

[107]Xiping, Zhang et al. 2009. "Influence of Baicalin on TNF-α MRNA, Caspase-3 and p-Selectin Expression in Pancreatic Tissue of Rats with Severe Acute Pancreatitis." *Indian Journal of Gastroenterology* 28(4): 131–35.

[108]Mosawy, Sapha, Denise E. Jackson, Owen L. Woodman, and Matthew D. Linden. 2013. "Inhibition of Platelet-Mediated Arterial Thrombosis and Platelet Granule Exocytosis by 3′,4′-Dihydroxyflavonol and Quercetin." *Platelets* 24(8): 594–604.

[109]Matsumura, Kazuaki et al. 2009. "Preservation of Platelets by Adding Epigallocatechin-3-O-Gallate to Platelet Concentrates." *Cell Transplantation* 18(5–6): 521–28.

[110]Kung, Po Hsiung et al. 2017. "HPW-RX40 Prevents Human Platelet Activation by Attenuating Cell Surface Protein Disulfide Isomerases." *Redox Biology* 13: 266–77. http://dx.doi.org/10.1016/j.redox.2017.05.019.

[111]Schulz, C., B. Engelmann, and S. Massberg. 2013. "Crossroads of Coagulation and Innate Immunity: The Case of Deep Vein Thrombosis." *Journal of Thrombosis and Haemostasis* 11(SUPPL.1): 233–41.

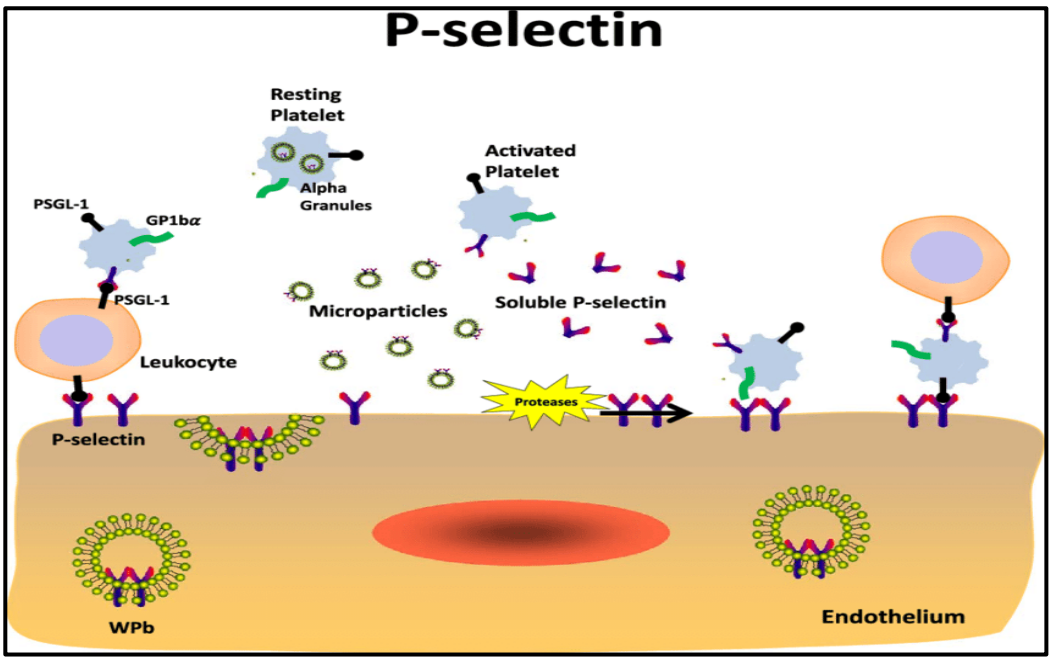
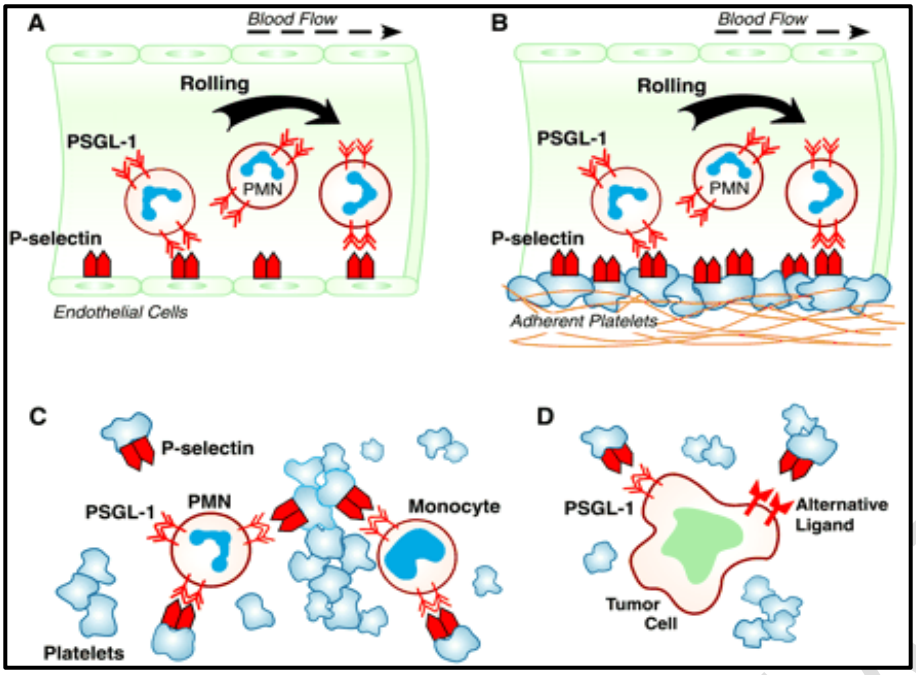


Figure :Compartments of P-selectin. P-selectin is stored in endothelial cells and platelets]

P-selectin is also very important in the recruitment and aggregation of platelets at areas of vascular injury. P-selectin has a functional role in tumour metastasis similar to E-selectin. P-selectin is expressed on the surface of both stimulated endothelial cells and activated platelets, and helps cancer cells invade into the bloodstream for metastasis and provides local multiple growth factors, respectively. P-selectin is expressed on activated platelets mediating platelet adhesion to monocytes and neutrophils. Furthermore, P-selectin has been shown to play an essential role in atherosclerotic lesion development[112]. P-selectin is incorporated into the intraluminal plasma membrane.

[112]Abdalruhman Gaiz, Almottesembellah et al. 2016. "The Green Tea Extract Epigallocatechin Gallate Inhibits Human Platelet Function but Not Plasma Coagulation." *International Journal of Prevention and Treatment* 5(2): 17–21. https://www.researchgate.net/publication/306396325.



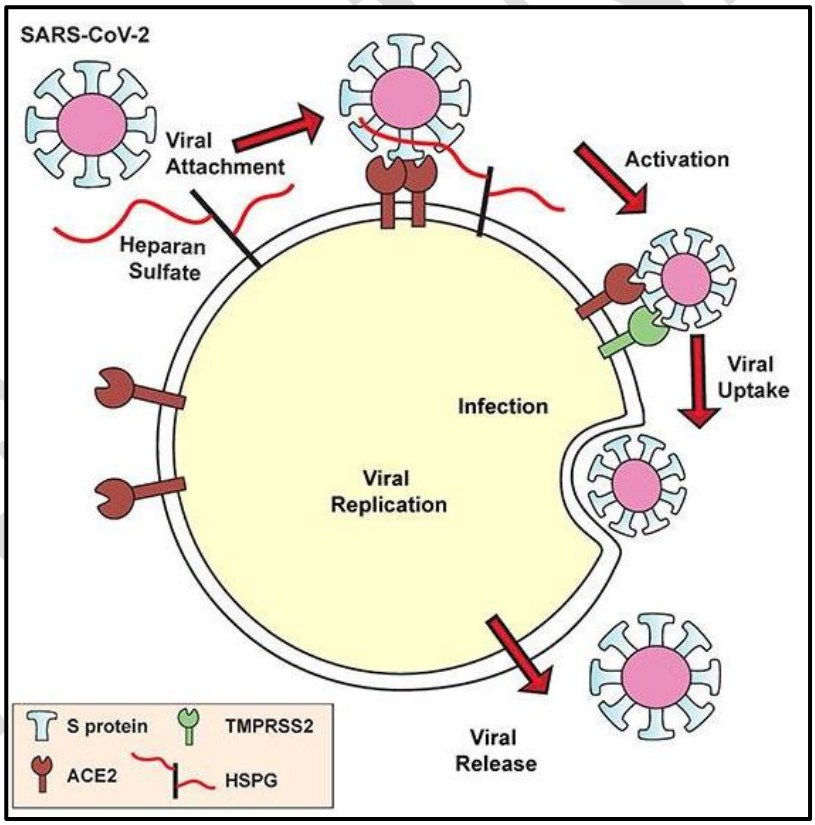
[Figure:- Cell–cell interactions mediated by P-selectin and PSGL-1. Binding of P-selectin to PSGL-1 mediates rolling of leukocytes on inflamed endothelial cells]

Role of Vedicinal: Our proposed formulation contains 6 compounds namely compounds 1, 2 & 7 are inhibitors of P-selectin domain. In this study, three confirmations of this enzyme have separately docked with Baicalin, Quercetin and EGCGwere found to offer good interaction with a binding energy and hydrogen bond interaction. P-selectin is one of the most intriguing and promising drug targets for SARS-CoV-2 drug development.

Pathway:28thHEPARAN SULFATE BINDING INHIBITORS

Heparan sulfate (**HS**) is a linear polysaccharide found in all animal tissues. It occurs as a proteoglycan (HSPG, i.e. Heparan Sulfate ProteoGlycan) in which two or three HS chains are attached in close proximity to cell surface or extracellular matrix proteins. It is in this form that HS binds to a variety of protein ligandsand regulates a wide range of biological activities, including developmental processes, angiogenesis, blood coagulation, abolishing detachment activity by GrB (Granzyme B), and tumour metastasis. HS has also been shown to serve as cellular receptor for a number of viruses, including the respiratory syncytial virus. The cellular heparan sulfate has a role in SARS-CoV-2 Infection, particularly when the virus attaches with ACE2.

Heparan sulfate is a member of the glycosaminoglycan family of carbohydrates and is very closely related in structure to heparin. Heparin, commonly known as an anticoagulant, is a highly sulfated form of HS which, in contrast to HS, is mainly found in mast cell secretory granules. Heparan sulfate binds with a large number of extracellular proteins. These are often collectively called the “heparin interactome” or "heparin-binding proteins".The functions of heparan sulfate binding proteins ranges from extracellular matrix components, to enzymes and coagulation factors, and most growth factors, cytokines, chemokines and morphogens[113].



[FIGURE : SARS-COV-2'S SPIKE PROTEIN MUST BIND BOTH THE ACE2 RECEPTOR AND HEPARAN SULFATE TO GAIN ENTRY INTO HUMAN CELLS.]

[113]Colpitts, Che C., and Luis M. Schang. 2014. “A Small Molecule Inhibits Virion Attachment to Heparan Sulfate- or Sialic Acid-Containing Glycans.” *Journal of Virology* 88(14): 7806–17.

SARS-CoV-2, the novel coronavirus that causes COVID-19, primarily uses ACE2 to enter these cells and establish respiratory infections. SARS-CoV-2 can't grab onto ACE2 without a carbohydrate called heparan sulfate, which is also found on lung cell surfaces and acts as a co-receptor for viral entry. The two approaches that can reduce the ability of SARS-CoV-2 to infect human cells cultured in the lab by approximately 80 to 90 percent: 1) removing heparan sulfate with enzymes or 2) using heparin as bait to lure and bind the coronavirus away from human cells. Heparin, a form of heparan sulfate, is already a widely used medication to prevent and treat blood clots to reduce virus infection[114].

The SARS-CoV-2 spike protein binds to heparin. The also drilled down to uncover the exact part of the SARS-CoV-2 spike protein that interacts with heparin the receptor binding domain. When heparin is bound, the receptor binding domain opens up and increases binding to ACE2. The virus, they found, must bind both heparan sulfate on the cell surface and ACE2 in order to get inside human lung cells grown.They found that enzymes that remove heparan sulfate from cell surfaces prevent SARS-CoV-2 from gaining entry into cells. Treatment with heparin also blocked infection. The heparin treatment worked as an anti-viral at doses currently given to patients[115].

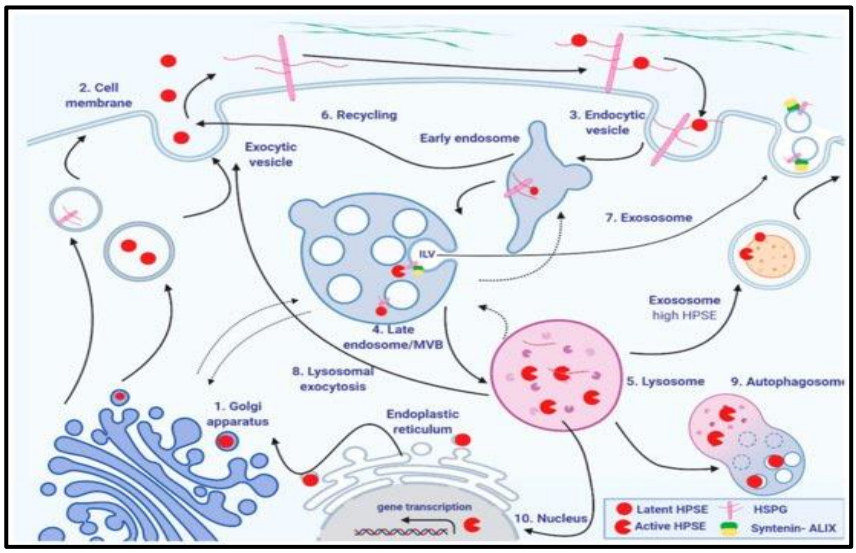


Figure 2. Schematic model of heparan sulfate proteoglycans and heparanase trafficking.

ACE2-mediated entry of SARS-Cov and CoV-2 requires the cell surface heparan sulfate (HS) as an assisting cofactor. HS is a negative charge-enriched linear polysaccharide molecule that is attached to several membrane and extracellular proteins collectively termed as heparan sulfate proteoglycans (HSPG). The cell surface HS can serve as an anchor to facilitate endocytosis of many, which include SARS-CoV-2-related coronaviruses[116].

[114]Connell, Bridgette J., and Hugues Lortat-Jacob. 2013. "Human Immunodeficiency Virus and Heparan Sulfate: From Attachment to Entry Inhibition." *Frontiers in Immunology* 4(NOV): 1–13.

[115]Ganesan, Shyamala et al. 2012. "Quercetin Inhibits Rhinovirus Replication in Vitro and in Vivo." *Antiviral Research* 94(3): 258–71.

[116]Veraldi, Noemi, Nawel Zouggari, and Ariane de Agostini. 2020. "The Challenge of Modulating Heparan Sulfate Turnover by Multitarget Heparin Derivatives." *Molecules* 25(2): 1–22.

Role of Vedicinal: Our proposed formulation contains 6 compounds namely compounds 1, 2, 3, 6, 7, 8&9 are TRP216(H1), SER212(H2), TRP215, GLU452(H1), PRO510(H2), ARG560(H3)THR565(H1), VAL558(H2), SER511PHE364(H1), TRP216(H2), HIS363, TRP305, PRO218, ALA409, GLU408, PHE461, LYS463(H1), TRP216(H2), TRP305(H3), PRO218, CYS394(H1), MET348(H2), SER349(H3), THR350(H4), TRP305(H1), THR156(H2), VAL303, TRP215, PRO159, TRP216, PRO109, SER59(H1), TYR480(H1), ARG471(H1), PRO109 and ARG61inhibitors of Heparan sulfate Inhibitors domain. In this study, three confirmations of this enzyme have separately docked with Baicalin, Quercetin, Luteolin and Glycyrrhizinwere found to offer good interaction with a binding energy and hydrogen bond interaction. Heparan sulfate Inhibitors is one of the most intriguing and promising drug targets for SARS-CoV-2 drug development.

Pathway :29thCCR5 INHIBITORS

C–C chemokine receptor 5(CCR5) is a cell membrane protein from G protein-coupled receptors (GPCR) family, which is an important modulator for leukocyte activation and mobilization.CCR5 receptor antagonists are a class of small molecules that antagonize the CCR5 receptor. The C-C motif chemokine receptor CCR5 is involved in the process by which HIV, the virus that causes AIDS, enters cells. Hence antagonists of this receptor are entry inhibitors and have potential therapeutic applications in the treatment of HIV infections.

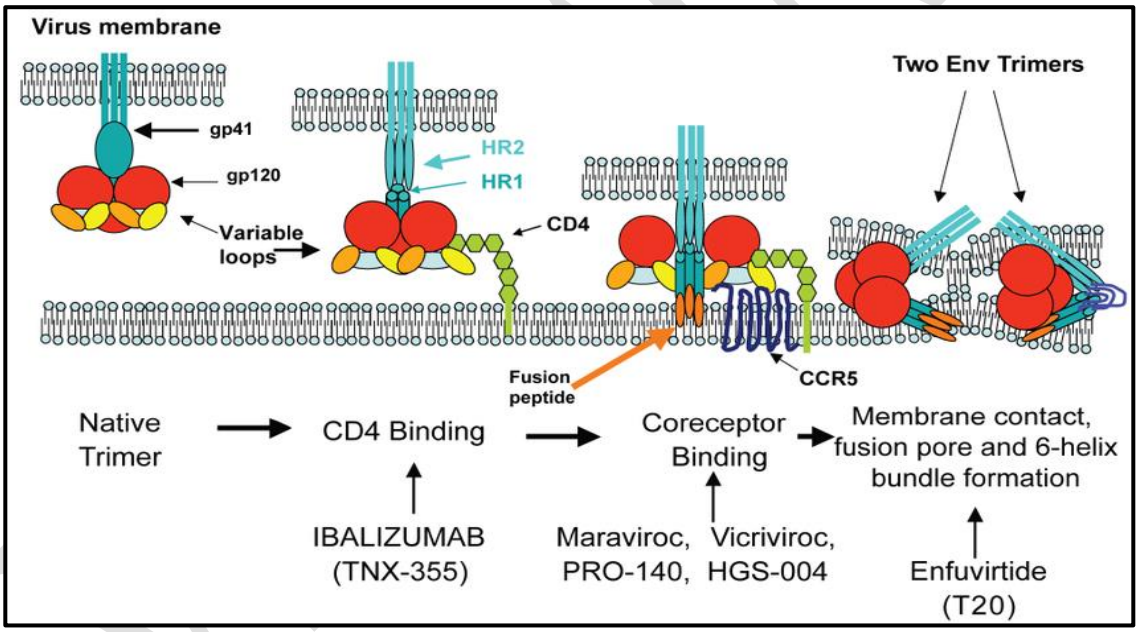
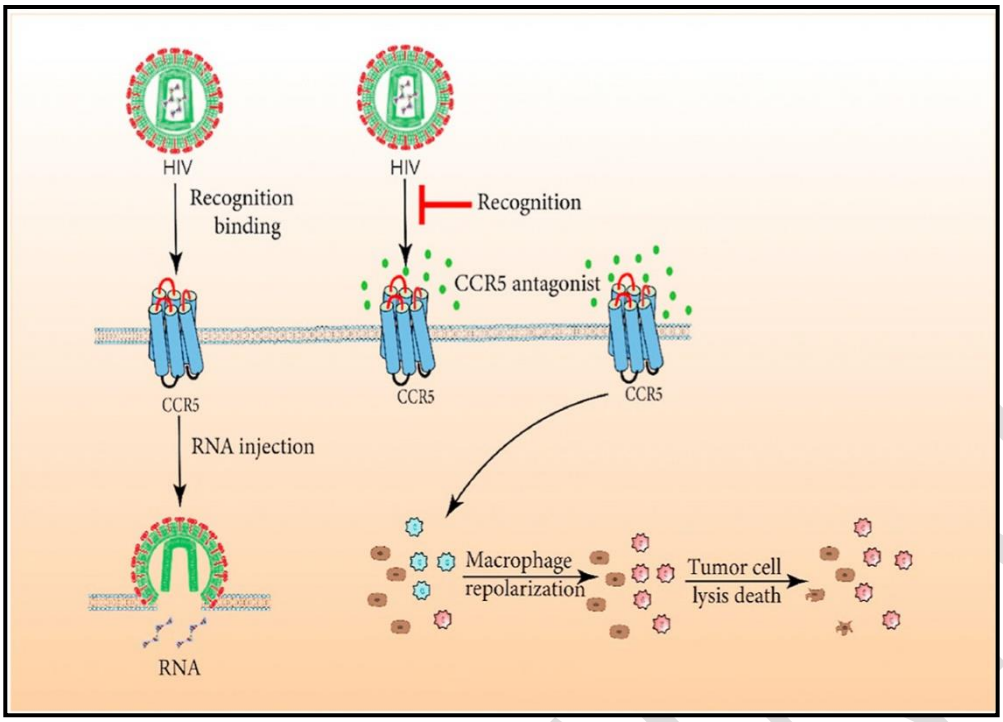


Figure :- CCR5 antagonists

The of CCR5's role in HIV infection, many pharmaceutical companies had already built a substantial collection of compounds that target GPCRs. Leronlimab is a humanized monoclonal antibody targeted against the CCR5 receptor found on T lymphocytes of the human immune system and many types of cancers. leronlimab restores immune function and impacts disease in COVID-19 patients. After treatment with leronlimab, these critically ill patients experienced reversed hyperimmune activation and inflammation, as well as reversed immunosuppression, thereby facilitating a more

effective immune response correlated with decreases in SARS-CoV-2 level in blood.Leronlimab is a drug candidate that is a CCR5 antagonist with the potential for multiple therapeutic indications. The CCR5 receptor appears to play a central role in modulating immune cell trafficking to sites of inflammation.



[Figure2: Advances of CCR5 antagonists]

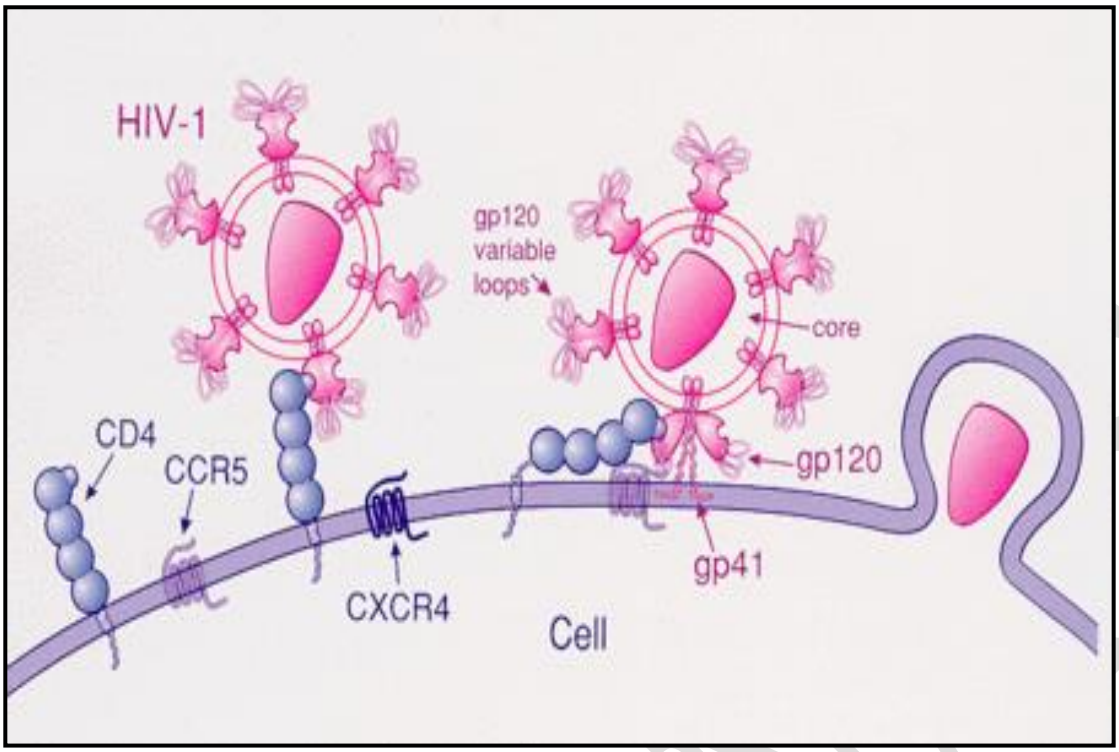
CCR5 and CXCR4 as the major co-receptors for HIV. These receptors belong to the seven transmembrane G-protein-coupled receptor (GPCR) family and are predominantly expressed on human T-cells, dendritic cells and macrophages, Langerhans cells.They play an important role as co-receptors that HIV type 1 (HIV-1) uses to attach to cells before viral fusion and entry into host cells. The coexpression of DPP4 and CCR5 may partially explain the association between DPP4 expression and HIV infection. In recent years, many studies have shown that CCR5 is closely related to the development of various cancers and inflammations to facilitate the discovery of CCR5 antagonists. There are many types of CCR5 antagonists, mainly including chemokine derivatives, non-peptide small molecule compounds, monoclonal antibodies, and peptide compounds[117]. The location of CCR5 receptors at the cell surface, both large and small molecules have the potential to interfere with the CCR5-viral interaction and inhibit viral entry into human cells[118].

CCR5 co-receptor antagonists prevent HIV-1 from entering and infecting immune cells by blocking CCR5 cell-surface receptor. Small molecule antagonists of CCR5 bind to a hydrophobic pocket formed by the transmembrane helices of

[117]Qi, Baowen et al. 2020. "Advances of CCR5 Antagonists: From Small Molecules to Macromolecules." *European Journal of Medicinal Chemistry* 208: 112819. https://doi.org/10.1016/j.ejmech.2020.112819.

[118]Zhong, Jixin et al. 2015. "Recent Advances in Dipeptidyl-Peptidase-4 Inhibition Therapy: Lessons from the Bench and Clinical Trials." *Journal of Diabetes Research* 2015.

the CCR5 receptor[119].



[Figure : HIV entry into CD4+ cell via CCR5 co-receptor]

Role of Vedicinal: Our proposed formulation contains 6 compounds namely compounds 1, 2, 3&7 are inhibitors of C–C chemokine receptor 5(CCR5)domain. In this study, three confirmations of this enzyme have separately docked with Baicalin, Quercetin, Luteolin and EGCG were found to offer good interaction with a binding energy and hydrogen bond interaction. C–C chemokine receptor 5(CCR5) is one of the most intriguing and promising drug targets for SARS-CoV-2 drug development

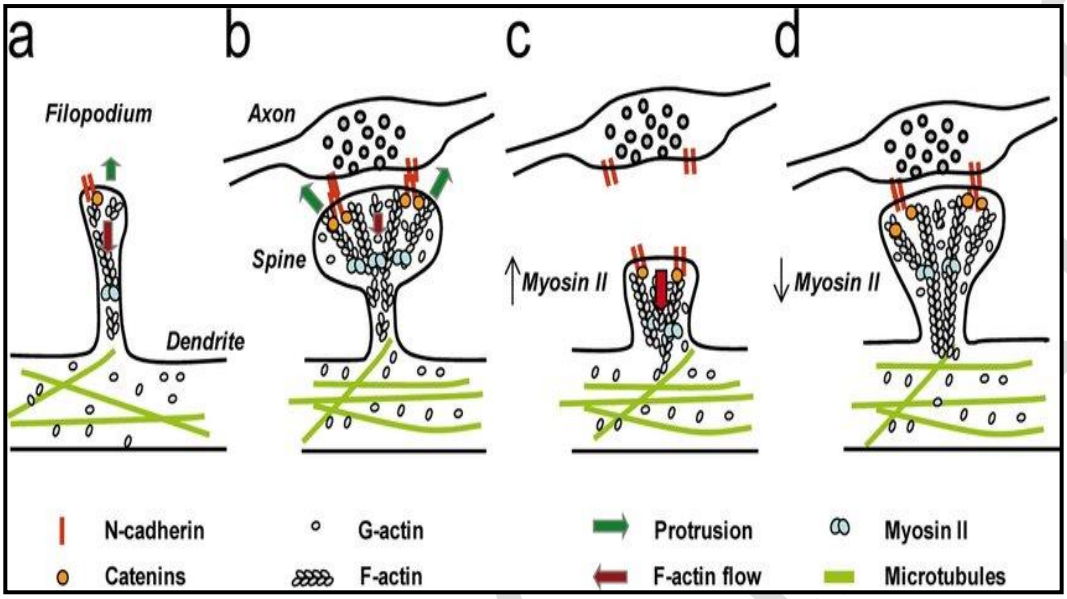
Pathway :30thMYOSIN II & FILOPODIA ADHESION INHIBITORS

Myosin II is a plus-end motor that localizes to the lamellum and retraction fibers. If anchored, this myosin can also mediate minus-end motility by moving entire actin filaments.The association of myosin II with F-actin results in the formation of stress fibers in interphase cells and the contractile ring in dividing cells. A cell protein called CK2 spurs the growth of tentacle-like protrusions known as filopodia. These filopodia contain viral particles and are likely used to poke holes in nearby cells, spreading SARS-CoV-2[120].

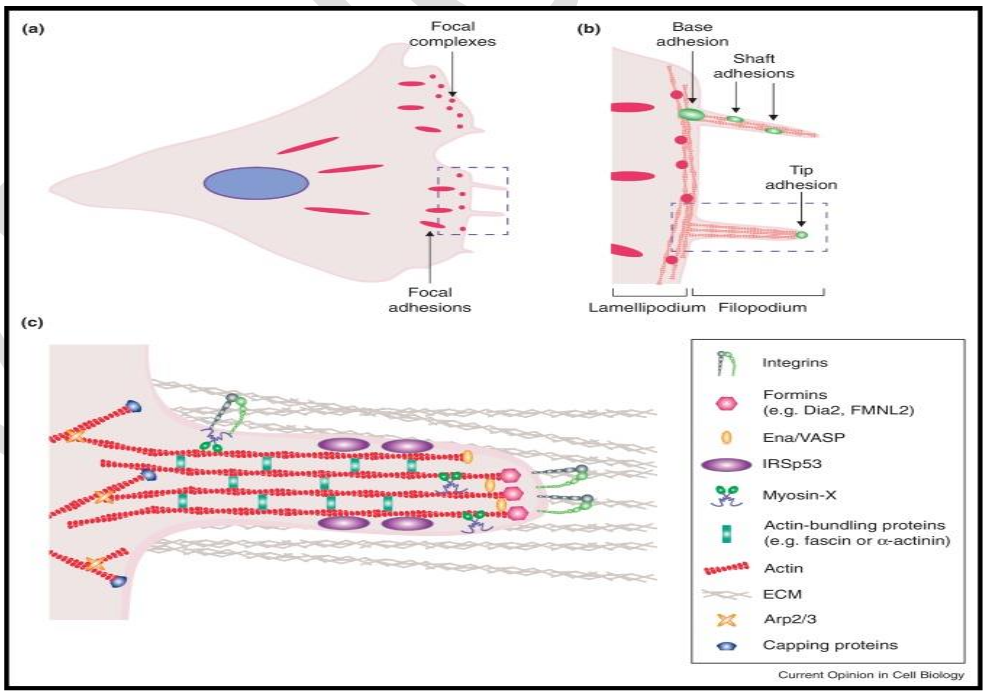
[119]Banerjee, Arinjay et al. 2021. "Predicting the Recombination Potential of Severe Acute Respiratory Syndrome Coronavirus 2 and Middle East Respiratory Syndrome Coronavirus." *Journal of General Virology* 101(12): 1251–60.

[120]Jacquemet, Guillaume, Hellyeh Hamidi, and Johanna Ivaska. 2015. "Filopodia in Cell Adhesion, 3D Migration and Cancer Cell Invasion." *Current Opinion in Cell Biology* 36: 23–31. http://dx.doi.org/10.1016/j.ceb.2015.06.007.

Myosin II also appeared to localize to the base of filopodia. Myosin II is clearly present in the cellular protrusions that promote virus motility. In the case of filopodia, myosin II may affect retrograde F-actin flow from the base of the filament. MyosinII is the predominant ATPase involved in virus cell surfing. Role for myosin II in virus entry, inhibitory effects were strictly associated with the microvilli-rich apical side of polarized epithelial cells. Transport along filopodia is mediated by the underlying actin cytoskeleton and is controlled by myosin II. Because myosin II is a plus end motor that apparently mediates minus end motility toward the cell body, it must regulate the movement of kinetics of ALV infection mediated by receptors. Myosin II clearly localizes to retraction fibers, it is absent from filopodia.



[Figure :-Model of Filopodium and Myosin II]



[Figure . Schematic representation of filopodia during cell migration on a planar substrate.

Virus cell surfing along filopodia is mediated by the underlying actin cytoskeleton and depends on functional myosin II. Any disruption of virus cell surfing significantly reduces viral infection. Viruses have been frequently observed in association with highly dynamic cell surface protrusions such as filopodia, or in the case of mucosal tissues, the dense meshwork of microvilli. Whether filopodia or microvilli actively contribute to infections is unclear. Upon interaction with filopodia, viruses undergo rapid actin- and myosin II– driven transport, "surfing" to entry sites at the cell body. Virus cell surfing along filopodia represents a novel mechanism by which viruses use host cell machineries for efficient infection. A novel process by which viruses induce rapid and directed trafficking along filopodia to reach the cell body for entry into cells. Transport along filopodia is mediated by the underlying actin cytoskeleton and is controlled by myosin II. Even in the case of short-lived filopodia that were observed to occasionally capture viral particles, particles were brought to the cell body by retrograde flow of actin filaments. Myosin II clearly localizes to retraction fibers, it is absent from filopodia. Myosin II may control actin filament movement at the base of filopodia by regulating actin filament disassembly.[121]

Filopodia induced by overexpression of myosin X, typical for cancer cells. Filopodia are ubiquitous cell extensions involved in cell motility, exploration of the microenvironment, and adhesion. These finger-like membrane protrusions help cells to determine the direction of movement, establish contacts with other cells, and capture inert particles or living objects (bacteria), which cells subsequently engulf. Filopodia are involved in numerous processes of embryonic development, as well as in cell migration in adult .

The presence and activity of myosin II affects force-induced filopodia growth and adhesion. Myosin II-generated force transmitted via actin core to formins at the filopodium tip can in principle stimulate actin polymerization, promoting filopodia growth and reinforcing adhesion. Myosin II does not localize to the filopodia tips or shafts, but is often seen at the proximal ends of the filopodia[122].Phosphorylation of myosin II regulatory light chain (MRLC) is critical event for many cellular processes including muscle contraction, mytosis, migration, and exocytosis. The activity of non-muscle myosin II is largely regulated by phosphorylation of its regulatory myosin light chain (MRLC).[123]

Myosin II& Filopodia Adhesion

Role of Vedicinal: Our proposed formulation contains 6 compounds namely compounds 1, 3,&7 are inhibitors of trans-membrane Furin Inhibitors domain. In this study, three confirmations of this enzyme have separately docked with Baicalin, Luteolin and EGCG were found to offer good interaction with a binding energy and hydrogen bond interaction. Trans-membrane Furin Inhibitors is one of the most intriguing and promising drug targets for SARS-CoV-2 drug development.

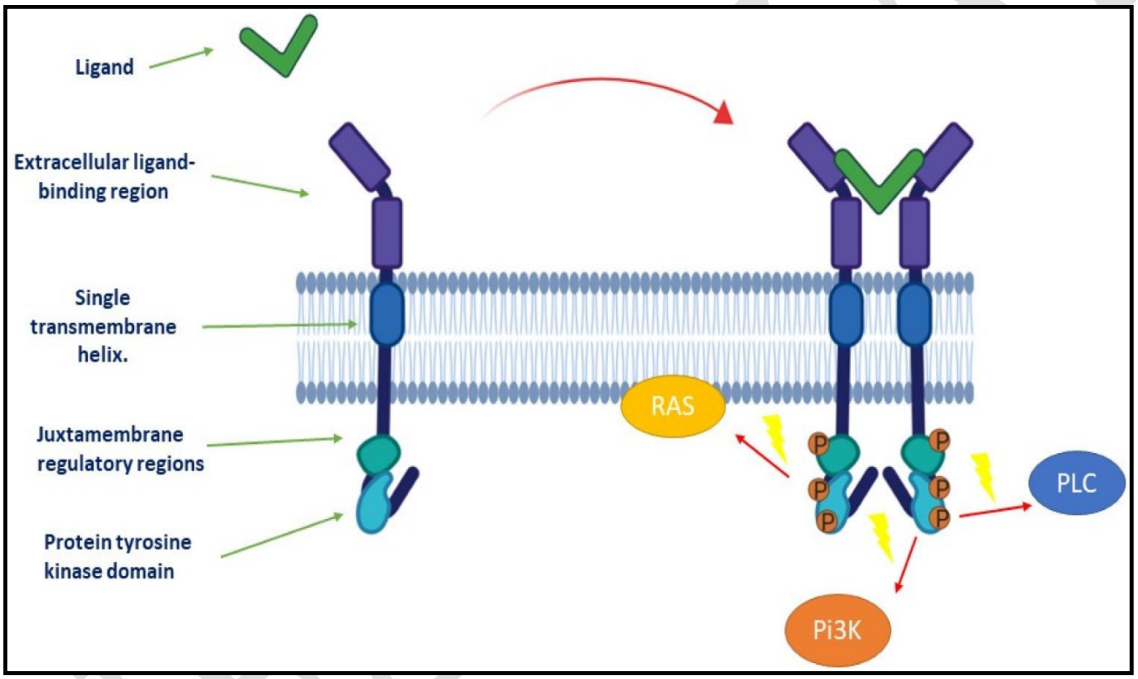
[121]Lehmann, Maik J. et al. 2005. "Actin- and Myosin-Driven Movement of Viruses along Filopodia Precedes Their Entry into Cells." *Journal of Cell Biology* 170(2): 317–25.

[122]Alieva, N. O. et al. 2019. "Myosin IIA and Formin Dependent Mechanosensitivity of Filopodia Adhesion." *Nature Communications* 10(1).

[123]Umeda, Daisuke, Hirofumi Tachibana, and Koji Yamada. 2004. "Tea Catechin, Epigallocatechin-3-Gallate Suppresses Myosin II Regulatory Light Chain Phosphorylation." *BioFactors* 21(1–4): 387–89.

Pathway:31stTYROSINE KINASE INHIBITORS

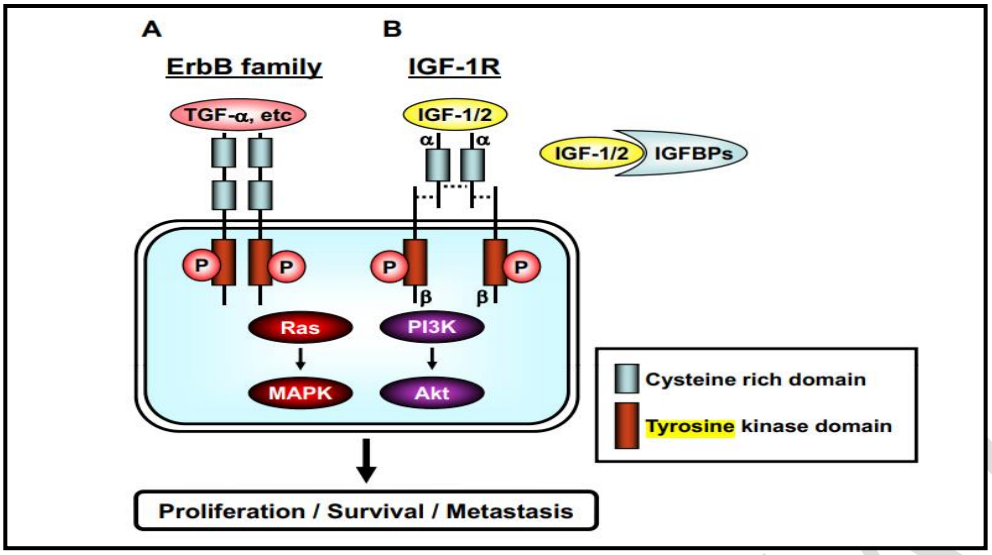
The targets in oncological therapy are, among others, tyrosine kinases, important mediators of signalling pathways whose impaired expression is observed in many types of cancer. The properties of curcumin and its derivatives in the treatment of cancers directed to signalling pathways of tyrosine kinases and confronts the problem of low assimilation of curcumin with potential therapeutic effects. The tyrosine kinases play an important role in the process of tumorigenesis. Due to their location in the cell, they are divided into: transmembrane receptor tyrosine kinases (RTK) and cytoplasmic non-receptor tyrosine kinases (NRTK). Ligands that cause tyrosine kinase receptor dimerization are extracellular signal molecules e.g. growth factors such as EGF or PDGF. The activity of tyrosine kinases is associated with several stages of cancer development and progression. The role of tyrosine kinases in cell signalling is, among others, the stimulation of growth, cell proliferation and inhibition of apoptosis. Hence, the genetic or epigenetic change in kinase expression is often responsible for the loss of cell growth control and gives it an oncogenic nature. It appears that nearly all cancers exhibit disturbances in the expression and thus the function of at least one receptor tyrosine kinase.



[Figure . Activation of tyrosine kinase receptor]

Receptor tyrosine kinases are important targets of drug action. The majority of presently investigated potential therapeutics targeted against TKs can be divided into two groups: the first one comprises small molecule inhibitors that are targeted to the ATP binding site of the intracellular tyrosine kinase domain and the second group are monoclonal antibodies that act directly on RTK and by recruiting immune cells for RTK expressing cells . The activity of tyrosine kinases can also be blocked indirectly by the modification of their chaperone proteins, mainly Hsp90 (Heat Shock Proteins), which are necessary to maintain the active conformation of the kinases. Mechanism of tyrosine kinase activity and led to the emergence of new therapeutic methods using synthetic chemical compounds with the properties of kinase

inhibitors. The level of phosphorylation of the cellular tyrosine kinase is tightly controlled by the antagonistic effects of tyrosine kinases and tyrosine phosphatases[124].



[Figure . Schematic representation]

Src tyrosine kinase (Src) is a ubiquitously expressed non receptor tyrosine kinase belonging to the Src family of kinases. Src family kinases are known for their roles in the progression of cancer; however they are also involved in inflammation-related signalling pathways, proliferation, and chemotaxis. Relating a particular cellular response, such as growth rate, to changes in cellular protein phosphorylation in response to a particular tyrosine kinase stimulator like EGF or a tyrosine kinase inhibitor like luteolin is a rather crude measure of intracellular signalling[125].Piperine can inhibit the activity of enzyme EGFR tyrosine kinase, which is one of the key targets of potential chemo preventive agents[126].

Quercetin was found to inhibit the activity of a tyrosine kinase which was thought to be responsible for the transformation of non-malignant fibroblast cells to sarcoma cells. Flavonoids have been found to inhibit the activity of protein tyrosine kinases. Tyrosine kinase inhibitors, such as genistein and herbimycin A, can block the effect of growth factors on growth factor-dependent cell lines. Tyrosine kinase activity can also repress the metastatic potential of tumour cells bearing this gene[127].Hepatocyte growth factor (HGF), also known as scatter factor (SF), and its receptor, the c-Met tyrosine kinase, play roles in cancer invasion and metastasis in a wide variety of tumor cells[128].

[124]Golonko, A. et al. 2019. "Curcumin as Tyrosine Kinase Inhibitor in Cancer Treatment." *European Journal of Medicinal Chemistry* 181.

[125]Jelić, Dubravko et al. 2016. "Baicalin and Baicalein Inhibit Src Tyrosine Kinase and Production of IL-6." *Journal of Chemistry* 2016.

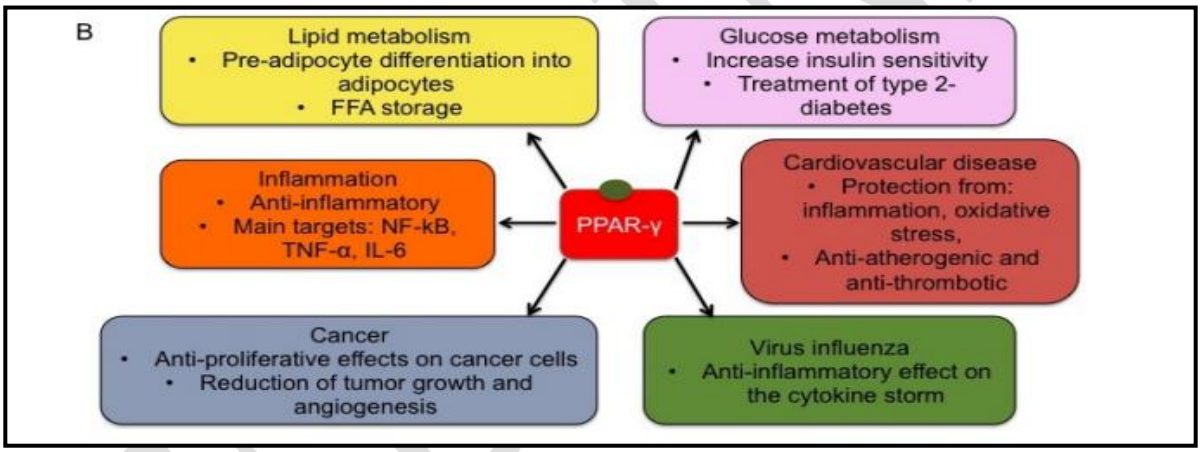
[126]Rather, Rafiq A., and Madhulika Bhagat. 2018. "Cancer Chemoprevention and Piperine: Molecular Mechanisms and Therapeutic Opportunities." *Frontiers in Cell and Developmental Biology* 6(FEB): 1–12.

[127]Huang, Y. T. et al. 1999. "Effects of Luteolin and Quercetin, Inhibitors of Tyrosine Kinase, on Cell Growth and Metastasis-Associated Properties in A431 Cells Overexpressing Epidermal Growth Factor Receptor." *British Journal of Pharmacology* 128(5): 999–1010.

The receptor tyrosine kinases (RTKs) are one of the critical targets of EGCG to inhibit cancer cell growth. Phytochemicals exert an anti-carcinogenic effect by modulating the activities of various types of receptor tyrosine kinases (RTKs) and their downstream specific cell signalling pathways which are associated with the expression of the genes involved in cell proliferation and apoptosis. All family members of RTK have an extracellular ligand-binding domain, a membrane-spanning region and a cytoplasmic protein tyrosine kinase domain[129].

Pathway 32nd: PPAR GAMMA ACTIVATORS

Peroxisome proliferator-activated receptor γ (PPARγ) is a ligandactivated transcription factor belonging to the nuclear receptor superfamily that is crucial for the regulation of adipogenesis, lipid metabolism and glucose homeostasis. They can be activated in the cytoplasm by a specific ligand and form specific complexes with a transcription factor, retinoic acid X receptor (RXR). The metabolic substrate switch seems to involve changes in mRNA level of genes implicated in the transport and metabolism of glucose and fatty acids, which are primarily regulated by a class of nuclear receptors called peroxisome proliferator-activated receptors (PPARs). PPARα is a key regulator of myocardial fatty acid uptake and oxidation in the heart. During pathological growth of heart failure, down-regulation of FAU genes correlates with decreased PPARα level. PPARα with medium chain triglycerides is able to prevent the reduction of FAU and reverse the development of cardiac hypertrophy. Similar to PPARα, PPARβ/δ is also highly expressed in cardiac myocytes and necessary for maintaining myocardial lipid homeostasis. Cardiac PPARβ/δ level was repressed by pressure overload in a hypoxia dependent pathway. baicalin-induced high expression of PPARα presents no significant lipotoxicity[130].



[Figure :-Function of PPAR]

The specific complexes combine with the peroxisome proliferator elements (PPRE), which are in the target gene promoter to regulate transcription of several genes involved in glucose metabolism, lipid metabolism, inflammation,

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[128]*Lee, Wei Jiunn et al. 2006. "Inhibitory Effect of Luteolin on Hepatocyte Growth Factor/Scatter Factor-Induced HepG2 Cell Invasion Involving Both MAPK/ERKs and PI3K-Akt Pathways." Chemico-Biological Interactions 160(2): 123–33.*

[129]*Shimizu, Masahito, Yohei Shirakami, and Hisataka Moriwaki. 2008. "Targeting Receptor Tyrosine Kinases for Chemoprevention by Green Tea Catechin, EGCG." International Journal of Molecular Sciences 9(6): 1034–49.*

[130]*Zhang, Yanqing et al. 2017. "Baicalin Attenuates Cardiac Dysfunction and Myocardial Remodeling in a Chronic Pressure-Overload Mice Model." Cellular Physiology and Biochemistry 41(3): 849–64.*

and other processes. The PPARs site can function to silence lipoprotein lipase (LPL) gene transcription in the presence of specific modulating factors. Many of the functions of PPARs are associated with pathways of lipid transport and metabolism. PPARs have 3 subtypes, α, β/δ, and γ;PPARγ predominates in adipose tissue, macrophages, and monocytes, and has also been identified in the liver, heart, skeletal muscle, and other organs (tissues). Its involvement in glucose metabolism is through improving insulin sensitivity, and plays a key role in adipogenesis and glucose homeostasis[131].

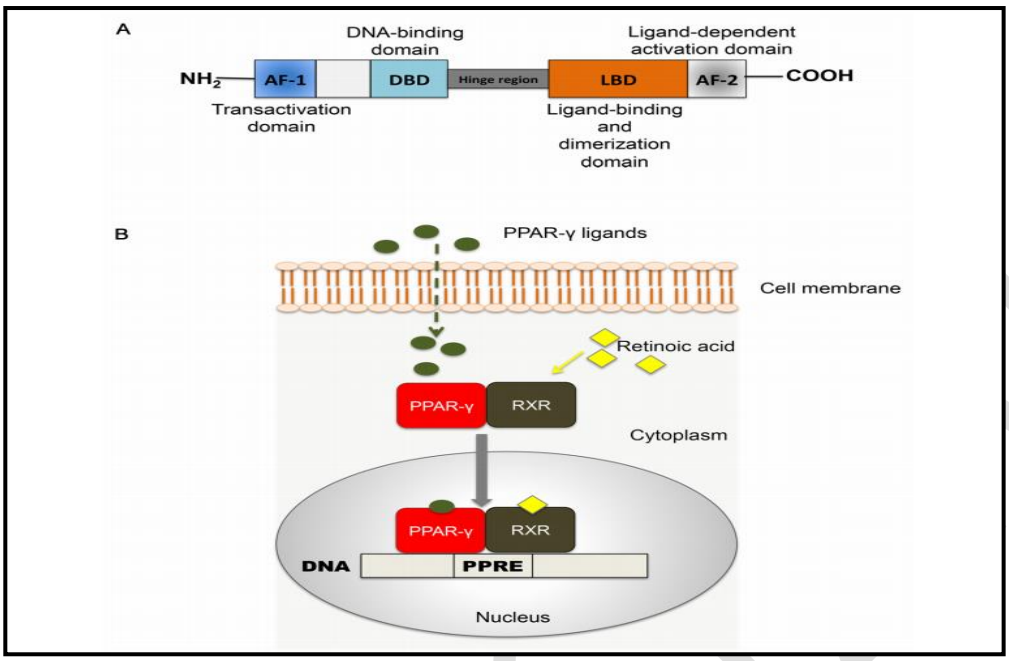


Figure : some proliferator-activated receptors (PPAR)

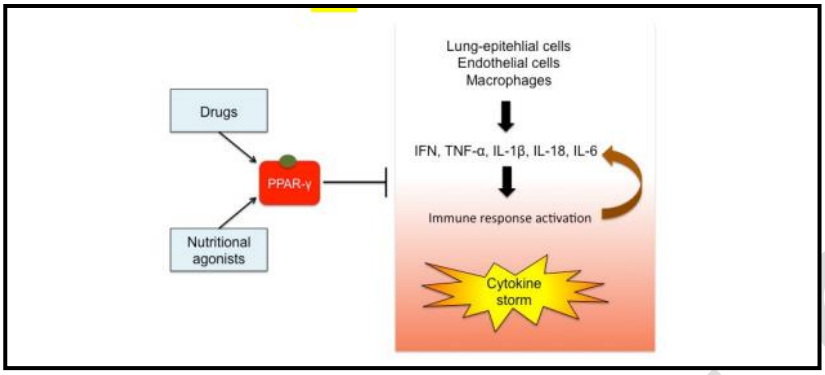
PPARγ has also been demonstrated to be a negative regulator of obesity-associated inflammatory responses and a key activator of insulin sensitivity. PPARγ inhibits the expression of pro-inflammatory genes by interfering with signal-dependent activation of the NF-κB pathway PPARγ, a key anti-inflammatory transcription factor, plays a significant role in the transcriptional regulation of metabolic gene expression. PPARγ is also a key therapeutic target for type 2 diabetes. The compounds that could affect PPARγ activity possess a huge potential for developing preventive and therapeutic agents for diabetes. PPARγ, as a target of other natural compounds such as phytol and abietic acid, has also been reported[132].

PPAR full agonists, such as thiazolidinediones (TZDs), are effective insulin sensitizers and anti-inflammatory agents, but their use is limited by adverse side effects. Luteolin is a flavonoid with anti-inflammatory actions that binds PPAR but, unlike TZDs, does not promote adipocyte differentiation. The three PPAR subtypes have different ligand specificity and tissue distribution. PPAR agonists such as the thiazolidinediones (TZDs) are efficient and clinically useful insulin

[131]Cai, Ying et al. 2012. "Effects of Rutin on the Expression of PPARγ in Skeletal Muscles of Db/Db Mice." *Planta Medica* 78(9): 861–65.

[132]Ding, Li, Daozhong Jin, and Xiaoli Chen. 2010. "Luteolin Enhances Insulin Sensitivity via Activation of PPARΓ Transcriptional Activity in Adipocytes." *Journal of Nutritional Biochemistry* 21(10): 941–47. http://dx.doi.org/10.1016/j.jnutbio.2009.07.009.

sensitizers and are under investigation for the treatment of inflammation with potential applications in atherosclerosis, inflammatory colitis, arthritis, and bowel disease. PPAR may also be a useful target in ocular conditions[133].



[Figure . Effects of PPAR-γ activation on cytokine storm]

The three PPAR isoforms share a common structure, but manifest different tissue distribution, target genes, and functions[134]. PPAR-γ stimulates pre-adipocyte differentiation into adipocytes and regulates the insulin sensitivity in peripheral tissues and the storage of fatty acids, through the modulation of genes involved in fatty acid release, transport, and storage into mature adipocytes[135].Piperine acted as an agonist of peroxisome proliferator that activated receptor-γ (PPAR-γ) and blocked the activation of protein kinase B (AKT)[136].PPARγ plays a pivotal role in regulation of hematopoietic stem cell (HSC) proliferation, osteoclastogenesis and monocyte differentiation. PPARγ ligands have been shown to suppress cellular growth by induction of apoptosis in different human lymphoma and leukemic cell lines[137].

Role of Vedicinal: In addition to modulating lipid and glucose metabolism, peroxisome proliferator-activated receptors (PPAR) play important roles in antagonizing core inflammatory pathways such as NF-kappaB, AP1, and STAT. Their role in regulating inflammatory responses caused by pulmonary pathogens is receiving increasing attention, setting the stage for the discovery of novel applications for anti-diabetic and lipid-lowering phytocompound 2, 4, 5, 6 and 8.

Pathway33th: SYNCYTIUM / SYNCYTIA FORMATION INHIBITORS

[133]Puhl, Ana C. et al. 2012. "Mode of Peroxisome Proliferator-Activated Receptor γ Activation by Luteolin." *Molecular Pharmacology* 81(6): 788–99.

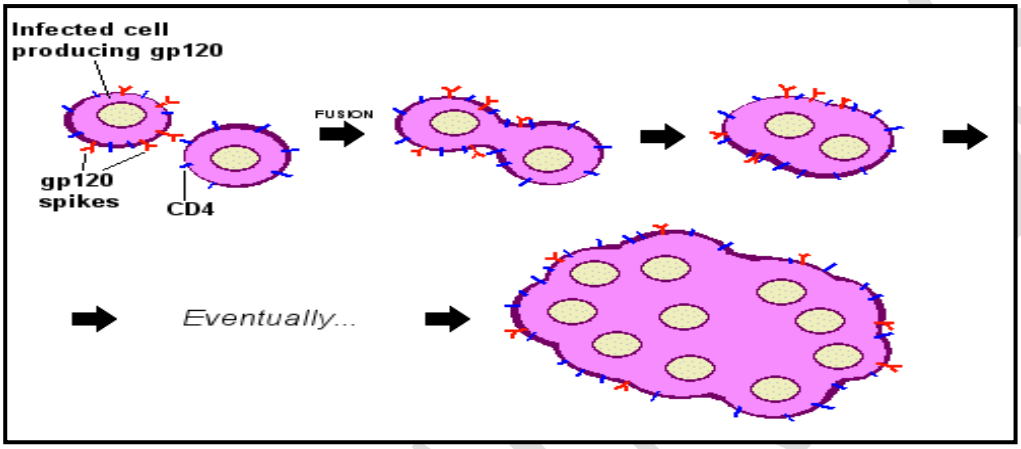
[134]Ciavarella, Carmen, Ilenia Motta, Sabrina Valente, and GianandreaPasquinelli. 2020. "Pharmacological (or Synthetic) and Nutritional Agonists of PPAR-γ as Candidates for Cytokine Storm Modulation in Covid-19 Disease." Molecules 25(9): 1–15.

[135] Yan, Jie et al. 2019. "Piperine Alleviates Doxorubicin-Induced Cardiotoxicity via Activating PPAR- γ in Mice." PPAR Research 2019.

[136] Ma, Zhen Guo et al. 2017. "Piperine Attenuates Pathological Cardiac Fibrosis Via PPAR-γ/AKT Pathways." EBioMedicine 18(2016): 179–87. http://dx.doi.org/10.1016/j.ebiom.2017.03.021.

[137] Abbasi, Parvaneh et al. 2015. "The Effect of Baicalin as A PPAR Activator on Erythroid Differentiation of CD133+ Hematopoietic Stem Cells in Umbilical Cord Blood." Cell Journal 17(1): 15–26.

Syncytium may be formed by the fusion of two or more cells, forming a *giant cell*. An example of syncytium can be found in skeletal muscles, which is essential since it allows rapid coordinated contraction of muscles along the entire length. An epithelium or tissue characterized by cytoplasmic continuity, or a large mass of cytoplasm not separated into individual cells and containing many nuclei[138]. Syncytium formation is normal in the development of the mammalian placenta, and the syncytin genes producing syncytin 1 and 2 stem from two human endogenous retroviruses. Syncytium formation leading to the creation of giant multinucleated cells in the placenta makes this tissue impermeable and generates mother–child immune tolerance. Syncytin genes are hypomethylated and therefore functionally active in mammalian placenta, whereas they are hypermethylated, and thus silenced, in other tissues, were syncytium formation may cause various diseases, including schizophrenia, multiple sclerosis, and diabetes type 1[139].



[Figure :- SYNCYTIA]

An important role for furin in activating S[140].Ongoing vaccine development efforts primarily focus on the coronavirus transmembrane spike (S) glycoprotein, which extends from the viral surface and mediates host cell entry. The spike glycoprotein consists of two subunits, subunit S1 and subunit S2. S1 is responsible for attachment to a host molecule on the cell membrane, and S2 facilitates the fusion between the cell and virus membrane and between neighbouring cells, producing cell–cell fusion, called a syncytium.

[138]De Maio, Flavio et al. 2020. "Improved Binding of SARS-CoV-2 Envelope Protein to Tight Junction-Associated PALS1 Could Play a Key Role in COVID-19 Pathogenesis." *Microbes and Infection* 22(10): 592–97. https://doi.org/10.1016/j.micinf.2020.08.006.

[139]Zupin, Luisa et al. 2020. "SARS-CoV-2 and the next Generations: Which Impact on Reproductive Tissues?" *Journal of Assisted Reproduction and Genetics* 37(10): 2399–2403.

[140]Mille, Jean Kaoru, and Gary R. Whittaker. 2014. "Host Cell Entry of Middle East Respiratory Syndrome Coronavirus after Two-Step, Furin-Mediated Activation of the Spike Protein." *Proceedings of the National Academy of Sciences of the United States of America* 111(42): 15214–19.

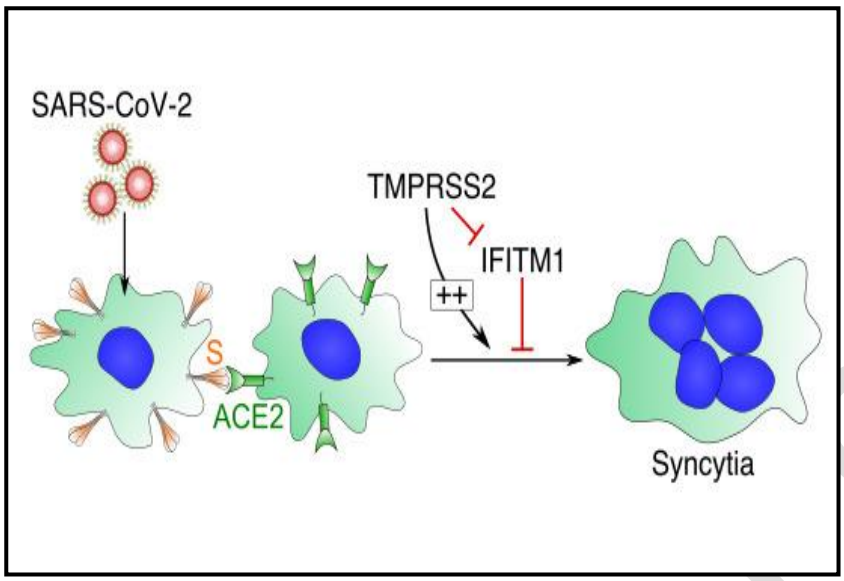


Figure :- SYNCYTIA formation by SARS-COV-2

A virus to evolve, it needs to develop a strategy to fuse itself with the cell membrane of the host and to induce host cell–cell fusion. Both mechanisms facilitate virus endocytosis and invasion of neighbouring cells and evasion of the innate antiviral immune system. The type of cell formed by membrane–virus or cell–cell fusion is called a syncytium. Syncytium formation is typical for coronavirus in general, and SARS-CoV-2 is no exception. Several viruses use the human syncytin genes to fuse themselves with the cell membrane of the host and to induce cell–cell fusion in the infiltrated tissues[141].

SARS-CoV-2 infected cells express the Spike protein (S) at their surface and fuse with ACE2-positive neighbouring cells. Expression of S without any other viral proteins triggers syncytia formation. Interferon-induced transmembrane proteins (IFITMs), a family of restriction factors that block the entry of many viruses, inhibit S-mediated fusion, with IFITM1 being more active than IFITM2 and IFITM3[142]. Cell infected with SARS-CoV-2 fuse with neighbouring cells to form syncytia. This process is accelerated by the TMPRSS2 protease and restricted by interferon-induced transmembrane proteins (IFITMs).SARS-CoV-2 infected cells can fuse with neighbouring cells to form syncytia. IFITM proteins, particularly IFITM1, restrict syncytia formation. TMPRSS2 protease accelerates syncytia formation and reverts the inhibitory effects of the IFITMs[143].

SARS-CoV-2 infected cells form large syncytia in culture. Syncytia may thus be considered as a frequent feature of severe COVID19. It will be worth determining whether the syncytia are also generated in mild cases and whether severe

[141]Pruimboom, Leo. 2020. "Methylation Pathways and SARS-CoV-2 Lung Infiltration and Cell Membrane-Virus Fusion Are Both Subject to Epigenetics." *Frontiers in Cellular and Infection Microbiology* 10(May): 1–5.

[142]Mehla, Rajeev, Shalmali Bivalkar-Mehla, and Ashok Chauhan. 2011. "A Flavonoid, Luteolin, Cripples HIV-1 by Abrogation of Tat Function." *PLoS ONE* 6(11).

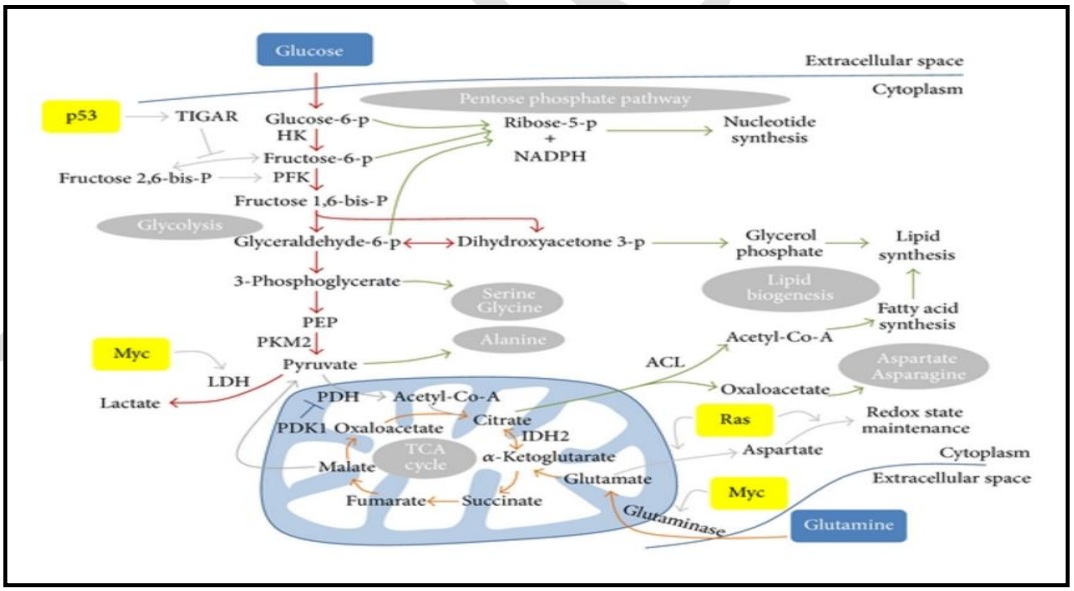
[143]Buchrieser, Julian et al. 2020. "Syncytia Formation by SARS-CoV-2-infected Cells." *The EMBO Journal* 39(23).

or critical cases are linked to polymorphisms in IFITMs, as already reported for Flu and in other IFN-related genes. The way for the future assessment of the role played by syncytia in viral persistence and dissemination, the destruction of alveolar architecture, and immune or inflammatory responses[144].

Role of Vedicinal: Our proposed formulation contains 6 compounds namely compounds 1, 2 & 3 are inhibitors of SyncytiumInhibitors domain. In this study, three confirmations of this enzyme have separately docked with Baicalin, Quercetin and Luteolinwere found to offer good interaction with a binding energy and hydrogen bond interaction. Syncytium Inhibitors is one of the most intriguing and promising drug targets for SARS-CoV-2 drug development.

Pathway34th:GLYCOLYSIS & GLUTAMINOLYSIS INHIBITORS

Tumor cells produce large quantities of lactate even when sufficient oxygen is present, a phenomenon referred to as Warburg effect or aerobic glycolysis, PKM2 is highly expressed in proliferating cells like tumor and embryonic cells. PKM2 is one of the four isoform of pyruvate kinase- PKL, PKR, PKM1 and PKM215. The decrease in glucose consumption and lactate production impedes the growth of cancer cells because glycolytic flux provides for anabolic synthesis in cancer cells to produce macromolecules for daughter cancer cells. Furthermore, intermediates of glycolysis acts as precursors or intermediates of cross-talking anabolic pathway like pentose phosphate pathway.High rates of glycolysis in cancer also serve the purpose of rapid ATP production, as in case of muscle during heavy exercise. Therefore, it is fathomable that high glycolytic rate is a life-line of dividing cancer cells. Inhibition of Warburg effect results in decreased anabolism and therefore the viability of cancer cells is negatively affected by curcumin and silencing of PKM2 as all these factors converge to suppress macromolecular synthesis[145].

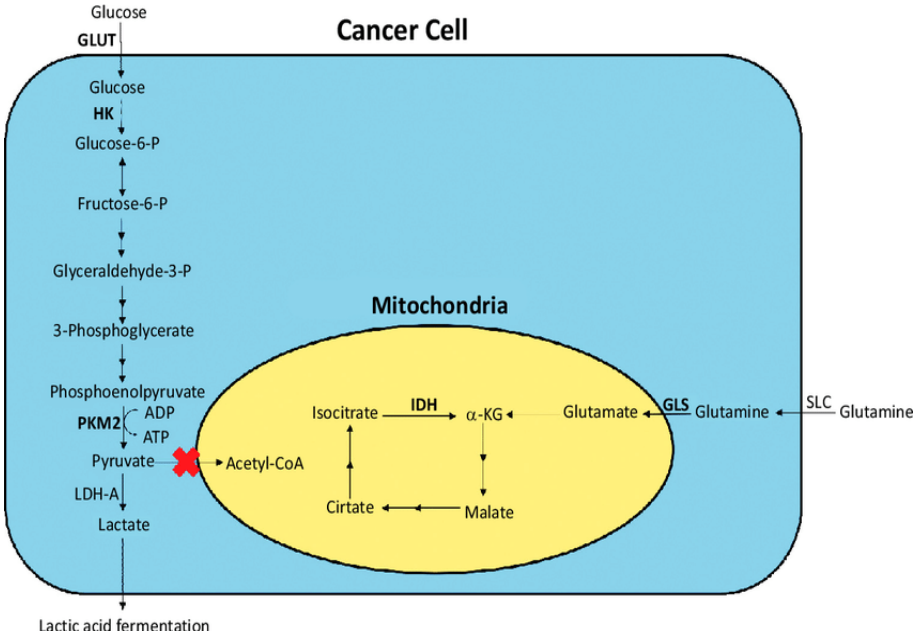


[144]Wang, Qian, Yu Tian Wang, Shao Ping Pu, and Yong Tang Zheng. 2004. "Zinc Coupling Potentiates Anti-HIV-1 Activity of Baicalin." *Biochemical and Biophysical Research Communications* 324(2): 605–10.

[145]Siddiqui, Farid Ahmad et al. 2018. "Curcumin Decreases Warburg Effect in Cancer Cells by Down-Regulating Pyruvate Kinase M2 via MTOR-HIF1α Inhibition." *Scientific Reports* 8(1): 2–10.

[Figure :- Glycolysis and glutaminolysis Overview]

Glycolysis and glutaminolysis are two of the most important pathways for cancer cells. Increased glucose uptake, together with reduced glycolytic flux, accumulates glycolytic intermediates for synthesis of biomolecules such as nucleotides, amino acids, and lipids.



[Figure :- Glycolysis and glutaminolysis in cancer cells]

Glycolysis and glutaminolysis are essential for virus replication and blocking these metabolic pathways caused significant reduction in virus production. The SARS-CoV-2 dysregulates PI3K/Akt/mTOR and HIF-1 signalling in infected cells. Glycolysis and glutaminolysis are essential for virus replication and metabolic perturbations of these processes can impede SARS CoV-2 and could be an attractive antiviral strategy.Both DNA and RNA viruses rewire host cell metabolism by altering central carbon metabolic pathways such as glycolysis, gluconeogenesis, PPP, TCA cycle, amino acid synthesis/degradation, and lipid synthesis. Viruses can target glycolysis by regulating glucose transporters' expression, which is also vital for immune cell activation during host cellularresponse. Glycolysisfavours SARS-CoV-2 infection and replication. The inhibition of glutaminolysis has a larger effect on viral replication and production compared to the inhibition of the glycolysis in lung cell model. Glutaminolysis is a process of converting glutamine to TCA cycle intermediates and also essential for biosynthesis of proteins, lipids and nucleic acids[146].

Cancer cells consume a larger amount of glucose, maintain a much higher rate of glycolysis and convert majority of glucose into lactic acid even in the presence of oxygen compared to that of normal cells[147]. Low oxygen levels are a

[146]Krishnan, Shuba et al. 2021. "Implications of Central Carbon Metabolism in SARS-CoV-2 Replication and Disease Severity." *bioRxiv*: 2021.02.24.432759. http://biorxiv.org/content/early/2021/02/24/2021.02.24.432759.abstract.

[147]Das, Laxmidhar, and Manjula Vinayak. 2014. "Long Term Effect of Curcumin in Regulation of Glycolytic Pathway and Angiogenesis via Modulation of Stress Activated Genes in Prevention of Cancer." *PLoS ONE* 9(6): 1–12.

defining characteristic of solid tumors, and the ATP necessary for survival and proliferation of cancer cells is derived from glycolysis rather than from oxidative phosphorylation's in this hypoxic environment[148]. Autophagy also plays important role in regulating glycolytic metabolism. Autophagy and high levels of glycolysis were prevalent in liver cancer. Autophagy regulation is an important research area through regulating cell glycolysis and cell mobility. Quercetin successfully blocked cell glycolysis, fundamentally blocking the energy supply of tumor cell migration.

Quercetin was able to suppress metastatic colorectal cancer in an experimental in vivo metastasis model. Quercetin treatment effectively inhibited tumor metastasis and glycolysis. At the same time, cell autophagy was induced through the inhibition of phosphorylation of AKT. In conclusion, this study demonstrated that quercetin effectively suppressed cell invasion and migration in breast cancer cells. Quercetin may suppressed cell mobility by blocking cell glycolysis[149]. EGCG strongly reduced pancreatic cancer cell growth by suppressing glycolysis in a ROS-dependent manner[150].

Role of Vedicinal: Our proposed formulation contains 6 compounds namely compounds 2, 3, 6, 7& 8 are inhibitors of Glycolysis & Glutaminolysis Inhibitors domain. In this study, three confirmations of this enzyme have separately docked with Quercetin, Luteolin, Curcumin, EGCG and Piperine were found to offer good interaction with a binding energy and hydrogen bond interaction. Glycolysis & Glutaminolysis Inhibitors is one of the most intriguing and promising drug targets for SARS-CoV-2 drug development.

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[148]Du, Gang Jun et al. 2008. "Luteolin as a Glycolysis Inhibitor Offers Superior Efficacy and Lesser Toxicity of Doxorubicin in Breast Cancer Cells." *Biochemical and Biophysical Research Communications* 372(3): 497–502.

[149]Jia, Lijun et al. 2018. "Quercetin Suppresses the Mobility of Breast Cancer by Suppressing Glycolysis through Akt-MTOR Pathway Mediated Autophagy Induction." *Life Sciences* 208: 123–30. https://doi.org/10.1016/j.lfs.2018.07.027.

[150]Wei, Ran, Robert M. Hackman, Yuefei Wang, and Gerardo G. Mackenzie. 2019. "Targeting Glycolysis with Epigallocatechin-3-Gallate Enhances the Efficacy of Chemotherapeutics in Pancreatic Cancer Cells and Xenografts." *Cancers* 11(10): 1–20.

Chapter3:Management of Covid 19 & Prevention of long Covid

Pathway 35: INTERLEUKIN-6 INHIBITORS

Cytokine release with coronavirus disease (COVID-19) infection and possible role of IL-6 inhibitors. *IL* interleukin, *INFγ* interferon gamma, *TNFα* tumor necrosis factor alpha. International guidelines do include IL-6 inhibitors as one of the options available for severe or critically ill patients. There has been increased interest in evaluating these drugs with a series of clinical trials being registered and conducted in different countries. The level of investigation though perhaps needs to be further intensified as there is a need to focus on therapeutic options that can prove to be 'life-saving' as the number of COVID-19 fatalities worldwide keeps increasing alarmingly. IL-6 inhibitors could be one such treatment option, with generation of more evidence and completion of a larger number of systematic studies .[151]

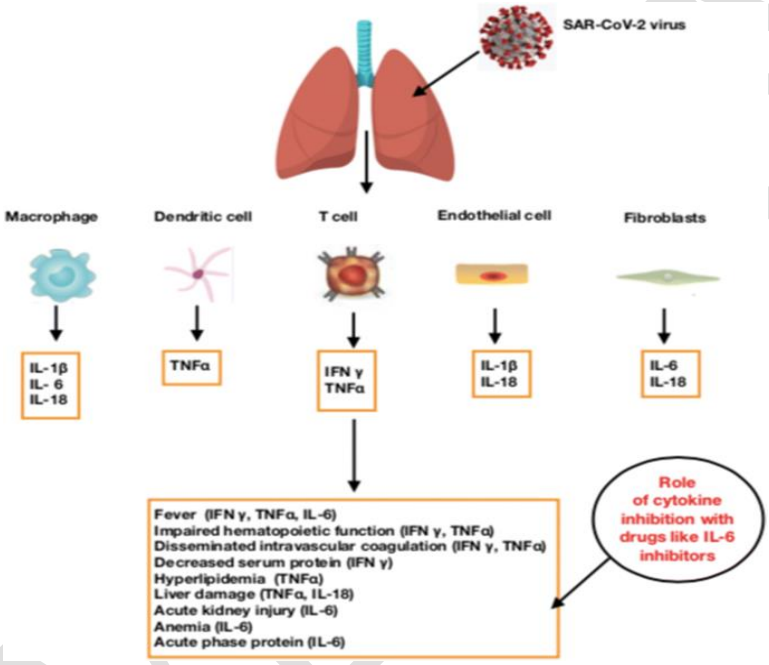

Fig :INTERLEUKIN-6 INHIBITION PATHWAY

Role of Vedicinal: Elevated interleukin-6 (IL-6), a major mediator of the inflammatory response, has been implicated in androgen receptor (AR) activation, cellular growth and differentiation, plays important roles in the development and progression of disease.Hesperidin, Luteolin,Rutin, Quercetin negatively regulatespro-inflammatory cytokines downstream of NF-κB, includingIL-6 .[152]Baicalein, Curcumin, Epigallocatechin-3-gallate, Piperine, Glycyrrhizin

[151] Atal S, Fatima Z. IL-6 Inhibitors in the Treatment of Serious COVID-19: A Promising Therapy? Pharmaceut Med. 2020 Aug;34(4):223-231. doi: 10.1007/s40290-020-00342-z. PMID: 32535732; PMCID: PMC7292936.

[152] Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol.* 2014;6(10):a016295. Published 2014 Sep 4. doi:10.1101/cshperspect.a016295

inhibited IL-6-mediated phosphorylation of signaling proteins, such as Jak, STAT3, MAPK, and Akt, also inhibited the expression of their target genes, such as bcl-xl the All 9 phytocompounds inhibit IL-6 expression and suppress IL-6-mediated signals.[153]

Pathway36 : MACROPHAGE POLARIZATION AND CCL2 REGULATORS

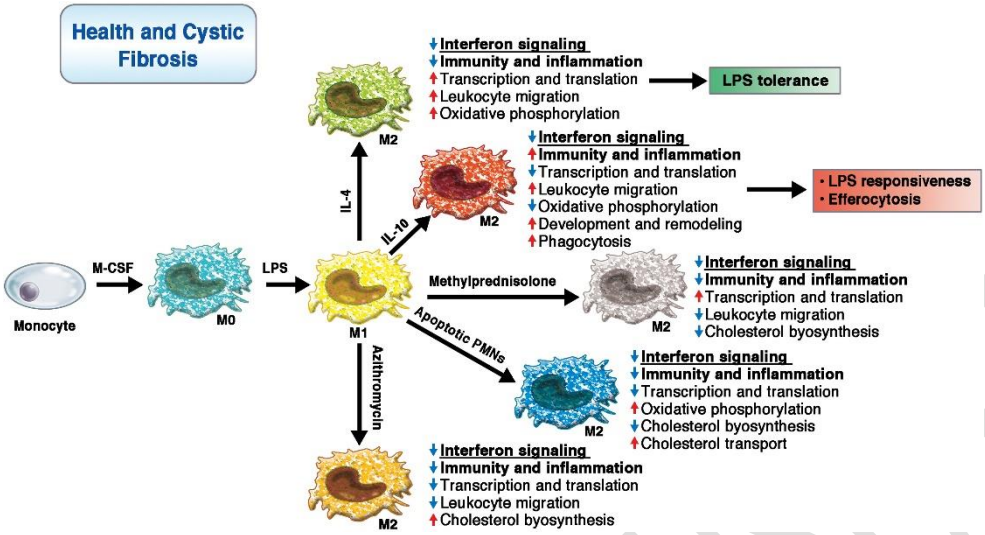

Fig: MACROPHAGE POLARIZATION AND CCL2 REGULATORS Pathway

Upon infection, monocytes migrate to the tissues where they become infected resident macrophages, allowing viruses to spread through all organs and tissues[154].The SARS-CoV-2-infected monocytes and macrophages can produce large amounts of numerous types of pro-inflammatory cytokines and chemokines. SARS-CoV-1 infection results that limits Dendritic Cell trafficking and T cell activation and increase airway hypersensitivity, thus exacerbating SARS-associated fibrosis. Cov infections have demonstrated that delayed IFN-I signaling and inflammatory monocytes-macrophages promote lung cytokine and chemokine levels, vascular leakage, and impaired antigen-specific T cell responses, culminating in lethal disease. the systemic hyper-inflammation defined as macrophage activation syndrome (MAS), or cytokine storm, requires an increase in choline consumption to synthesize phosphatidylcholine and stimulate phagocytosis, organelle biogenesis, secretory functions, and endocytosis blocking of the CCL2/CCR2 axis using a

[153] Domitrović, R., Potočnjak, I. A comprehensive overview of hepatoprotective natural compounds: mechanism of action and clinical perspectives. *Arch Toxicol* **90,** 39–79 (2016). https://doi.org/10.1007/s00204-015-1580-z

[154] Jafarzadeh Leila, Masoumi Elham, Fallah-Mehrjardi Keyvan, Mirzaei Hamid Reza, Hadjati Jamshid

TITLE=Prolonged Persistence of Chimeric Antigen Receptor (CAR) T Cell in Adoptive Cancer Immunotherapy: Challenges and Ways Forward JOURNAL=Frontiers in Immunology VOLUME=11 YEAR=2020PAGES=702

URL=https://www.frontiersin.org/article/10.3389/fimmu.2020.00702 DOI=10.3389/fimmu.2020.00702 ISSN=1664-3224

Vabret N, Britton GJ, Gruber C, Hegde S, Kim J, Kuksin M, Levantovsky R, Malle L, Moreira A, Park MD, Pia L, Risson E, Saffern M, Salomé B, Esai Selvan M, Spindler MP, Tan J, van der Heide V, Gregory JK, Alexandropoulos K, Bhardwaj N, Brown BD, Greenbaum B, Gümüş ZH, Homann D, Horowitz A, Kamphorst AO, Curotto de Lafaille MA, Mehandru S, Merad M, Samstein RM; Sinai Immunology Review Project. Immunology of COVID-19: Current State of the Science. Immunity. 2020 Jun 16;52(6):910-941. doi: 10.1016/j.immuni.2020.05.002. Epub 2020 May 6. PMID: 32505227; PMCID: PMC7200337.

CCR2 antagonist inhibits the recruitment of inflammatory monocytes, infiltration of the tumor-associated macrophages (TAMs) and M2 macrophage polarization Non-human primate (NHP) studies and patient data of CoV have also shown that virus spike-specific immunoglobulin G (IgG) responses can exacerbate acute lung injury due to repolarization of alveolar macrophages into pro-inflammatory phenotypes and enhanced recruitment of inflammatory monocyte via CCL2 and IL-8 .[155]

Role of Vedicinal: Phytocompound 1, 2, 3, 6, 7 and 9 could exhibit an anti-inflammatory reaction via inhibiting the polarization of macrophages into M1 type. Mechanistically, it was the result of the down-regulation in the activation of the Notch-1 signalling pathway. curcumin was found to inhibit NF-κB signalling in macrophages, as well as the subsequent production of cytokines/chemokines [156]. Glycyrrhizin Ameliorate Ischemia Reperfusion Lung Injury through Downregulate TLR2 Signaling Cascade in Alveolar Macrophage.[157]Epigallocatechin-3-gallate (EGCG), Quercetin, Curcumin, Hesperidininhibits IL-1β-stimulation CC/CXC chemokine.. Piperine also reduced activation of signal transducer and activator of transcription (STAT)-1. In addition, activation of STAT-1 was inhibited in IFN-alpha/beta-treated cells by piperine .[158]Baicalin,Luteolin and Rutin a major bioactive flavonoid, has been reported to attenuate chemokine-regulated leukocyte trafficking .[159]

Pathway 37 : PRO-INFLAMMATORY CYTOKINES SUPPRESSOR

155

[156] Zhou P, Li Q, Su S, et al. Interleukin 37 Suppresses M1 Macrophage Polarization Through Inhibition of the Notch1 and Nuclear Factor Kappa B Pathways. *Front Cell Dev Biol*. 2020;8:56. Published 2020 Feb 14. doi:10.3389/fcell.2020.00056

[157] Fei L, Jifeng F, Tiantian W, Yi H, Linghui P. Glycyrrhizin Ameliorate Ischemia Reperfusion Lung Injury through Downregulate TLR2 Signaling Cascade in Alveolar Macrophages. Front Pharmacol. 2017 Jun 16;8:389. doi: 10.3389/fphar.2017.00389. PMID: 28670282; PMCID: PMC5472719.

[158] Gi-Sang Bae, Min-Sun Kim, Won-Seok Jung, Sang-Wan Seo, Seung-Won Yun, Sung Gyu Kim, Rae-Kil Park, Eun-Cheol Kim, Ho-Joon Song, Sung-Joo Park,Inhibition of lipopolysaccharide-induced inflammatory responses by piperine,European Journal of Pharmacology,Volume 642, Issues 1–3,2010,Pages 154-162,ISSN 0014-2999,https://doi.org/10.1016/j.ejphar.2010.05.026.

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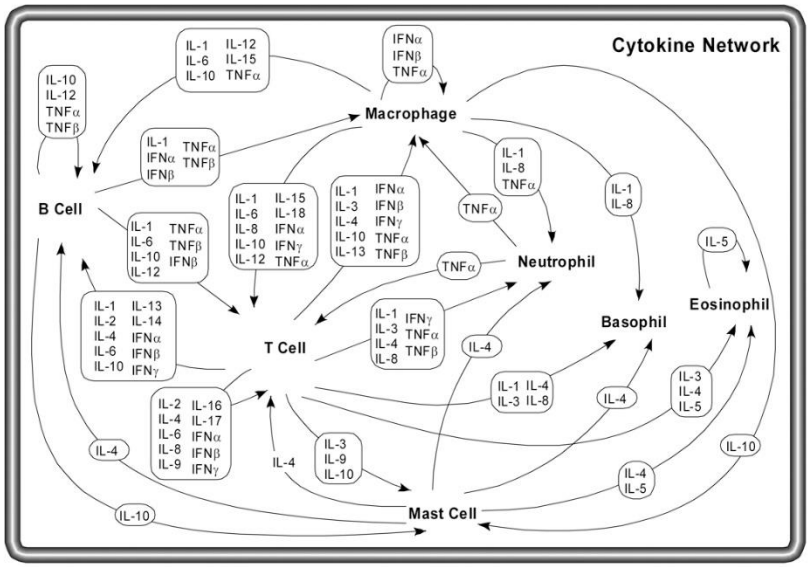

Fig:Cytokine Network

Cytokine network plays major role in inflammatory response. Several cell types participate as part of the immune system, including B cells, T cells, macrophages, mast cells, neutrophils, basophils and eosinophils.[160]. Each of these cell types has a distinct role in the immune system, and communicates with other immune cells using secreted cytokines. Macrophages phagocytose foreign bodies and are antigen-presenting cells, using cytokines to stimulate specific antigen dependent responses by B and T cells and non-specific responses by other cell types . T cells secrete a variety of factors to coordinate and stimulate immune responses to specific antigen, such as the role of helper T cells in B cell activation in response to antigen. The proliferation and activation of eosinophils, neutrophils and basophils respond to cytokines as well.

Role of Vedicinal: All of our selected compounds have shown very good ability to down regulate the inflammatory cytokines which are responsible for producing the cytokine storm in the body. Reducing the cytokine storm is the most important strategy to combat the SARS-CoV- 2. Curcumin, Glycyrrhizin, Luteolin, Quercetin, Piperine suppression of cytokine release and cytokine storm[161],[162]Baicalin, Epigallocatechin-3-gallate, Glycyrrhizin, Piperine, Rutin and Hesperidin was found to reveal a potent suppressive effect on cytokine production in. Baicalin is primarily converted by the human intestinal flora into deglycosylated baicalein and the methylated aglycone oroxylin A, oroxylin A has anti-inflammatory properties, associated with its inhibition of NO, cytokines, chemokines and growth factors in PIC-induced macrophages via the calcium-STAT pathway.[163]

Pathway 38:TNFα REDUCERS

[160] (Zhang JM, and An J. (2007). Cytokines, inflammation, and pain. International anesthesiology clinics, 45(2):27–37.)

[161] Midura-Kiela MT, Radhakrishnan VM, Larmonier CB, Laubitz D, Ghishan FK, Kiela PR. Curcumin inhibits interferon-γ signaling in colonic epithelial cells. Am J Physiol Gastrointest Liver Physiol. 2012 Jan 1;302(1):G85-96. doi: 10.1152/ajpgi.00275.2011. Epub 2011 Oct 28. PMID: 22038826; PMCID: PMC3345961.

[162] Leyva-López N, Gutierrez-Grijalva EP, Ambriz-Perez DL, Heredia JB. Flavonoids as Cytokine Modulators: A Possible Therapy for Inflammation-Related Diseases. Int J Mol Sci. 2016 Jun 9;17(6):921. doi: 10.3390/ijms17060921. PMID: 27294919; PMCID: PMC4926454.

[163] Dinda B, Dinda S, DasSharma S, Banik R, Chakraborty A, Dinda M. Therapeutic potentials of baicalin and its aglycone, baicalein against inflammatory disorders. Eur J Med Chem. 2017 May 5;131:68-80. doi: 10.1016/j.ejmech.2017.03.004. Epub 2017 Mar 6. PMID: 28288320.

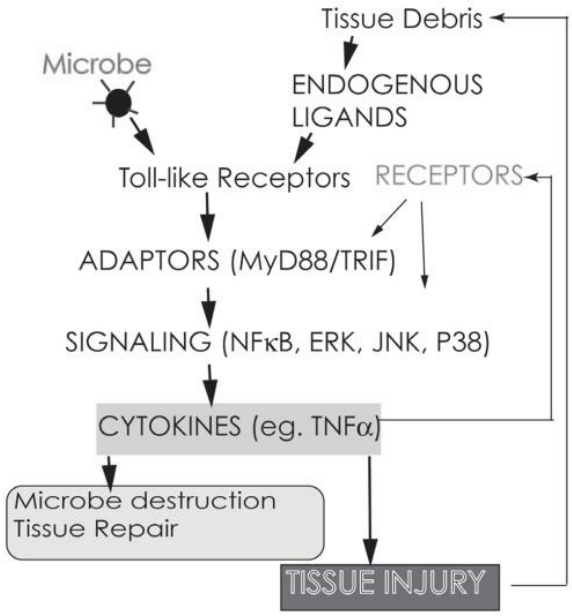

Fig: **TNFα REDUCERS**

TNFα is a pleiotropic cytokine produced by many different types of cells in the body. However, cells of the monocytic lineage—such as macrophages, astroglia, microglia, Langerhans cells, Kupffer cells, and alveolar macrophages—are the primary synthesizers of TNFα.14,15 TNFα acts through transmembrane receptors: TNF receptor 1 (TNFR1), also known as p55 or p60, and TNF receptor 2 (TNFR2), also known as p75 or p80[164]. Binding of TNFα onto TNFR1 is considered to be an irreversible mechanism, whereas binding of TNFα onto TNFR2 has both rapid on and off kinetics (Parameswaran, and Patial, 2010). TNFR2 act as a "ligand passer" to TNFR1 in some cells, increasing the local concentration of TNFα at the cell surface through rapid ligand binding and dissociation. In addition to its role in the initiation and perpetuation of inflammation, efferocytosis plays a crucial role in clearing neutrophils, this role of TNFα is especially important in the resolution of inflammation.

Role of Vedicinal: Cytokine storm may be the central inducer of apoptosis of alveolar epithelial cells, which leads to rapid progression in severe group patients. Given the similarities of clinical features and pathogenesis between toxic epidermal necrolysis (TEN) and COVID-19 Our proposed formulation contains 8 compounds that can reduce the virus-specific lung immunopathology. Piperine significantly inhibited the production of cytokines, increase in the secretion of Th-1 cytokines (IFN-γ and IL-2)[165]. Quercetin, EGCG, Hesperidin can prevent the TNF-induced cytotoxicity. The relative potency for suppression of tumor necrosis factor (TNF)-induced nuclear factor-kappaB (NF-kappa B) activation was highest for Curcumin .[166]Luteolin, Rutin and Baicalin significantly decreased TNF-α productions in human peripheral blood .

[164] Parameswaran N, Patial S. Tumor necrosis factor-α signaling in macrophages. Crit Rev Eukaryot Gene Expr. 2010;20(2):87-103. doi: 10.1615/critreveukargeneexpr.v20.i2.10. PMID: 21133840; PMCID: PMC3066460.

[165] Pradeep CR, Kuttan G. Effect of piperine on the inhibition of nitric oxide (NO) and TNF-alpha production. Immunopharmacol Immunotoxicol. 2003 Aug;25(3):337-46. doi: 10.1081/iph-120024502. PMID: 19180797.

[166] Sandur SK, Pandey MK, Sung B, Ahn KS, Murakami A, Sethi G, Limtrakul P, Badmaev V, Aggarwal BB. Curcumin, demethoxycurcumin, bisdemethoxycurcumin, tetrahydrocurcumin and turmerones differentially regulate anti-inflammatory and anti-proliferative responses through a ROS-independent mechanism. Carcinogenesis. 2007 Aug;28(8):1765-73. doi: 10.1093/carcin/bgm123. Epub 2007 May 23. PMID: 17522064.

Pathway 39: MAST CELLS STABILIZERS

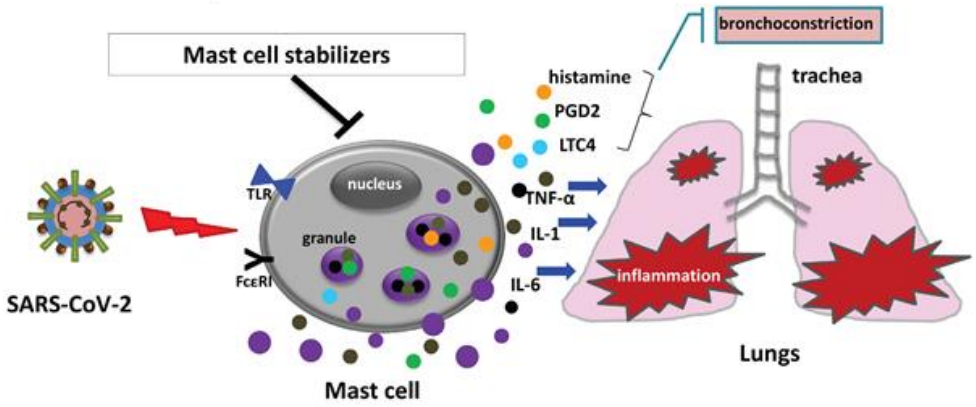

Fig: **MAST CELLS STABILIZERS Involvement**

Mast cell deficiency resulted in a damped systemic inflammatory response, greatly attenuated multiple organ injury, and more stable hemodynamics in HS/T. Thus, mast cells appear to be a critical component of the initial host response to severe injury and survival following sepsis induction [167]. Prevalence of severe Covid-19 is similar to that of mast cell activation syndrome (MCAS) .[168]Within the purely synthetic class of inhibitors, particular attention has been devoted to the inhibition of important signalling molecules including spleen TK and JAK3. The statin class of cholesterol-lowering drugs as well as nilotinib, a TK inhibitor, are just some examples of clinically used drugs that have been evaluated for their anti-allergic properties Here, we examine each approach under investigation, summarize the test data generated and offer suggestions for further preclinical evaluation before their therapeutic potential can be realized. The diversity of natural products evaluated range from simple phenols, alkaloids, terpenes to simple amino acids. While in some cases their precise mode of action remains unknown it has nevertheless sparked interest in the development of synthetic derivatives with improved pharmacological properties .[169]

Role of Vedicinal: 8 of our compounds work on mast cell stabilization. Our compounds have powerful anti-inflammatory and anti-oxidant properties. Several studies suggest that our compounds inhibit protease-activated receptors (PAR), which play a role in inflammation and PAR2 and 4-mediated human mast cell activation. Curcumin, Hesperidin alleviated both the IgE-mediated and calcium ionosphere A23187-and reduced the release of the allergic

[167] Nihan Kilinc A, Sugiyama N, Reddy Kalathur RK, Antoniadis H, Birogul H, Ishay-Ronen D, George JT, Levine H, Kumar Jolly M, Christofori G. Histone deacetylases, Mbd3/NuRD, and Tet2 hydroxylase are crucial regulators of epithelial-mesenchymal plasticity and tumor metastasis. Oncogene. 2020 Feb;39(7):1498-1513. doi: 10.1038/s41388-019-1081-2. Epub 2019 Oct 30. PMID: 31666683.

[168] Afrin LB, Weinstock LB, Molderings GJ. Covid-19 hyperinflammation and post-Covid-19 illness may be rooted in mast cell activation syndrome. *Int J Infect Dis*. 2020;100:327-332. doi:10.1016/j.ijid.2020.09.016

[169] Finn, D.F. and Walsh, J.J. (2013), Twenty-first century mast cell stabilizers. Br J Pharmacol, 170: 23-37. https://doi.org/10.1111/bph.12138

mediators .[170]Piperine inhibited Th2/Th17 responses and mast cells activation ,Rutin, Glycyrrhizin, Epigallocatechin-3-gallate, Baicalein, Oroxylin A, Luteolin Quercetin inhibits mast cell degranulation[171],[172].These compounds also known to inhibit the release of histamine from the mast cells thereby stabilizing the mat cells

Pathway 40: T CELLS STABILIZERS

Antigen presentation subsequently stimulates the body's humoral and cellular immunity, which are mediated by virus-specific B and T .[173]T cell content was decreased in aortic abdominal aneurysms (AAA) .CD4+ and CD8+ T cells in the peripheral blood of SARS-CoV-2-infected patients significantly is reduced, whereas its status is excessive activation, as evidenced by high proportions of HLA-DR (CD4 3.47%) and CD38 (CD8 39.4%) double-positive .FOXP3 is to suppress the function of NFAT and NFkappaB and this leads to suppression ofexpression of many genes including IL-2 and effector T-cell cytokines .Similarly, the acute phase response in patients with SARS-CoV is associated with severe decrease of CD4+ T and CD8+ T cells. In absence of antigen, CD4+ and CD8+ memory T cells can persist for four years in a part of SARS-CoV recovered individuals and can perform T cell proliferation, DTH response and production of IFN-γ .CD3+ T cells, CD4+ T cells, CD8+ T cells, and natural killer cells were significantly decreased in patients with COVID-19, T-cell subset counts were related to the severity and prognosis of COVID-19, suggesting that the counts of CD8+ T and CD4+ T cells can be used as diagnostic markers. In some cases, it is seen Regulatory T cells inhibit T cell proliferation and decrease demyelination in mice chronically infected with a coronavirus .[174]

Role of Vedicinal: All of 9 our compounds contain the compound which have immune system enhancing activity.Curcumin inhibits Th1 cytokine profile in CD4+ T cells by suppressing interleukin-12 production in macrophages also downregulate the expression of various proinflammatory cytokines including TNF, IL-1, IL-2, IL-8, IL-12, and chemokines[175], [176].Quercetin, Luteolin, Hesperidin is able to modulate immune response, T effector

[170] Hosokawa J, Suzuki K, Nakagomi D, Tamachi T, Takatori H, Suto A, Nakajima H. Role of calcium ionophore A23187-induced activation of IkappaB kinase 2 in mast cells. Int Arch Allergy Immunol. 2013;161 Suppl 2:37-43. doi: 10.1159/000350357. Epub 2013 May 29. PMID: 23711852.

[171] Zheng YY, Ma YT, Zhang JY, and Xie X. (2020) COVID-19 and the cardiovascular system, Nature Reviews Cardiology 17 :259–260.

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[173] Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, Ren R, Leung KSM, Lau EHY, Wong JY, Xing X, Xiang N, Wu Y, Li C, Chen Q, Li D, Liu T, Zhao J, Liu M, Tu W, Chen C, Jin L, Yang R, Wang Q, Zhou S, Wang R, Liu H, Luo Y, Liu Y, Shao G, Li H, Tao Z, Yang Y, Deng Z, Liu B, Ma Z, Zhang Y, Shi G, Lam TTY, Wu JT, Gao GF, Cowling BJ, Yang B, Leung GM, Feng Z. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. N Engl J Med. 2020 Mar 26;382(13):1199-1207. doi: 10.1056/NEJMoa2001316. Epub 2020 Jan 29. PMID: 31995857; PMCID: PMC7121484.

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[176]Xiaoqiang Chai, Longfei Hu, Yan Zhang, Weiyu Han, Zhou Lu, Aiwu Ke, Jian Zhou, Guoming Shi, Nan Fang, Jia Fan, Jiabin Cai, Jue Fan, Fei Lan bioRxiv 2020.02.03.931766; doi: https://doi.org/10.1101/2020.02.03.931766

differentiation is reduced and T regulatory cells are induced .[177].Quercetin has an anti-inflammatory activity which reduces the severity of lupus nephritis signs in lupus mice by initially increasing the CD4+CD25+ and FOXP3+ Tregs numbers .Furthermore, EGCG, Piperine, Glycyrrhizin suppresses specific antigen driven T cell response and affects CD4+ T cell differentiation . Baicalin and Oroxylin A the metabolite derived from baicalin regulate the generation of Tregs in non-small cell .There are several studies that shows that selected compounds (compounds) have good T cell stabilization activity which will help in strengthening the immune system of COVID-19 patients.

Pathway 41: Nrf2 ACTIVATORS

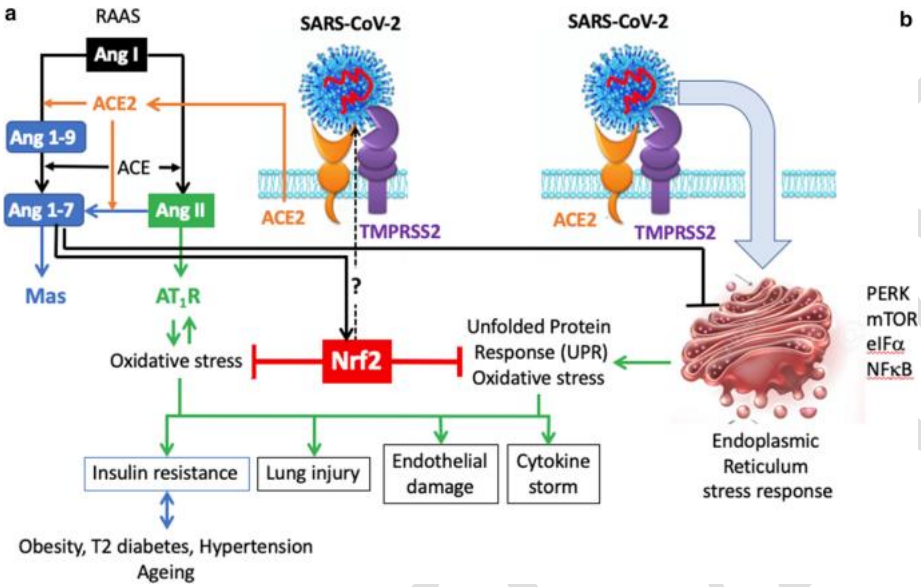

Fig: Nrf2 ACTIVATORS

Low to moderate concentrations of RNOS are known to activate the antioxidant defense systems, such as NF-E2-related factor 2 (Nrf2) .Controlled regulation of inflammatory cycle is very important to guarantee a balanced immune response without developing chronic inflammation. One of the major mediators of the resolution of inflammation is the transcription factor: the nuclear factor erythroid 2-like 2 (Nrf2) . Nrf2 induces the expression of antioxidants as well as cytoprotective genes, which provoke an anti-inflammatory expression profile, and is crucial for the initiation of healing. In view of this fundamental modulatory role, Nrf2 expression/activation is known to affect inflammatory processes will facilitate development of therapeutic approaches to prevent Nrf2 dysregulation and ameliorate chronic inflammatory diseases[178] [179]. The mechanism involves decrease of the infiltration of several pro-inflammatory cells, the inhibition of ROS production, the reduction of the NF-κB pathway, the down-regulation of NLRP3 inflammasome, and the potentiation of Nrf2 induction (the nuclear factor erythroid-2 related factor 2) and HO-1. Nrf2 activators an important medication that's have a role in reduce viral pathogenesis via inhibit virus entry through induce SPLI gene expression as

[177] Mlcek J, Jurikova T, Skrovankova S, Sochor J. Quercetin and Its Anti-Allergic Immune Response. Molecules. 2016 May 12;21(5):623. doi: 10.3390/molecules21050623. PMID: 27187333; PMCID: PMC6273625.

[178] Vomund S, Schäfer A, Parnham MJ, Brüne B, von Knethen A. Nrf2, the Master Regulator of Anti-Oxidative Responses. Int J Mol Sci. 2017 Dec 20;18(12):2772. doi: 10.3390/ijms18122772. PMID: 29261130; PMCID: PMC5751370.

[179] Alice Pasini, Joseph Lovecchio, Giulia Ferretti, Emanuele Giordano, "Medium Perfusion Flow Improves Osteogenic Commitment of Human Stromal Cells", Stem Cells International, vol. 2019, Article ID 1304194, 10 pages, 2019. https://doi.org/10.1155/2019/1304194

well as inhibit TRMPSS2, upregulation of ACE2 that's make a competition with the virus on binding site, induce gene expression of anti-viral mediators such as RIG-1 and INFs, induce anti-oxidant enzymes .[180]

Role of Vedicinal: Our proposed formulation contains 8 compounds that can up regulate the Nrf2 thereby providing the immune boosting to the hosts. Hesperidin, Rutin, Quercetin, Curcumin inhibited the activation of NLRC4, and boosted the expression of Heme Oxygenase-1 (HO-1.Baicalin, Epigallocatechin gallate, Piperine, Glycyrrhizin and Oroxylin A metabolite of baicalin upregulates NRF2 .[181]All the selected compounds have strong documented evidence on up-regulation of Nrf2.[182]

Pathway 42 : NLRP-3 and CASPASE-1 INHIBITORS

[180] Wan Hasan WN, Kwak MK, Makpol S, Wan Ngah WZ, Mohd Yusof YA. Piper betle induces phase I & II genes through Nrf2/ARE signaling pathway in mouse embryonic fibroblasts derived from wild type and Nrf2 knockout cells. BMC Complement Altern Med. 2014 Feb 23;14:72. doi: 10.1186/1472-6882-14-72. PMID: 24559113; PMCID: PMC3936848.

[181] Liu PF, Han FG, Duan BB, Deng TS ,Xianglin H and Zhao MQ. (2013). Purification and antioxidant activities of baicalin isolated from the root of huangqin (Scutellariabaicalensisgcorsi). Journal of Food Science and Technology. 50(3):615–619.

[182] Sadhukhan P, Ugurlu MT, Hoque MO. Effect of COVID-19 on Lungs: Focusing on Prospective Malignant Phenotypes. *Cancers (Basel)*. 2020;12(12):3822. Published 2020 Dec 18. doi:10.3390/cancers12123822

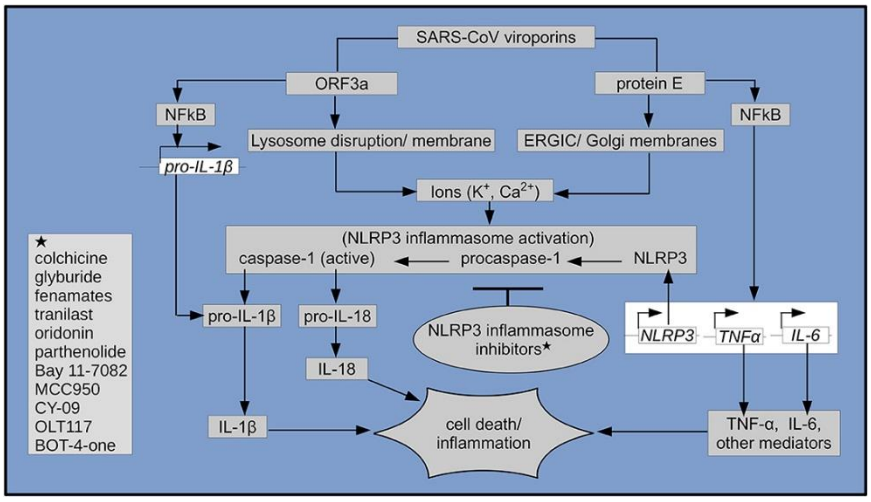

Fig: NLRP-3 and CASPASE-1 INHIBITORS

SARS-CoV encoded viroporins, and ORF3a activate the NLRP3 inflammasome and assembly. This leads to activation of inflammatory cascade involving cytokines such as IL-1β, IL-6, TNF, and other mediators as part of the host inflammatory responses to SARS-CoV infection and contribute to tissue damage .
[183]

Role of Vedicinal: Inflammasome in which the sensor molecule such as NLRP3 oligomerizes and then the adaptor protein ASC is recruited into the complex, followed by incorporation of caspase-1, which then exerts its catalytic activity on the pro-inflammatory cytokines that after their release perpetuate the inflammatory response,.[184]Phytocompound 1, 2, 3, 5, 6, 7, 8 and 9 down regulated NLRP3 and CASPASE 1. Baicalin, Luteolin, Epigallocatechin-3-Gallate, Quercetin alleviates NLRP3 inflammasome activation and directs macrophage polarization. Curcumin, Rutin, Hesperidin, Piperine suppresses NLRP3 expression in inflammasome .Glycyrrhizin inhibits NLRP3 inflammasome activation .[185]

[183] Shah A. Novel Coronavirus-Induced NLRP3 Inflammasome Activation: A Potential Drug Target in the Treatment of COVID-19. *Front Immunol*. 2020;11:1021. Published 2020 May 19. doi:10.3389/fimmu.2020.01021

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Pathway 43 :STAT 3 PHOSPHORYLATION SUPPRESSOR

STAT3 exhibits a proviral function in several viral infections, including those of HBV, HCV, HSV-1, varicella zoster virus, human CMV and measles virus. Viral components induce signal transducer and activator of transcription 1 (STAT1) dysfunction and compensatory hyperactivation of STAT3. The IFN released from infected cells binds to IFN receptors on neighboring cells, alerting them to a viral attack. The IFN-I and IFN-II receptors are almost ubiquitously expressed, while IFN-III receptors are only expressed on cells lining the epithelial barrier (Matsuyama et al., 2020). The engagement of IFN-I and IFN-III receptors activates various members of the JAK and STAT families, and specific transcription factor complexes are formed. For example, STAT1 interacts with STAT2 and IRF-9 to constitute the transcription factor complex "IFN-stimulated gene factor 3" (ISGF3). In contrast, IFN-II activates JAK1 and JAK2, producing a phosphorylated STAT1 homodimer known as "γ-interferon activation factor" (GAF). Interestingly, both ISGF3 and GAF can be evoked by all IFNs. In any case, before they can exert their transcription factor activity, the ISGF3 and GAF complexes must be transported to the nucleus and the subsequent upregulation of the interferon-stimulated gene products (ISGs). Karyopherin-α1 (KPNA1) is essential for the nuclear transport of STAT1, and the interaction between STAT1 and KPNA1 (STAT1/KPNA1) involves a nonclassical nuclear localization signal (NLS). In addition to IFN signaling, STAT proteins are involved in signal transduction for other families of cytokines, including IL-6 .[186]

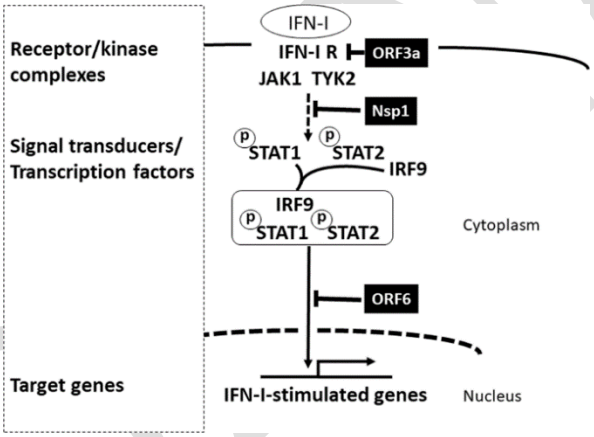

Fig: STAT 3 PHOSPHORYLATION SUPPRESSOR

Role of Vedicinal: STAT3 can be activated by IL-6 and IL-10 family cytokines and growth factors, Activation of p38 MAPK pathway induces STAT3 dephosphorylation to promote replication. Thus, the role of STAT3 in virus replication is uncertain and seems to function as a proviral or antiviral factor in a virus-specific manner.Compounds 1, 2, 3,6, 7, 8 and 9 supress the STAT3 phosphorylation. Baicalin, Quercetin, EGCG, and Glycyrrhizin Modulates Cytokine Expression and Inhibits TLR2 Expression and STAT3 Activation .[187], [188]. Luteolin, Hesperetin, Piperine and Curcumin inhibit hepatic stellate cell activation via suppression of the stat3 pathway .

[186] . Hojyo S, Uchida M, Tanaka K, et al. How COVID-19 induces cytokine storm with high mortality. *Inflamm Regen*. 2020;40. DOI:10.1186/s41232-020-00146-3. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

[187] Susmitha GD, Miyazato K, Ogura K, Yokoyama S, Hayakawa Y. Anti-metastatic Effects of Baicalein by Targeting STAT3 Activity in Breast Cancer Cells. Biol Pharm Bull. 2020;43(12):1899-1905. doi: 10.1248/bpb.b20-00571. PMID: 33268707.

[188] Tao Y, Zhan S, Wang Y, Zhou G, Liang H, Chen X,and Shen H. (2018) Baicalin, The major component of traditional Chinese medicine *Scutellariabaicalensis* induces colon cancer cell apoptosis through inhibition of oncomic RNAs. Sci Rep 8:14477

Pathway 44: ALPHA ANTI TRYPSIN ACTIVATOR

α1AT inhibits SARS-CoV-2 entry at physiological concentrations and suppresses viral replication in cell lines and primary cells including human airway epithelial cultures [189] [190]The entry of SARS-CoV2 into host cells is through the binding of viral S-protein to angiotensin converting enzyme 2 (ACE2) located on host cells, which is mediated by the transmembrane serine protease 2 (TMPRSS2). A1AT inhibits TMPRSS2, thus, reduces SARS-CoV-2 infection. In addition, A1AT can reduce acute inflammatory responses, cell death, neutrophil elastase trap formation, coagulative activity, and dysregulated immune responses .Insufficient anti-inflammation, anti-cell death, anti-protease, and anticoagulation effects of α1-antitrypsin could increase the likelihood of severe acute lung injury

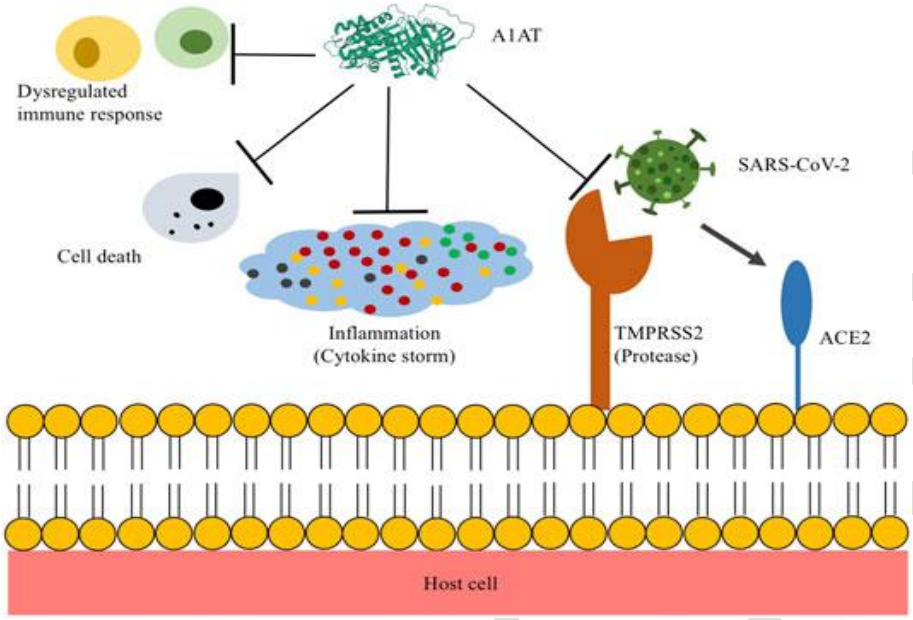

Fig: ALPHA ANTI TRYPSIN ACTIVATOR

Role of Vedicinal: Alpha-1-antitrypsin (AAT) is the most abundant circulating serine protease inhibitor. Phytocompound 2,6 and 7 activated physiological AAT concentrations inhibited virus production and reduced virus replication in freshly infected peripheral blood mononuclear cells, and blocked infection**.** EGCG, Curcumin, Quercetin reverses human neutrophil elastase-induced migration in A549 cells by directly binding to HNE and by regulating α1-AT .[191]

,

Pathway 45: C REACTIVE PROTEIN (CRP) SUPRESSORS

[189] Wettstein, L., Weil, T., Conzelmann, C. *et al.* Alpha-1 antitrypsin inhibits TMPRSS2 protease activity and SARS-CoV-2 infection. *Nat Commun* **12,** 1726 (2021). https://doi.org/10.1038/s41467-021-21972-0

[190] Bai Y, Yao L, Wei T, et al. Presumed Asymptomatic Carrier Transmission of COVID-19. *JAMA.* 2020;323(14):1406–1407. doi:10.1001/jama.2020.2565

[191] Xiaokaiti, Y., Wu, H., Chen, Y. *et al.* EGCG reverses human neutrophil elastase-induced migration in A549 cells by directly binding to HNE and by regulating α1-AT. *Sci Rep* **5,** 11494 (2015).

https://doi.org/10.1038/srep11494

CRP levels are correlated well with the severity of symptoms of patients with COVID-19; therefore, it may be a suitable marker in assessing a patient's conditions together with other clinical findings. The elevated levels of CRP might be linked to the overproduction of inflammatory cytokines in severe patients with COVID-19. Cytokines fight against the microbes but when the immune system becomes hyperactive, it can damage lung tissue. Thus, CRP production is induced by inflammatory cytokines and by tissue destruction in patients with COVID-19. In conclusion, elevated level of CRP may be a valuable early marker in predicting the possibility of disease progression in non-severe patients with COVID-19[192], which can help health workers to identify those patients an early stage for early treatment. Besides, COVID-19 patients with elevated levels of CRP need close monitoring and treatment even though they did not develop symptoms to meet the criteria for the severe disease course .

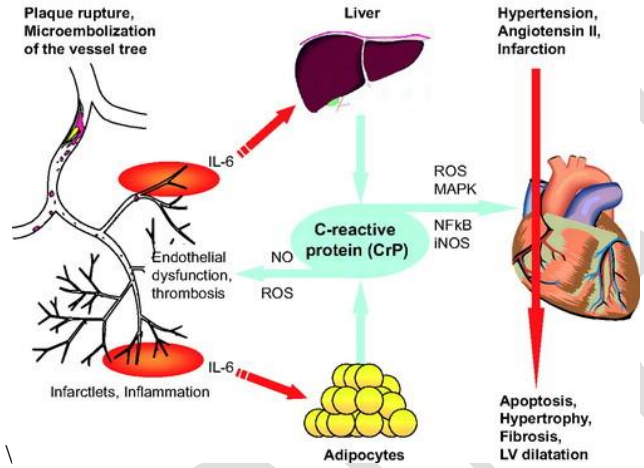

Fig: C REACTIVE PROTEIN (CRP) SUPRESSORS

Role of Vedicinal: Phytocompounds 1, 2, 4, 6, 7, and 9 markedly reduce serum levels of CRP. The CRP-lowering effect of these molecules is more pronounced as their lipid lowering effect. Baicalin, Curcuminoids, Glycyrrhizin, plays major role as C-Reactive Protein lowering .Epigallocatechin gallate Quercetin, Rutin treatment significantly reduced expression of TNF-α and CRP .[193]

[192] Lavillegrand, JR., Garnier, M., Spaeth, A. et al. Elevated plasma IL-6 and CRP levels are associated with adverse clinical outcomes and death in critically ill SARS-CoV-2 patients: inflammatory response of SARS-CoV-2 patients. Ann. Intensive Care 11, 9 (2021). https://doi.org/10.1186/s13613-020-00798-x

[193] Sahebkar A. Are curcuminoids effective C-reactive protein-lowering agents in clinical practice? Evidence from a meta-analysis. Phytother Res. 2014 May;28(5):633-42. doi: 10.1002/ptr.5045. Epub 2013 Aug 7. PMID: 23922235.

Pathway 46 : CREATINE KINASE

CK, an enzyme present in the muscle will be markedly elevated. Rhabdomyolysis has been associated with a variety of viral and bacterial infections. The common reported acute viral infection associated with rhabdomyolysis include influenza, parainfluenza, Epstein-Barr virus, Cytomegalic virus, Herpes Simplex virus, and Human Immunodeficiency Virus as well as other respiratory tract pathogens. elevated CK and rhabdomyolysis seem to be the presenting symptom in Covid 19 .

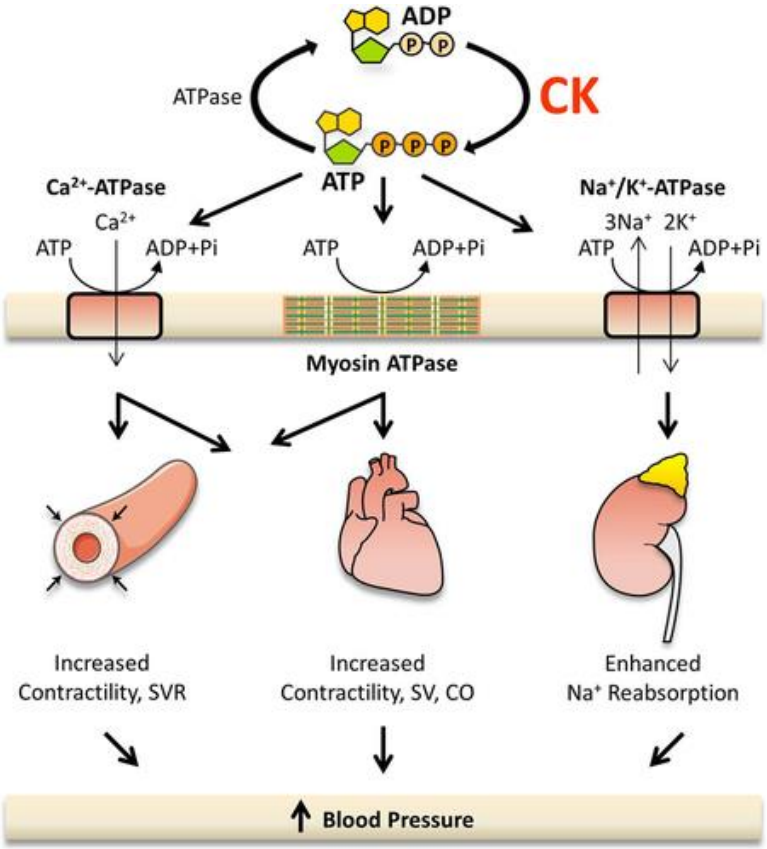

Fig :CREATINE KINASE Pathway

Role of Vedicinal: Our studies indicate that phytochemical Baicalin significantly reduced the infarct size and related myocardial enzymes namely CK, CK-MB, LDH and cTnT. Similarly, Glycyrrhizin, EGCG, Quercetin, and Hesperidin exhibited CK reducing activity .Baicalin, Curcumin attenuates acute myocardial infarction of rats via mediating CK[194] .

[194] Kyo R, Nakahata N, Sakakibara I, Kubo M, Ohizumi Y. Baicalin and baicalein, constituents of an important medicinal plant, inhibit intracellular Ca2+ elevation by reducing phospholipase C activity in C6 rat glioma cells. J Pharm Pharmacol. 1998 Oct;50(10):1179-82. doi: 10.1111/j.2042-7158.1998.tb03331.x. PMID: 9821667.

Pathway 47: RAISINGANTIBODY LEVEL

The sensitivity of IgG was significantly higher than that of IgM, where IgG tests performed 100% of sensitivity. The seroconversion of IgG against SARS-CoV-2 was earlier than that of IgM (3 days vs 6 days after onset), then maintained at high level longer than IgM. IgG maintained positive longer than 50 days, but IgM converted to negative around 36 days after onset. IgG antibody could generally keep positive for a long period. IgG, values significantly increased to during 11 to 20 days after onset, and then maintained relatively high [195].

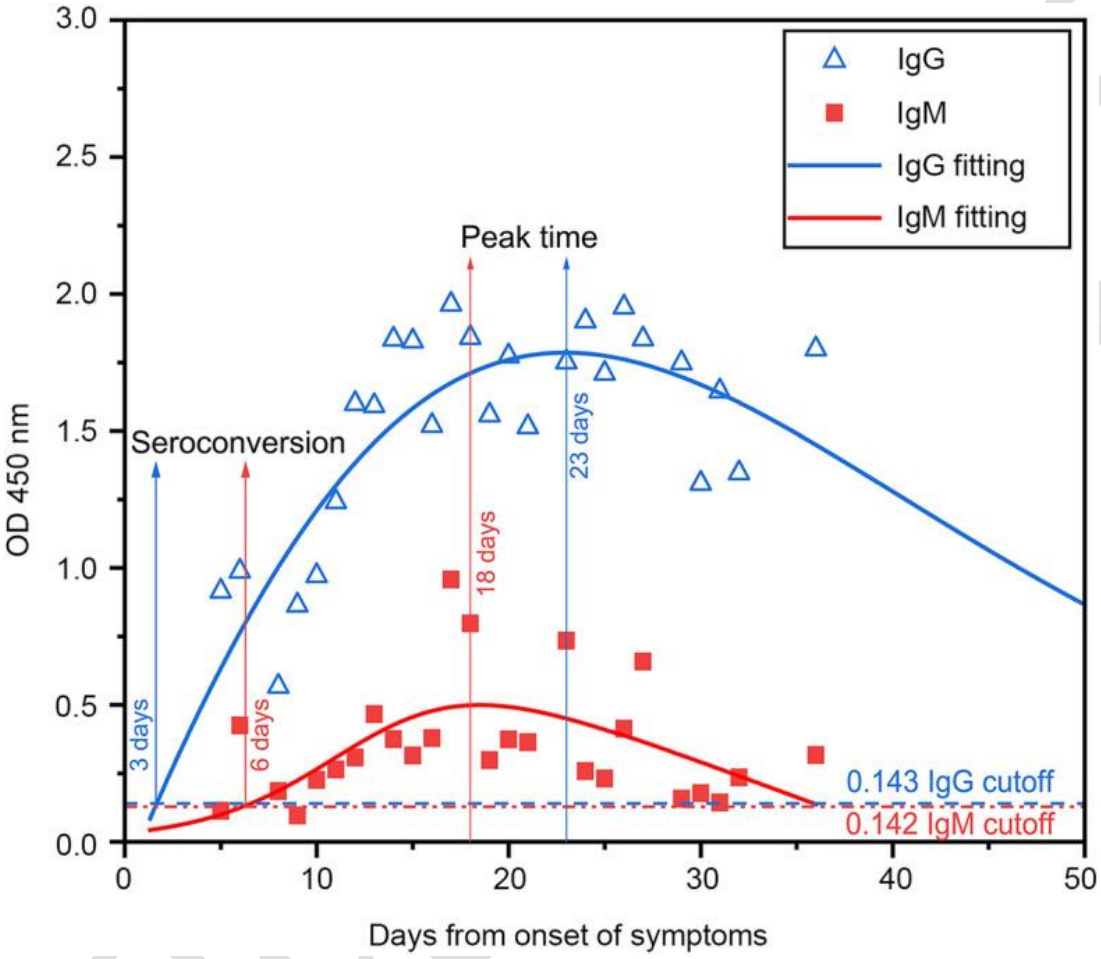

Fig:Antibody Developement

Role of Vedicinal: All 9 phytochemicals exerted their action through immunomodulatory effects such as increased proliferation of immune cells, modulation of cytokines, and increased antibody titers. Baicalin, Cucurmin, Epigallatocatechin gallate, hesperdin, exponentially proliferate and differentiate: B-cells turn into plasma cells, a sort of

[195] Chan KH, Sonnenberg K, Niedrig M, Lam SY, Pang CM, Chan KM, Ma SK, Seto WH, Peiris JSM. Use of antibody avidity assays for diagnosis of severe acute respiratory syndrome coronavirus infection. *Clin Vaccine Immunol.* 2007;14:1433–1436. doi: 10.1128/CVI.00056-07. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

antibody factories that release thousands of antibodies into the bloodstream .Luteolin, Piperine, Rutin, Quercetin and Glycyrrhizin assessed by higher levels of serum .[196]

Pathway 48 :LUNG TISSUE DAMAGE ALLEVIATORS

Lungs as target of COVID-19 infection, SARS-CoV2 infection and simultaneous ineffectiveness of the chemotherapeutic regime. Individuals suffering from lung cancer generally have impaired alveolar function with upregulated expression of ACE2 receptor, immunosuppressive cytokines, and impaired function of cytotoxic immune cells .

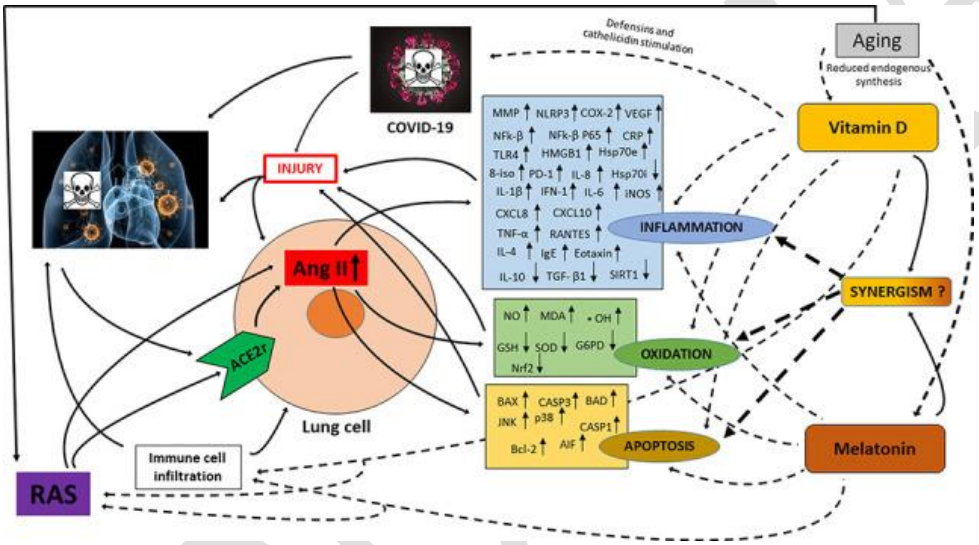

Fig : LUNG TISSUE DAMAGE ALLEVIATORS

Role of Vedicinal: 8 of our compounds help in alleviating the lung tissue damage[197].The reason behind the lung tissue damage is inflammation led by cytokine storm. Our compounds are able to alleviate the lung tissue damage. In fact our compounds also helps in boosting the lung cells**.** Epigallocatechin Gallate, Hesperidin, Quercetin causes a increase in the serum levels of antibodies. Baicalin, Curcumin, Glycyrrhizin and Oroxylin-A modulates the inflammatory response and regulating macrophage cytokines production Rutin, Luteolin .[198]has potential beneficial effects including anti-inflammation, antioxidation, anti-hyperlipidemia, and expression of VCAM-1 and iNOS, and NFκB activation

[196] Alberts B, Johnson A, Lewis J, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002. B Cells and Antibodies. Available from: https://www.ncbi.nlm.nih.gov/books/NBK26884/

[197] The major Cheng K, Yang A, Hu X, Zhu D, Liu K. Curcumin Attenuates Pulmonary Inflammation in Lipopolysaccharide Induced Acute Lung Injury in Neonatal Rat Model by Activating Peroxisome Proliferator-Activated Receptor γ (PPARγ) Pathway. Med Sci Monit. 2018 Feb 26;24:1178-1184. doi: 10.12659/msm.908714. PMID: 29480285; PMCID: PMC5839073

[198] Tseng CT, Sbrana E, Iwata-Yoshikawa N, Newman PC, Garron T, Atmar RL, Peters CJ, Couch RB. Immunization with SARS coronavirus vaccines leads to pulmonary immunopathology on challenge with the SARS virus. PLoS One. 2012;7(4):e35421. doi: 10.1371/journal.pone.0035421. Epub 2012 Apr 20. Erratum in: PLoS One. 2012;7(8). doi:10.1371/annotation/2965cfae-b77d-4014-8b7b-236e01a35492. PMID: 22536382; PMCID: PMC3335060.

Pathway 49: PROTECTING NEURONAL TISSUE

Neurochemical evidence of neuronal injury and glial activation in patients with moderate and severe COVID-19 .[199]Anti-inflammatory herbal medicine and its constituents are being proved to be a potent neuroprotector against various brain pathologies. Phytochemical based antioxidants may have neuroprotective (preventing apoptosis) and neuroregenerative roles, by reducing or reversing cellular damage and by slowing progression of neuronal cell loss [200]. The Anti-oxidants are found in dietary phytochemicals (such as polyphenols, quinones, flavonoids, catechins, coumarins, terpenoids) and the smaller molecules like ascorbic acid (Vitamin C), alpha-tocopherol (Vitamin E), beta-carotene vitamin-E, and supplements .[201]Though their mode of action is not yet completely elucidated, and clinical trials involving them are still relatively scarce, anti-oxidants offer a promising approach in the control or slowing down progression of neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, ischaemic and haemorrhagic stroke . Viral infection-induced colon inflammation, gut microbial imbalance, and α-synuclein upregulation are of particular interest with regard to the interplay between the gastrointestinal tract and the central nervous system (microbiome-gut-brain axis) .Identification of molecules such as natural compounds that are able to modulate astrocyte biology might open an important avenue to halt the negative effects of aging and to restore the injured brain.

Role of Vedicinal: Since the components present in all our compounds are small molecules and they easily cross the blood brain barrier. Once the components cross the blood brain barrier, they will start exerting their Anti-SARS-CoV-2 activity through above-described pathways and reduce the viral load present in the brain. Secondly, they will also provide the support with respect reduced cytokine storm, inflammation, histamine etc. Moreover, the specific interactions of flavonoids with receptors within the ERK and PI3-kinase/Akt signalling pathways have been reported to augment the expression of neuromodulator and nuero-protective proteins as well as enhance the number and strength of different types of neurons. Concomitantly, their beneficial effects on the cerebrovascular system can improve the cognitive performance of individuals via an enhancement in blood flow and stimulation of neurogenesis in brain. Baicalin, Curcumin, Hesperidin, Oroxylin A act as a Neuroprotective Agent[202]. Epigallocatechin-3-gallate, Glycyrrhizin, Quercetin provides robust neuroprotection in brain . Piperine has shown potent acetylcholine and amyloidogenic inhibitors with significant neuroprotective activity .Rutin has demonstrated the neuroprotective effect on brain ischemia .

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[199] Kanberg N, Ashton NJ, Andersson LM, Yilmaz A, Lindh M, Nilsson S, Price RW, Blennow K, Zetterberg H, Gisslén M. Neurochemical evidence of astrocytic and neuronal injury commonly found in COVID-19. Neurology. 2020 Sep 22;95(12):e1754-e1759. doi: 10.1212/WNL.0000000000010111. Epub 2020 Jun 16. PMID: 32546655.

[200] Zhang, X., Tan, Y., Ling, Y. *et al.* Viral and host factors related to the clinical outcome of COVID-19. *Nature* **583,** 437–440 (2020). https://doi.org/10.1038/s41586-020-2355-0

[201] Kumar GP, Khanum F. Neuroprotective potential of phytochemicals. *Pharmacogn Rev*. 2012;6(12):81-90. doi:10.4103/0973-7847.99898

[202] Hajialyani M, Hosein Farzaei M, Echeverría J, Nabavi SM, Uriarte E, Sobarzo-Sánchez E. Hesperidin as a Neuroprotective Agent: A Review of Animal and Clinical Evidence. Molecules. 2019 Feb 12;24(3):648. doi: 10.3390/molecules24030648. PMID: 30759833; PMCID: PMC6384806.

Pathway 50 :PROTECTING KIDNEY TISSUE

Kidney is a major target for SARS-CoV-2, post-infection observed strong complement C5b-9 (membrane attack complex) deposition in renal tubules of six patients with SARS-CoV-2 infection, suggesting activation of the complement pathway. An interaction between angiotensin II (AngII) overactivity, innate/adaptive immune and complement pathways, and the coagulation system could influence AKI severity and outcomes. Inflammation-induced erythrocyte aggregation (reflected as elevated erythrocyte sedimentation rate) and heme-mediated pathology may worsen oxidative stress, inflammation, and complement activation, to aggravate microvascular injury. Further, organ crosstalk between the injured lung, the heart, and the kidney can worsen pathology. SARS-CoV-2 nucleocapsid protein was observed in tubular structures in the kidneys and nucleocapsid protein–positive inclusion bodies were also observed in the cytoplasm. Similarly of virus-like particles observed in podocytes and renal tubular epithelial cells by electron microscopy, and SARS-CoV-2 nucleoprotein antibody-stained renal tubular epithelia positive. The main binding site for SARS-CoV-2, like SARS-CoV, is the ACE2 protein, which is expressed in the kidney much more than the lungs. ACE2 is expressed on the brush border apical membrane of the proximal tubule, where it colocalizes with angiotensin-converting enzyme (ACE), and is also present at lower levels in podocytes[203]. It is conceivable that the virus could enter the kidney by invading podocytes first, and thus gain access to the tubular fluid and subsequently bind to ACE2 in the proximal tubule. In primary human airway epithelia, ACE2 is expressed apically, and SARS-CoV-2 infection predominantly occurs on the apical surface, but infection can occur on the basolateral surface at low efficiency

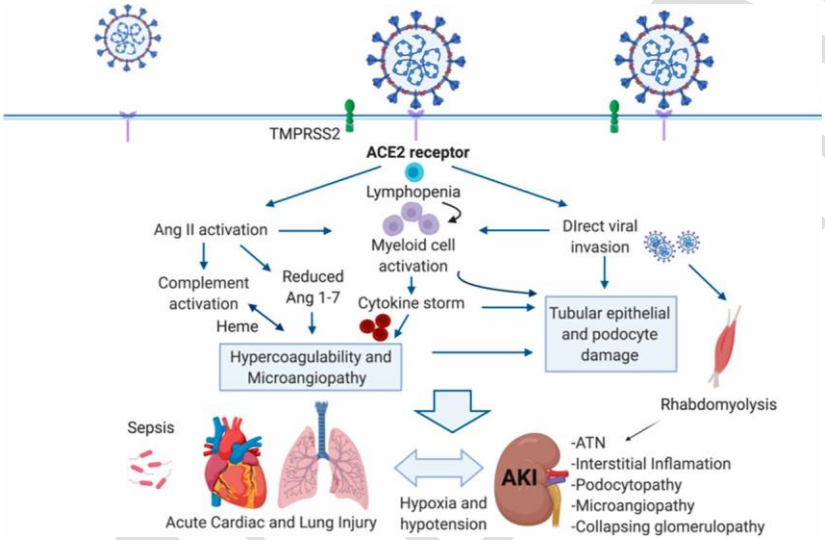

Fig: KIDNEY TISSUE

Role of Vedicinal: All of our 9 compounds contain the compounds which have proven nephro-protective activity. Baicalin, Luteolin, Hesperidin, Rutin, prevents or ameliorates adverse effects on the kidney compounds attenuate the hyperglycemia-disrupted renal endothelial barrier function, urinary microalbumin excretion, and glomerular hyperfiltration that results from a reduction of podocyte injury, a determinant factor for albuminuria in diabetic nephropathy.Curcumin, Piperine, Quercetin has a potential treatment for COVID-19-induced acute kidney injury EGCG acts against a broad spectrum of kidney diseases, including acute kidney injury, cisplatin-induced nephrotoxicity, kidney stone disease, glomerulonephritis, lupus nephritis, renal cell carcinoma, diabetic nephropathy, CKD, and renal

[203] Pfister F, Vonbrunn E, Ries T, et al. Complement Activation in Kidneys of Patients With COVID-19. *Front Immunol*. 2021;11:594849. Published 2021 Jan 29. doi:10.3389/fimmu.2020.594849

fibrosis .[204] Glycyrrhizin exerts protective effects in rats with NS, reducing the excretion of Upr, Ch, BUN, sCr, and mean arterial blood pressure, and also decreasing expression of LN, FN, Col, TGF?1 and CTGF in the kidney. Renal function is improved and the severity of NS is lessened . All compounds are currently being used for the treatment of kidney damage by several factors hence we believe our formulation will greatly help to alleviate kidney damage problems.

Pathway 51: PROTECTING CARDIO-VASCULAR SYSTEM

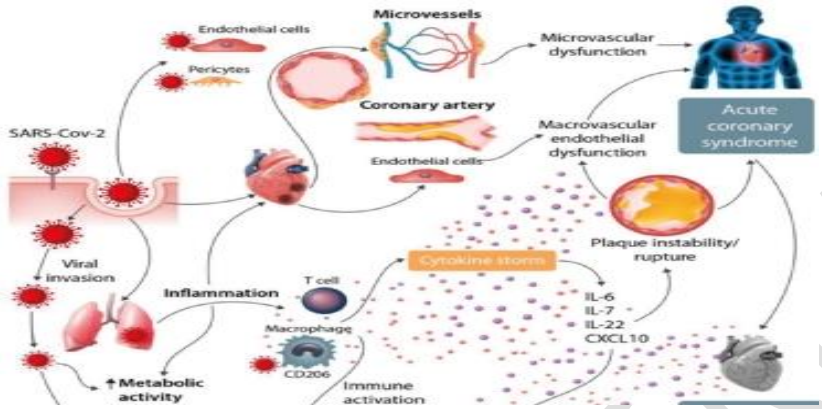

Fig: CARDIO-VASCULAR SYSTEM

EC are responsible for the supply of tissues with oxygen by synthesis and release of relaxing and contracting factors modulating blood flow rate, like nitric oxide (NO) which is the major antiplatelet agents . [205]Angiotensin-converting enzyme 2 (ACE2) is a membrane-bound aminopeptidase that has a vital role in the cardiovascular and immune systems . ACE2 is involved in heart function and the development of hypertension and diabetes mellitus. SARS-CoV-2 and MERS-CoV have similar pathogenicity, and the myocardial damage caused by infection with these viruses undoubtedly increases the difficulty and complexity of patient treatment. The levels of biomarkers of myocardial injury were significantly higher in patients treated in the ICU than in those not treated in the ICU, suggesting that patients with severe symptoms often have complications involving acute myocardial injury. In addition, among the confirmed cases of SARS-CoV-2 infection reported by the National Health Commission of China (NHC), some of the patients first went to see a doctor because of cardiovascular symptoms.[206]

[204] Gu YY, Zhang M, Cen H, et al. Quercetin as a potential treatment for COVID-19-induced acute kidney injury: Based on network pharmacology and molecular docking study. PLoS One. 2021;16(1):e0245209. Published 2021 Jan 14. doi:10.1371/journal.pone.0245209

[205] Knowles RB, Warner TD. Anti-platelet drugs and their necessary interaction with endothelial mediators and platelet cyclic nucleotides for therapeutic efficacy. Pharmacol Ther. 2019;193:83-90. doi:10.1016/j.pharmthera.2018.08.004

[206] (Zheng YY, Ma YT, Zhang JY, and Xie X. (2020) COVID-19 and the cardiovascular system, Nature Reviews Cardiology 17 :259–260).

Role of Vedicinal: All of our 9 compounds inhibitsthe inflammation and blood clotting by Disturbing NADPH Oxidase 4 and Reactive Oxygen Species-sensitive NOD-, LRR- and pyrin domain-containing protein 3 Inflammasome, by increasing nitric acid bio availability and reducing oxidative stress, by increasing Glomerular Filtration Rate and consequently reducing plasma creatinine and urea-nitrogen concentration. Baicalin, Curcumin improves vascular endothelial function by increasing nitric oxide bioavailability and reducing oxidative stress .Epigallocatechin-3-gallate (EGCG), Hesperidin, Luteolin has exhibitedpreventive effects against cardiovascular disease.. Glycyrrhizin, Quercetin potential protective role in hypertension, ischemic heart disease and stroke .Rutin plays important role for therapeutic benefit in cardiovascular diseases . [207]

Pathway 52: PROTECTING MYOCARDIAL TISSUE

Myocarditis can be caused by different infectious causal agents, following infection patients may develop lymphocytic, eosinophilic, or giant cell/granulomatous myocardial inflammation. It can lead to infectious dilated cardiomyopathy, a disease frequently requiring cardiac transplantation. Although acute viral myocarditis is frequently sub-clinical and recovery may be spontaneous, treatment of chronic myocarditis is presently unsatisfactory. Depending on the etiology, treatment may be antiviral or immunosuppressive .[208]

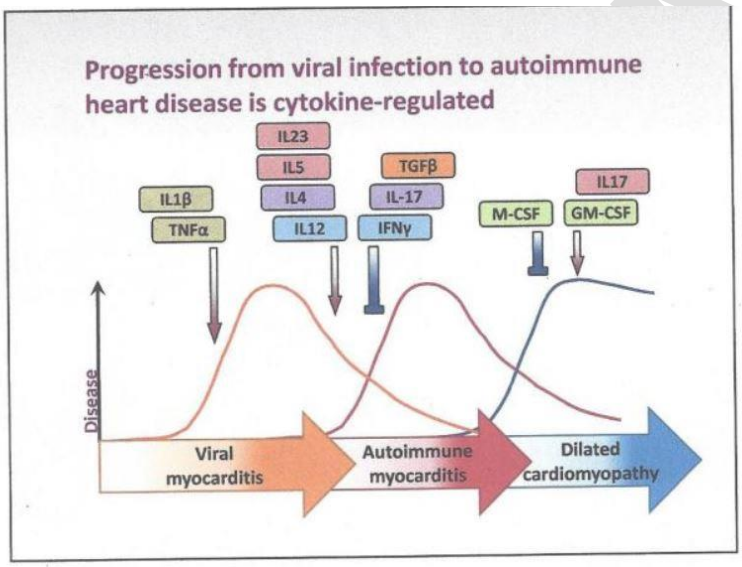

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Fig: **MYOCARDIAL TISSUE**

Different viruses produce the same outcome suggests that the role of infection may be non-specific amplification ("adjuvant effect") as well as providing an antigen specific stimulus. The studies reported that in the absence of continued viral presence and the production of heart specific autoantibodies, immunosuppressive therapy may be the most effective treatment of patients with lymphocytic inflammatory cardiomyopathy .[209]

[207] Mas-Capdevila A, Teichenne J, Domenech-Coca C, Caimari A, Del Bas JM, Escoté X, Crescenti A. Effect of Hesperidin on Cardiovascular Disease Risk Factors: The Role of Intestinal Microbiota on Hesperidin Bioavailability. Nutrients. 2020 May 20;12(5):1488. doi: 10.3390/nu12051488. PMID: 32443766; PMCID: PMC7284956.

[208] Pandey S, Rajasurya V. Nonviral Myocarditis. [Updated 2020 Jun 3]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK536928/

[209] Rose NR. Viral myocarditis. *Curr Opin Rheumatol*. 2016;28(4):383-389.

In SARS-CoV-2, the possible pathophysiology of arrhythmias includes direct injury to cardiomyocytes disrupting the plasma membrane and electrical conduction, infection of the pericardium causing massive edema, ischemia from microvascular disease due to possible infection of the pericytes, re-entrant arrhythmias due to myocardial fibrosis or scars; and proinflammatory cytokines predisposing to arrhythmogenicity. Proinflammatory cytokines (eg, IL-6) might cause displacement of plakoglobin, a desmosomal protein, from the cardiomyocyte membrane. This could be arrhythmogenic, as inadequate cell-to-cell adherence is postulated to damage the cell membrane, leading to cardiac cell death and fibrofatty replacement . [210]

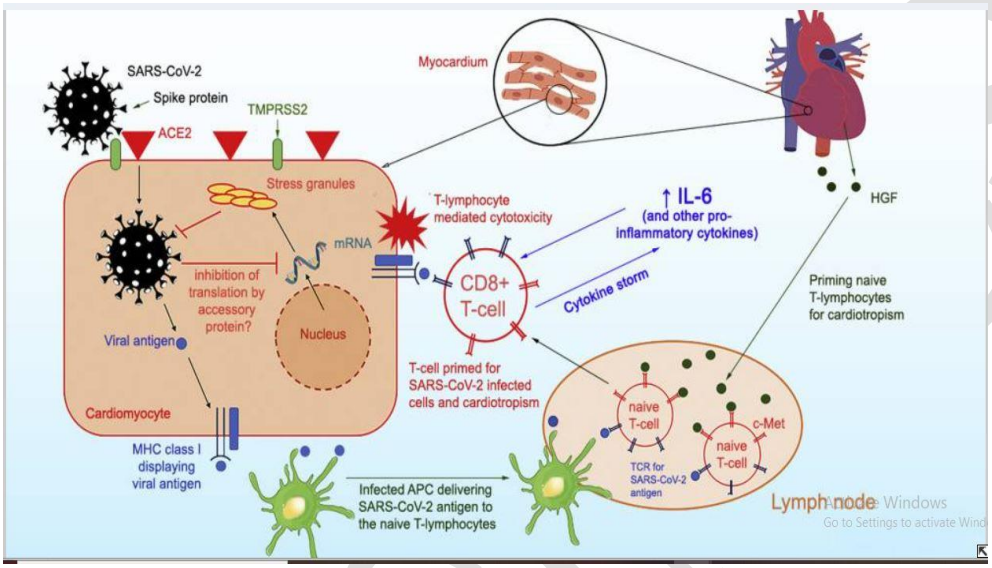


Fig:Myocardial infection

Role of Vedicinal: Myocardial cells are a potential target for SARS-CoV-2 and myocarditis has been reported,Phytocompound 1, 2, 3, 4, 5, 6, 7 and 8 prevented viral myocarditis by down regulating the cytokine storm caused during the inflammation. Baicalin, Curcumin, Epigallocatechin gallate attenuates acute myocardial infarction via mediating the mitogen-activated protein kinase pathway .Glycyrrhizin, Hesperidin, Luteolin mitigates the myocardial inflammatory response and myocardial inflammation through antioxidant anti-inflammatory mechanisms.[211] Piperine, Quercetin, Rutin reverses posttraumatic cardiac dysfunction by reducing cardiomyocyte apoptosis through the suppression of TNF-α increases, ROS overproduction and Ca2+ overload in cardiomyocytes, representing a potential preventive approach for the treatment of secondary cardiac injury .[212]

doi:10.1097/BOR.0000000000000303

[210] Siripanthong B, Nazarian S, Muser D, et al. Recognizing COVID-19-related myocarditis: The possible pathophysiology and proposed guideline for diagnosis and management. *Heart Rhythm*. 2020;17(9):1463-1471. doi:10.1016/j.hrthm.2020.05.001

[211] Liu X, Gu J, Fan Y, Shi H, Jiang M. Baicalin attenuates acute myocardial infarction of rats via mediating the mitogen-activated protein kinase pathway. Biol Pharm Bull. 2013;36(6):988-94. doi: 10.1248/bpb.b13-00021. Epub 2013 Mar 30. PMID: 23546333.

[212] Jing Z, Wang Z, Li X, Li X, Cao T, Bi Y, Zhou J, Chen X, Yu D, Zhu L, Li S. Protective Effect of Quercetin on Posttraumatic Cardiac Injury. Sci Rep. 2016 Jul 29;6:30812. doi: 10.1038/srep30812. PMID: 27470932; PMCID: PMC4965739.

Pathway 53 : BDNF AND REMYELINATION AGONIST, MYELIN SHEET PROTECTOR

Myelin-specific multiple sclerosis antibodies cause complement-dependent oligodendrocyte loss and demyelination .The process of demyelination is largely immune-mediated, as immunodeficient mice (RAG1(-/-) mice) do not develop demyelination upon infection; however, demyelination develops if these mice are reconstituted with either JHMV-immune CD4 or CD8 T cells . [213]Remyelination, which is usually a spontaneous endogenous process, is achieved when myelin-producing oligodendrocytes are generated from oligodendrocyte precursor cells (OPCs). Brain-derived neurotrophic factor (BDNF) mimetics to promote myelin repair in the brain. Angiotensin-converting enzyme 2 (ACE2) causes the production of angiotensin (1-7) Ang-(1-7) from angiotensin-2 (AngII). Ang (1-7) causes the production of Mas protein, which leads to the formation of brain-derived neurotrophic factor (BDNF), as the main protein in neurogenesis, and inhibits occurrences of oxidative stress, inflammation and apoptosis and also cause modulation of the mood-related disorder. Ang (1-7) causes activation of AT2R, which is angiotensin receptor type-2. Activation of AT2R plays a critical role in the management of the normal function of brain vascular endothelial. According to some indirect evidence, it seems the infection by the COVID-19 virus can cause distributing ACE-2/Mas/BDNF signaling pathway and can have unknown neurological and mental sequels. eNOS: Endothelial-derived nitric oxide syntheses; NO: Nitric oxide; MDA: Malondialdehyde; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; GR: Glutathione reductase; TNF-α: Tumor necrosis factor-alpha; IL-1β: Interleukin 1-β . [214]

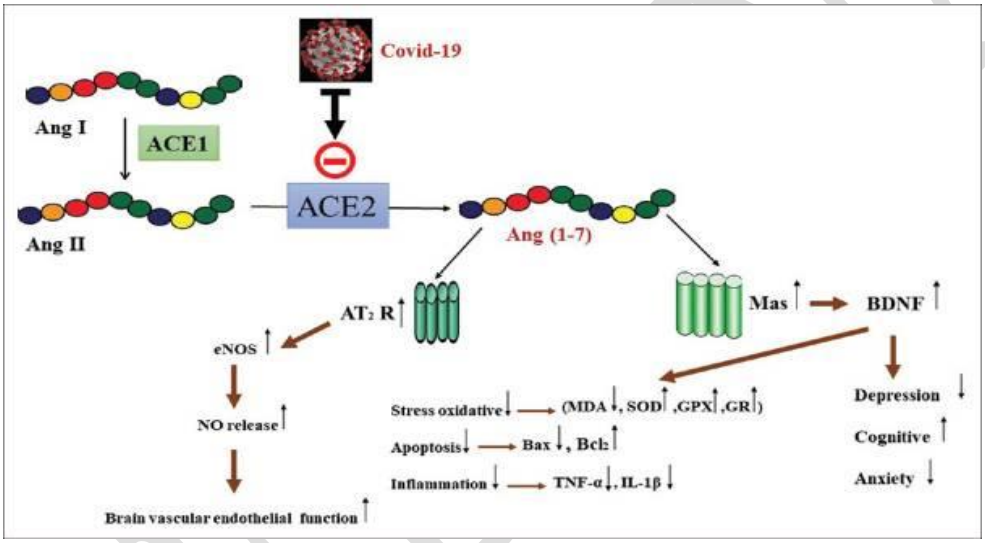


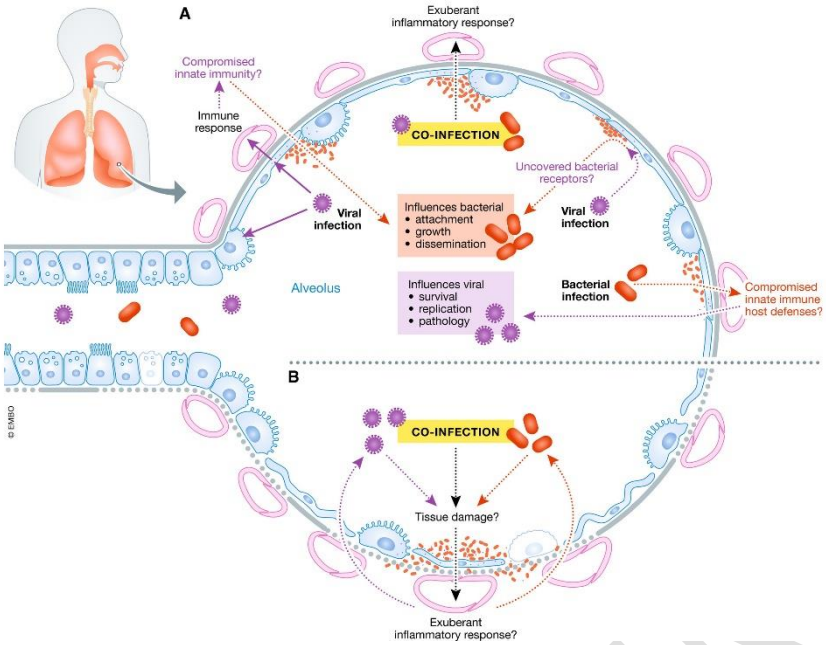
Fig: BDNF AND REMYELINATION AGONIST, MYELIN

Role of Vedicinal: Phytocompound 1, 2, 3, 5, 6, 8, 7 and 9 act as myelin sheet protector and in some case shown to exhibit remyelination properties. This plays vital role in protection against neuronal damage caused by covid-19. Baicalin, curcumin, Epigallocatechin gallate activate BDNF and assist in remyelination of nerve tissue. Glycyrrhizin, Hesperidine, Piperine promotes neural repair by directly driving functional remyelination .Luteolin, Quercetin decreases

[213] Bergmann CC, Lane TE, Stohlman SA. 2006. Coronavirus infection of the central nervous system: Host-virus stand-off. *Nat Rev Microbiol* 4:121–132. [PMC free article] [PubMed] [Google Scholar]

[214] Motaghinejad M, Gholami M. Possible Neurological and Mental Outcomes of COVID-19 Infection: A Hypothetical Role of ACE-2\Mas\BDNF Signaling Pathway. *Int J Prev Med.* 2020;11:84. Published 2020 Jul 9. doi:10.4103/ijpvm.IJPVM_114_20

secondary damage is through iron chelation and improves recovery of motor function after acute traumatic spinal cord injury .[215]



Role of Vedicinal: 8 of our compounds help in reducing the bacterial co-infections. There are abundant studies which shows that the selected compounds have very broad spectrum anti-microbial, anti-fungal and anti-viral activities. Hence these broad-spectrum components from our herbal compounds will help in reducing bacterial co-infections. Also, our compounds enhance immunity of the patients which will also help in fighting the bacterial co-infections.

[215] Tian J , Li X , Zhao L , Shen P , Wang Z , Zhu L , Li C , Su C , Zhang Y . Glycyrrhizic acid promotes neural repair by directly driving functional remyelination. Food Funct. 2020 Jan 29;11(1):992-1005. doi: 10.1039/c9fo01459d. PMID: 31808502.

Pathway 54: PROTECTING AGAINST THROMBOSIS

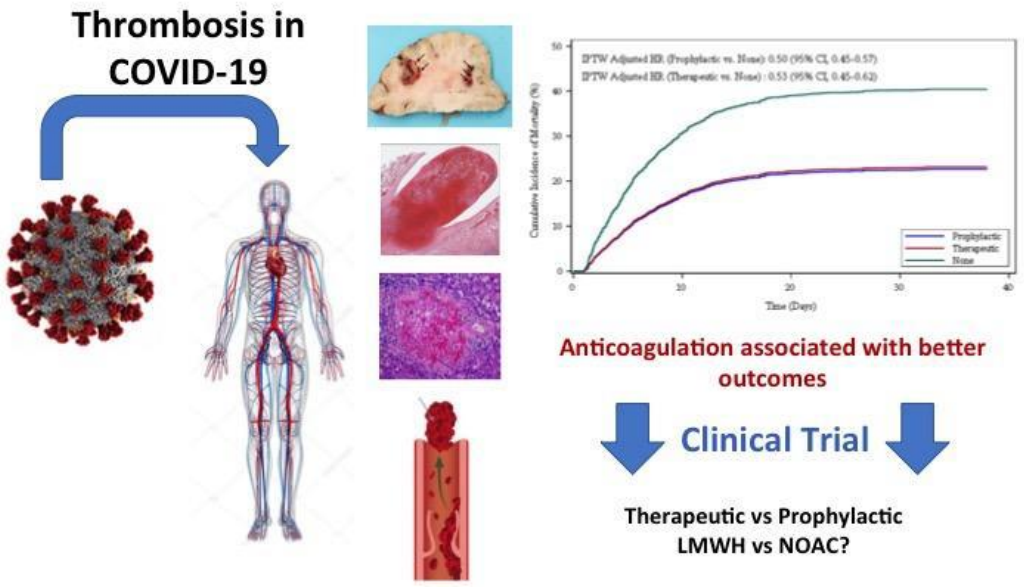


Fig: THROMBOSIS Pathway

COVID19 infection, is often associated with hypercoagulability and disseminated intravascular coagulation (DIC). COVID19 anticoagulation recommendations in children, Pediatric Blood and cancer, e28485. This hypercoagulability is manifested as progressive lung and kidney disease, pulmonary emboli (PE), venous thrombotic events (VTE), recurrent line obstruction, and stroke in adults. Research consistently reports a diversity of abnormal hemostatic laboratory results in SARS CoV2infected adults . Blood clotting is subsequently attenuated by a plethora of inhibitors that prevent excessive clot formation and eventual thrombosis, functional endothelium is essential to maintain hemostasis and prevent thrombosis [216]

. Most often, fibrinogen and D-dimer are elevated and correlated with acute inflammatory markers such as C-reactive protein. Prothrombin time prolongation is often seen. Covid patients often exhibit a platelet count that is only mildly decreased, a partial thromboplastin time that is normal-to-mildly prolonged, and no signs of microangiopathy .Typical DIC can also be seen in adults and graded per the ISTH scoring system. The presence and severity of DIC in adults is suggested to have strong prognostic value. Rising D-dimer over time, reflecting increasing coagulation and fibrinolysis, is also associated with an increased mortality in adults . [217]

Role of Vedicinal: The studies showed that protein disulfideismomerase (PDI) is rapidly secreted though platelets and endothelial cells. Hence blocking PDI could be one of the major strategies to inhibit thrombosis.Antiplatelet, anticoagulant, and profibrinolytic activities of Baicalin, Luteolin, Piperine Antithrombotic activities of Curcumin,

[216] Asakura H, Ogawa H. COVID-19-associated coagulopathy and disseminated intravascular coagulation. *Int J Hematol.* 2021;113(1):45-57. doi:10.1007/s12185-020-03029-y

[217] Warkentin TE, Kaatz S. COVID-19 versus HIT hypercoagulability. *Thromb Res.* 2020;196:38-51. doi:10.1016/j.thromres.2020.08.017

Epigallocatechin gallate, Hesperidin Glycyrrhizin, Quercetin, Rutin are potent natural thrombolytic agent[218]. Even though all of our 9 compounds have anticoagulant properties, but one of our compounds contain the components which is the most potent inhibitor of PDI among 5,000 inhibitors screened by the scientists.

Pathway 55: PROTECTING PANCREATIC BETA CELLS

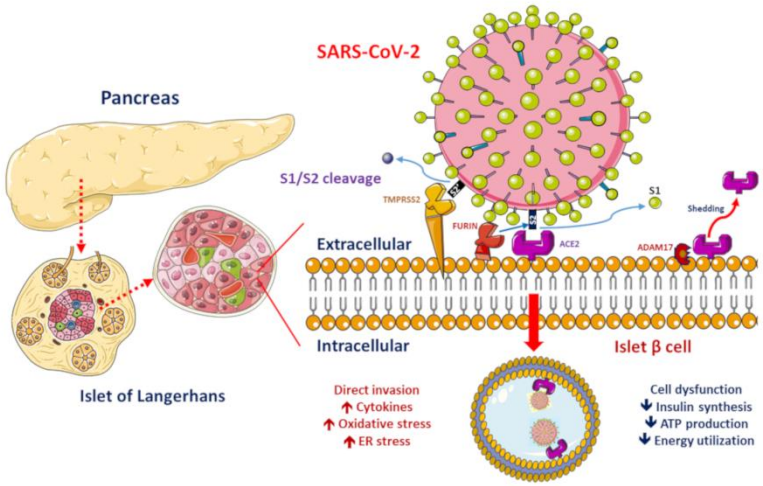


Fig: PANCREATIC BETA CELLS

Histopathological studies have reported organotropism of SARS-CoV-2 beyond the respiratory tract, including tropism to renal, myocardial, neurologic, pharyngeal tissues etc.Localization of ACE2 expression in the endocrine part of the pancreas suggests that SARS coronavirus enters islets using ACE2 as its receptor and damages islets causing acute diabetes .ACE2 and TMPRSS2 are present in lung alveolar epithelial type II cells, nasal goblet secretory cells, cholangiocytes, colonocytes, esophageal keratinocytes, gastrointestinal epithelial cells, pancreatic β-cells, and renal proximal tubules and podocytes[219]. These findings suggest that multiple-organ injury may occur at least in part due to direct viral tissue damage. The mechanism of extrapulmonary spread of SARS-CoV-2, whether hematogenous or otherwise, remains elusive.

Recent studies report acute pancreatitis associated with SARS-CoV-2 infection, 17% of 52 patients with COVID-19 had slightly abnormal amylase or lipase. Viral pancreatitis develops due to direct destruction of pancreatic acinar cells by inflammation and edema .[220]

Role of Vedicinal: Destruction of pancreatic islets is the major determinant for the onset of hyperglycemia and development of complications in insulin-dependent diabetic patients. Preventing beta cell degeneration, stimulating endogenous regeneration of islets, and islet transplantation will be of essential approaches for Covid 19 management, phytocompound 1, 3, 5, 6, and 7 have pancreatic beta cells protective property. Baicalin, Curcumin suppresses cytokine-

[218] Chiu HF, Venkatakrishnan K, Wang CK. The role of nutraceuticals as a complementary therapy against various neurodegenerative diseases: A mini-review. *J Tradit Complement Med*. 2020;10(5):434-439. Published 2020 Apr 1. doi:10.1016/j.jtcme.2020.03.008

[219] Ni, W., Yang, X., Yang, D. *et al.* Role of angiotensin-converting enzyme 2 (ACE2) in COVID-19. *Crit Care* **24,** 422 (2020). https://doi.org/10.1186/s13054-020-03120-0

[220] Hadi A, Werge M, Kristiansen KT, et al. Coronavirus Disease-19 (COVID-19) associated with severe acute pancreatitis: Case report on three family members. *Pancreatology*. 2020;20(4):665-667. doi:10.1016/j.pan.2020.04.021

induced pancreatic beta-cell damage. Epigallocatechin gallate (EGCG), Hesperidine, Luteolin reduces the intensity of pancreatic amyloid fibrils .[221]

Pathway 56 : PROTECTING LIVER TISSUE

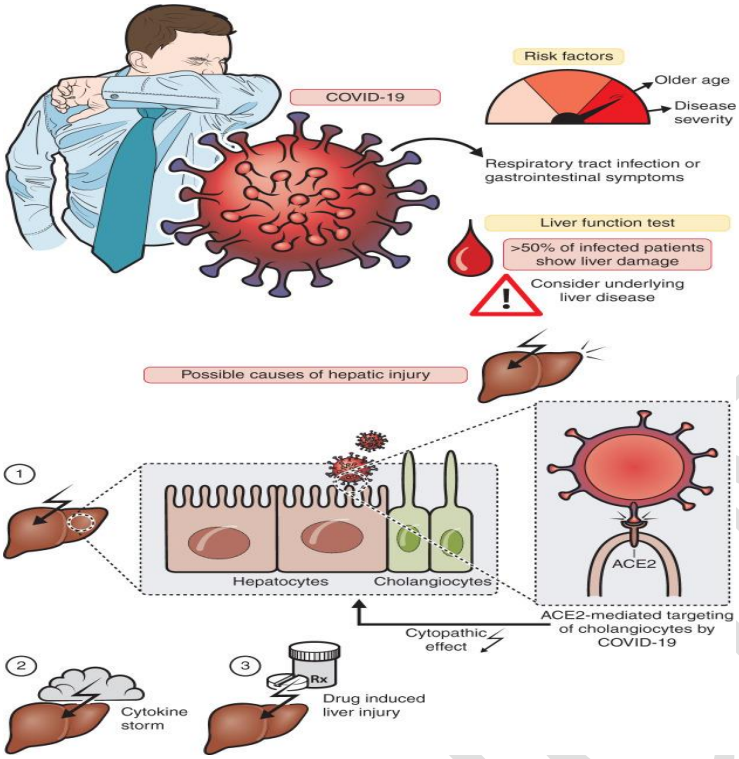


Fig:Liver Tissue Injury

Many of the COVID-19 patients in the various studies had a varying degree of deranged liver enzymes. The degree of injury was mild in most cases; and it appears to correlate with the severity of COVID-19 infection. Severe liver injury causing significant liver damage, liver failure, or death is uncommon. Deranged liver enzymes are not an uncommon finding in COVID-19 patients[222]. Usually, it is in the form of altered aminotransferases picked up during routine investigations. Seldom does it present with acute hepatitis. The cause of the liver injury is not clearly established, but most likely, it seems multifactorial, with a cytokine storm and immune dysregulation possibly playing a role so it could be hypoxia, hypotension, multiple drugs, direct viral effect, and ICU-related infections. In the majority of patients, the liver injury seems to be self-limiting, not requiring any specific intervention, and not associated with acute liver failure [223].

[221] Rashid K, Sil PC. Curcumin enhances recovery of pancreatic islets from cellular stress induced inflammation and apoptosis in diabetic rats. Toxicol Appl Pharmacol. 2015 Feb 1;282(3):297-310. doi: 10.1016/j.taap.2014.12.003. Epub 2014 Dec 23. PMID: 25541178.

[222] Ghoda A, Ghoda M. Liver Injury in COVID-19 Infection: A Systematic Review. *Cureus*. 2020;12(7):e9487. Published 2020 Jul 31. doi:10.7759/cureus.9487

[223] Alqahtani SA, Schattenberg JM. Liver injury in COVID-19: The current evidence. *United European Gastroenterol J*. 2020;8(5):509-519. doi:10.1177/2050640620924157

Liver function abnormalities – predominantly AST elevation – in COVID-19 appear to be frequent but not severe in most cases. Direct viral hepatotoxicity, DILI, 'bystander effects' during a systemic viral infection and potentially sepsis, or exacerbation of an underlying liver disease have to be considered. Ex vivo studies offer that SARS-CoV-2 can selectively target the liver, in particular cholangiocytes through ACE2, and thus hepatobiliary injury appears plausible.[224]

Role of Vedicinal:

Baicalin has therapeutic potential to cure liver fibrosis by way of *Pparγ*derepression mediated by suppression of canonical Wntsignaling .Curcumin protects against hepatic stellate cells activation and migration by inhibiting the CXCL12/CXCR4 biological axis in liver fibrosis. EGCG acts as a hepatoprotectant by reducing the serum levels of liver functional enzymes, increasing total anti-oxidative capacity, reducing pathological changes and apoptosis, as well as causing the movement of cells from the sub G1 to S or G2/M phase of the cell cycle Luteolin was able to significantly reduced neutrophil infiltration and pro-inflammatory cytokines level in MSU induced inflammation .Quercetin improves hepatic fibrosis reducing hepatic stellate cells and regulating pro-fibrogenic/anti-fibrogenic molecules balance [225].[226]

[224] Alqahtani, Saleh & Schattenberg, Jörn. (2020). Liver injury in COVID-19: The current evidence. United European Gastroenterology Journal. 8. 509-519. 10.1177/2050640620924157.

[225] Qiao H, Tong Y, Han H, Xu W, Ren Z, Ouyang J, Chen Y. A novel therapeutic regimen for hepatic fibrosis using the combination of mesenchymal stem cells and baicalin. Pharmazie. 2011 Jan;66(1):37-43. PMID: 21391433.

[226] Yang, M.D., Chiang, Y.-M., Higashiyama, R., Asahina, K., Mann, D.A., Mann, J., Wang, C.C. and Tsukamoto, H. (2012), Rosmarinic acid and baicalin epigenetically derepress peroxisomal proliferator-activated receptor γ in hepatic stellate cells for their antifibrotic effect. Hepatology, 55: 1271-1281. https://doi.org/10.1002/hep.24792

Pathway 57: TREATMENT OF BACTERIAL CO-INFECTION

Some mechanisms by which viral respiratory infections may predispose patients to bacterial infections to include failure immune response, viral-induced changes in epithelial cells, and the increased bacterial colonization 34 and summarize of the potential mechanisms responsible for bacterial coinfection with viral respiratory infections. This situation can explain why bacterial co-infections occur when the virus starts to be eradicated from the lungs of patients with COVID-19. This is accompanied by a shift in phagocytic activity of lung cells that mediate basal levels of innate protection via phagocytosis and pro-inflammatory cytokines formation to cells better attuned to antigen presentation and stimulation of adaptive immune reactions. Additionally, recently it has been found the microbiome diversity shapes our immune system. In line with this, the depletion of the gut microbiome hinders the immune system's ability to create a humoral response against viruses like the flu virus. However, this novel paradigm ultimately allows the development of new immune intervention approaches for the prevention and management of viral-bacterial co-infections in COVID-19 patients (Mirzaei et al., 2020). Although cytokines storms seem to be induced by both SARS-CoV-2 and influenza, given the early stage in our understanding regarding SARS-CoV-2 infection, to conclude that the immunomodulatory or immune suppressive effects of these two viruses are highly similar might be premature.

NK cells activated during bacterial infections contribute to bacteria elimination but also to disease pathogenesis. NK cell activation in these infections can occur both directly by sensing of bacteria through pattern recognition receptors and indirectly via bacterial stimulation of dendritic cells or macrophages (Straub et al., 2018). In a research it was demonstrated that NK cell activation is facilitated via IL-2, IL-18 and IFN-ß produced by dendritic cells (Straub et al., 2018). In a study demonstrated that NK cells in LPS-treated mice suppress clonal expansion of LCMV-specific CTLs by a NKG2D-independent or NCR1-independent but perforin-dependent mechanism. Thus TLR4-mediated immunoregulatory role of NK cells during viral-bacterial coinfections (Straub et al., 2018).

Few studies proved that influenza aggravated the pulmonary status of individuals with TB so that latent TB could become active, a closed cavity might open, and various lesions might progress, leading to further deterioration of pulmonary function. In this regard, a mouse coronavirus model demonstrated the ability to reactivate dormant MTB from CD271 + mesenchymal stem cells through the altruistic stem cell-based defense mechanism, predicting a potential increase of TB in SARS-CoV-2 era. Additionally, in a cohort of 49 patients with active TB and COVID-19, the diagnosis of COVID-19 preceded or was simultaneous (within seven days) with TB in 23 patients, raising the suspicion that SARS-CoV-2 infection might boost the development of active TB. However, this remains purely speculative as individuals with latent TB infection were not followed up over time (Crisan-Dabija et al., 2020). Researchers have identified a lung stem cell that repairs the organ's gas exchange compartment. They isolated and characterized these progenitor cells from mouse and human lungs and demonstrated they are essential to repairing lung tissue damaged by severe influenza and other respiratory ailments.

Malaria and COVID-19 have similar aspects and seem to have a strong potential for mutual influence. The similar and generic symptoms make it harder to achieve an immediate diagnosis. Healthcare systems and professionals will face a great challenge in case of a syndemic. In countries with a high burden of malaria. In patients with symptoms such as fever, fatigue, and headache, both malaria and COVID-19 tests should always be performed. According to WHO recommendations, in the case of challenges due to the COVID-19 pandemic a malaria diagnosis should be considered for all fever cases in endemic countries. On the other hand, patients with COVID-19-related symptoms that negative for malaria must undergo isolation to exclude COVID-19 until repetition of the virological sample. The WHO has called for ministries of health and national malaria control programs to ensure that malaria control efforts are not disrupted

while facing the COVID-19 response. Preparedness is the key to tackling any public health crisis, and malaria-endemic countries need to be prepared for the challenges COVID-19 could pose. From a global perspective, it is necessary to increase and join efforts in order to develop an effective vaccine and it make available for everyone, as this would be the most effective preventive measure for both diseases (Gennaro et al., 2020).

Dengue, a mosquito-borne viral infection, itself a great threat in the world, during the COVID-19 pandemic, dengue cases have increased in most of the dengue-endemic countries. Corona and dengue, both viruses are coexisting currently in the dengue-endemic countries. Therefore, the coinfection of these viruses to the patients has been reported recently in different dengue-endemic countries like Singapore, Thailand, India, and Bangladesh. Hence, it can be speculated that coinfection cases may be increased in the upcoming days as peak dengue season. Coinfection poses a challenge for accurate diagnosis and treatment, particularly when symptoms such as fever and aches are similar for several viral diseases like COVID-19 and dengue. In recent study it is reported that fever, cough, and headache were observed as the most common symptoms for patients with COVID-19 whereas fever, headache, and skin rash were observed for patients with dengue. Clinical and laboratory features of both dengue and COVID-19 are quite identical, and therefore, it is difficult to distinguish. The dengue-endemic countries are at the risk of possible coinfection and co-epidemics in where COVID-19 and dengue disease are coexisting, and the viruses are co-circulating (Miah, and Husna, 2020).

Acute disseminated encephalomyelitis (ADEM) is a rare demyelinating disease, often post-viral, and more commonly seen in children than adults. Clinically, it is heterogeneous, generally causing encephalopathy and multifocal deficits. MRI typically demonstrates FLAIR hyperintensities in deep white matter and at the grey/white matter interface. Post-contrast enhancement is not always present but is often punctate or rim enhancing. Diffusion restriction can be seen, especially early in the course of the disease. ADEM lesions can be hemorrhagic, but the etiology of the intraventricular hemorrhage in this case is unclear. Anticoagulation had not been utilized, blood pressure had been well controlled, there was no laboratory evidence of coagulopathy, and she had not suffered trauma.

The location and evolution of lesions on MRI, sparse contrast enhancement, clinical exam, and CSF are all consistent with an acute demyelinating event, and the clinical situation in this case, weeks after an acute viral infection, is favorable for the development of ADEM. ADEM has been reported in a child following a coronavirus infection. As more patients reach the weeks after initial infection with COVID-19, ADEM should be considered a potentially treatable cause of profound encephalopathy or multifocal neurological deficits.

Role of Vedicinals-9 compounds: 8 of our compounds help in reducing the bacterial co-infections. There are abundant studies which shows that the selected compounds have very broad spectrum anti-microbial, anti-fungal and anti-viral activities. Hence these broad-spectrum components from our herbal compounds will help in reducing bacterial co-infections. Also, our compounds enhance immunity of the patients which will also help in fighting the bacterial co-infections. Baicalin, Curcumin, Epigallocatechin-3-gallate has anti-infective activity against Gram-positive and Gram-negative bacterias .Glycyrrhizin, piperine possess antiviral and antimicrobial activities.Quercetin, Rutin impairs biofilm formation and exhibits high antibacterial activity

Pathway :58 MODULATING GUT BACTERIA AND GUT-BRAIN AXIS

The intestine involves the regulation of CNS function in various ways, such as nervous system, hormone system, and immune system. Increasing evidence shows that the SARS-CoV-2 can cause intestinal dysfunction, microbial imbalance, and immune disorder. Through the microbe-gut-brain axis, the intestine, especially the intestinal bacteria, is most likely to be the major approach for SARS-CoV-2 affecting the brain function. It is therefore likely that brain dysfunction would be secondary complication after SARS-CoV-2 infection [227]

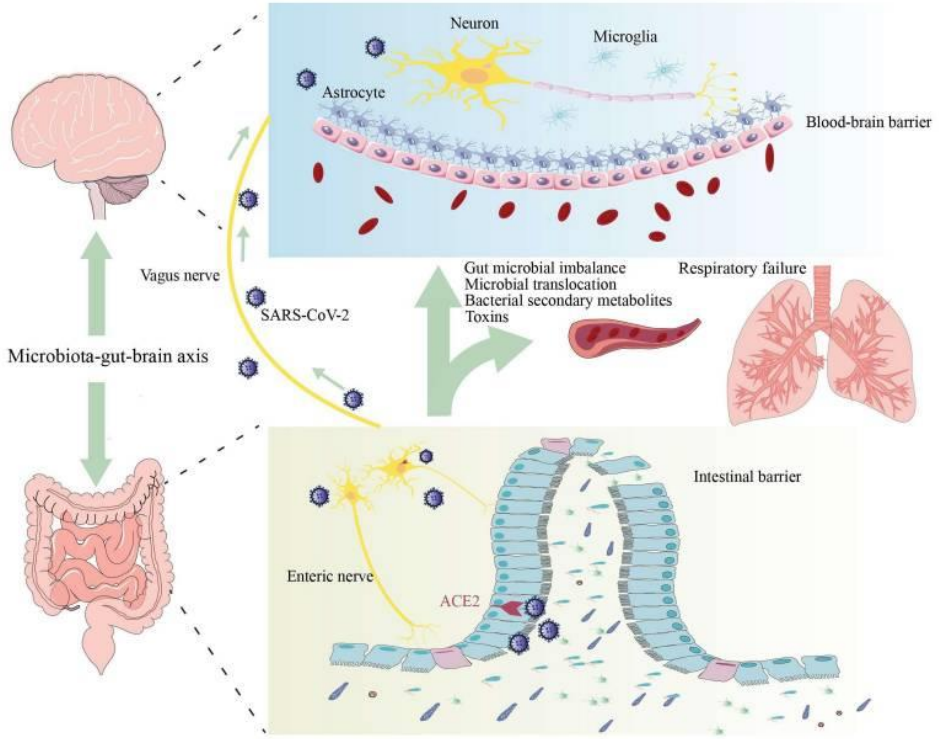


Fig:Gut BACTERIA AND GUT-BRAIN AXIS

Even though human coronavirus (CoV) is mainly responsible for the infections of the respiratory tract, some studies have shown CoV (in case of Severe Acute Respiratory Syndrome, SARS and Middle East Respiratory Syndrome, MERS) to possess potential to spread to extra-pulmonary organs including the nervous system as well as gastrointestinal tract (GIT). Patients infected with COVID-19 have also shown symptoms associated with neurological and enteric infection like disorders related to smell/taste, loss of appetite, nausea, emesis, diarrhea, and pain in the abdomen. In the present review, we attempt to evaluate the understanding of basic mechanisms involved in clinical manifestations of COVID-19, mainly focusing on interaction of COVID-19 with gut-brain axis . The absorption of polyphenols present in food depends mostly on the activity of intestinal microflora . Flavonoids could exert a protective role against obesity-associated pathologies by modulating inflammatory-related cellular events in the intestine and/or the composition of the microbiota populations.[228]

[227] Shi Y, Li Z, Yang C, Liu C. The role of gut-brain axis in SARA-CoV-2 neuroinvasion: Culprit or innocent bystander?. *Brain Behav Immun*. 2021;94:476-477. doi:10.1016/j.bbi.2021.01.024

[228] Shinu P, Morsy MA, Deb PK, Nair AB, Goyal M, Shah J, Kotta S. SARS CoV-2 Organotropism Associated Pathogenic Relationship of Gut-Brain Axis and Illness. Front Mol Biosci. 2020 Dec 22;7:606779. doi: 10.3389/fmolb.2020.606779. PMID: 33415126; PMCID: PMC7783391.

Role of Vedicinal:

Curative and immunoregulatory properties of baicalin and have direct practical and clinical relevance for the treatment of bacterial infection. Curcumin and its metabolites can have health benefits by eliminating intestinal microflora dysbiosis. In addition, curcumin undergoes enzymatic modifications by bacteria, forming pharmacologically more active metabolites than their parent, curcumin Epigallocatechin gallate resulted in stimulation of the beneficial bacteria Bacteroides, Christensenellaceae, and Bifidobacterium. Additionally, the pathogenic bacteria Fusobacterium varium, Bilophila, and Enterobacteriaceae were inhibited. Furthermore, changes in concentrations of metabolites, including 4-phenylbutyric acid and phenylacetic acid, were strongly correlated with changes in the abundance of specific gut microbiota[229] .Hesperidin, Glycyrrhizin, increase of chemokine and chemokine receptor genes expression that modulates B and T cell recruitment to lymphoid follicles .. piperine significantly enhanced the oral absorption of linarin in rats by inhibiting P-glycoprotein mediated cellular efflux during the intestinal absorption . Quecetin, Rutin assists in reshaping gut microbiota composition.[230]

Pathway 59: TREATMENT OF INTESTINAL INFLAMMATION

Flavonoids have clear anti-inflammatory properties, which have primarily been evaluated in non-intestinal models. At present, a growing body of evidence suggests that flavonoids could exert a protective role against obesity-associated pathologies by modulating inflammatory-related cellular events in the intestine and/or the composition of the microbiota populations .[231]

SARS-CoV-2 can cause gastrointestinal symptoms, such as vomiting, diarrhea, or abdominal pain during the early phases of the disease. Intestinal dysfunction induces changes in intestinal microbes, and an increase in inflammatory cytokines. Thus, diagnosing gastrointestinal symptoms that precede respiratory problems during COVID-19 may be necessary for improved early detection and treatment[232]. Growing evidence indicates the crosstalk between gut

[229] Pluta R, Januszewski S, Ułamek-Kozioł M. Mutual Two-Way Interactions of Curcumin and Gut Microbiota. *Int J Mol Sci*. 2020;21(3):1055. Published 2020 Feb 5. doi:10.3390/ijms21031055

[230] Zhang YH, Isobe K, Nagase F, Lwin T, Kato M, Hamaguchi M, Yokochi T, Nakashima I. Glycyrrhizin as a promoter of the late signal transduction for interleukin-2 production by splenic lymphocytes. Immunology. 1993 Aug;79(4):528-34. PMID: 8406577; PMCID: PMC1421919.

[231] Gil-Cardoso K, Ginés I, Pinent M, Ardévol A, Blay M, Terra X. Effects of flavonoids on intestinal inflammation, barrier integrity and changes in gut microbiota during diet-induced obesity. Nutr Res Rev. 2016 Dec;29(2):234-248. doi: 10.1017/S0954422416000159. Epub 2016 Nov 14. PMID: 27841104.

[232] Soriano, S., Moffet, B., Wicker, E. et al. Serum Amyloid A is Expressed in the Brain After Traumatic Brain Injury in a Sex-Dependent Manner. Cell Mol Neurobiol 40, 1199–1211 (2020). https://doi.org/10.1007/s10571-020-00808-3

microbiota and lung, that maintains host homeostasis and disease development with the association of immune system. This gut-lung interaction may influence the COVID-19 severity in patients with extrapulmonary conditions. its bidirectional relationship with immune system and lung. Dysbiosis in gut microbiota results in gut permeability leading to secondary infection and multiple organ failure. Conversely, disruption of the gut barrier integrity due to dysbiosis may lead to translocation of SARS-CoV-2 from the lung into the intestinal lumen via circulatory and lymphatic system .The use of prebiotics and probiotics to regulate the balance of the intestinal flora could be an effective treatment to reduce the risk of bacterial and viral infections . [233]

Role of Vedicinal: The curative and immunoregulatory properties of Baicalin have direct practical and clinical relevance for the treatment of enteritis in humans. Baicalein induces CD4+Foxp3+ T cells and enhances intestinal barrier function .EGCG Maintains Th1/Th2 Balance and Mitigates Ulcerative Colitis Induced by Dextran Sulfate Sodium through TLR4/MyD88/NF- κ B Signaling Pathway .Luteolin blocks NF-κB signalling and proinflammatory gene expression in intestinal epithelial cells and dendritic cells.[234] Modulation of innate immunity by natural plant products may represent an attractive strategy to prevent intestinal inflammation associated with dysregulated innate immune responses .Quercetin improves gut dysbiosis Rutin, one of the most abundant flavonoids in nature, has been shown to exert intestinal antiinflammatory effects in experimental models of colitis.

Pathway 60: TREATMENT OF ENCEPHALOMYELITIS

Acute disseminated encephalomyelitis (ADEM) is a rare demyelinating disease, often post-viral, and more commonly seen in children than adults. Clinically, it is heterogeneous, generally causing encephalopathy and multifocal deficits .MRI typically demonstrates FLAIR hyperintensities in deep white matter and at the grey/white matter interface. Post-contrast enhancement is not always present but is often punctate or rim enhancing. Diffusion restriction can be seen, especially early in the course of the disease. ADEM lesions can be hemorrhagic, but the etiology of the intraventricular hemorrhage in this case is unclear. Anticoagulation had not been utilized, blood pressure had been well controlled, there was no laboratory evidence of coagulopathy, and she had not suffered trauma.[235]

The location and evolution of lesions on MRI, sparse contrast enhancement, clinical exam, and CSF are all consistent with an acute demyelinating event, and the clinical situation in this case, weeks after an acute viral infection, is favorable for the development of ADEM. ADEM has been reported in a child following a coronavirus infection. As more patients reach the weeks after initial infection with COVID-19, ADEM should be considered a potentially treatable cause of profound encephalopathy or multifocal neurological deficits.[236]

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[233] ellieux Geoffroy, Sonneville Romain, Vledouts Sérafima, Jaquet Pierre, Rouvel-Tallec Anny, d'Ortho Marie-PiaCOVID-19-Associated Neurological Manifestations: An Emerging Electroencephalographic Literature JOURNAL=Frontiers in Physiology VOLUME=11 yEAR=2021PAGES=1866 DOI=10.3389/fphys.2020.622466 ISSN=1664-042X

234 Xue Bing, Liu Xuelei, Dong Wanwei, Liang Linlang, Chen Keyan, "EGCG Maintains Th1/Th2 Balance and Mitigates Ulcerative Colitis Induced by Dextran Sulfate Sodium through TLR4/MyD88/NF-κB Signaling Pathway in Rats", Canadian Journal of Gastroenterology and Hepatology, vol. 2017, Article ID 3057268, 9 pages, 2017. https://doi.org/10.1155/2017/3057268

[235] Filippi M, Rocca MA. Acute Disseminated Encephalomyelitis. *White Matter Diseases*. 2020;109-125. Published 2020 Feb 12. doi:10.1007/978-3-030-38621-4_5

[236] Parsons T, Banks S, Bae C, Gelber J, Alahmadi H, Tichauer M. COVID-19-associated acute disseminated encephalomyelitis (ADEM). *J Neurol*. 2020;267(10):2799-2802. doi:10.1007/s00415-020-09951-9

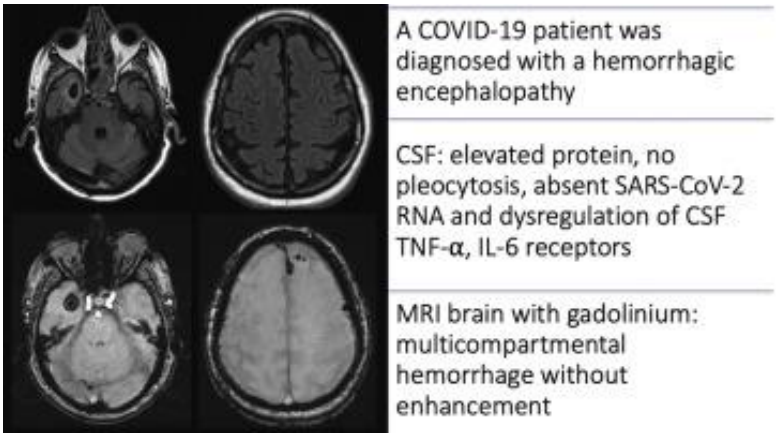

Fig: ENCEPHALOMYELITIS

Role of Vedicinal: Acute disseminated encephalomyelitis (ADEM) is a rare immune-mediated demyelinating disease that has been associated with vaccine and viral infections, including SARS-CoV-2 infection. Phytocompound 1, 2, 3, 7 and 9 support treatment of Encephalomyelitis by impeding the demyelination of nerve cells. Baicalin reduced infiltration of immune cells into the CNS, inhibited expression of proinflammatory molecules and chemokines and prevented Th1 and Th17 cell differentiation via STAT/NFκBsignaling pathways .Curcumin resulted in a decrease in IL-12-induced T cell proliferation and Th1 differentiation by inhibition of Janus kinase-STAT pathway use in the treatment encephalomyelitis and other Th1 cell-mediated inflammatory diseases .Glycyrrhizin, a Potential Drug for Autoimmune [237]Encephalomyelitis by Inhibiting High-Mobility Group Box .Luteolin also inhibits mast cells, as well as mast cell-dependent T cell activation, recently implicated in MS pathogenesis. and inhibit encephalomyelitis . Piperine alleviates encephalomyelitis in mice by targeting dihydroorotate dehydrogenase.Epigallocatechin-3-Gallate, Hesperidin,Quercetin, ameliorates Encephalomyelitis by Blocking IL-12 Signaling Through JAK-STAT Pathway in T Lymphocyte .[238]

[237] Natarajan C, Bright JJ. Curcumin inhibits experimental allergic encephalomyelitis by blocking IL-12 signaling through Janus kinase-STAT pathway in T lymphocytes. J Immunol. 2002 Jun 15;168(12):6506-13. doi: 10.4049/jimmunol.168.12.6506. PMID: 12055272.

[238] Kempuraj D, Tagen M, Iliopoulou BP, Clemons A, Vasiadi M, Boucher W, House M, Wolfberg A, Theoharides TC. Luteolin inhibits myelin basic protein-induced human mast cell activation and mast cell-dependent stimulation of Jurkat T cells. Br J Pharmacol. 2008 Dec;155(7):1076-84. doi: 10.1038/bjp.2008.356. Epub 2008 Sep 22. PMID: 18806808; PMCID: PMC2597265.

Pathway 61: MANAGING c

A large proportion of patients with COVID-19 requiring hospitalization and/or succumbing to the disease have had diabetes and other chronic conditions as underlying risk factors. In particular, individuals belonging to racial/ethnic minorities in the U.S. and other countries have been significantly and disproportionately impacted. Multiple and complex socioeconomic factors have long played a role in increasing the risk for diabetes and now for COVID-19.

ICU patients with SARS-CoV-2 infection, and no history of diabetes, a substantial number of patients had hyperglycemia, to a higher degree than would be expected by the stress of critical illness, lending credence to reports that speculated a tentative association between SARS-CoV-2 and hyperglycemia .[239]

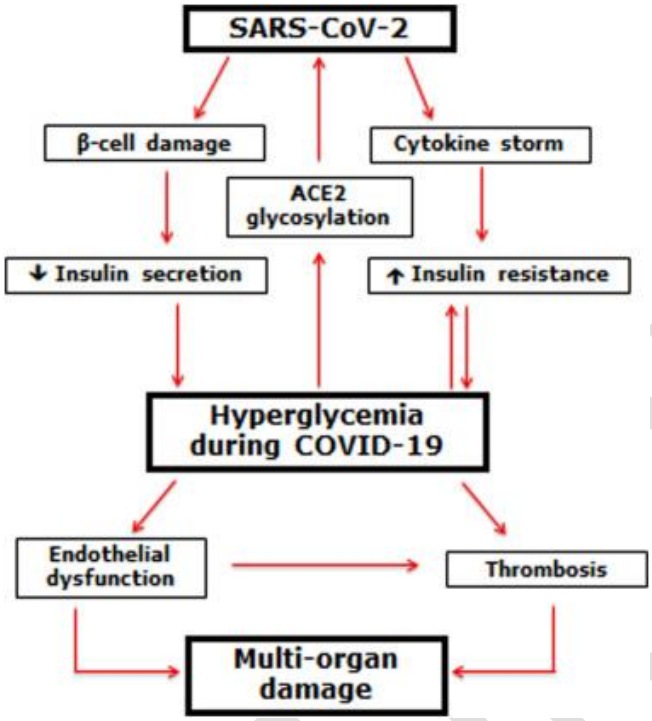

Fig:Hyperglycemia during covid 19

Role of Vedicinal: Phytocompound 1, 2, 3 and 7 help regulate blood sugar level in patients infected by Covid-19. This helps regulate hyperglycaemia which is major problem faced by doctors while treating patients. Baicalin administration attenuates hyperglycemia .Epigallocatechin gallate Ameliorates Hyperglycemia by Promoting the Translocation of Glucose Transporter 4 in the Skeletal Muscle .Luteolin inhibits hyperglycemia-induced proinflammatory cytokine production .Quercetin has Protective effect against hyperglycemia, oxidative stress and DNA damage .[240]

[239] Caballero AE, Ceriello A, Misra A, Aschner P, McDonnell ME, Hassanein M, Ji L, Mbanya JC, Fonseca VA. COVID-19 in people living with diabetes: An international consensus. J Diabetes Complications. 2020 Sep;34(9):107671. doi: 10.1016/j.jdiacomp.2020.107671. Epub 2020 Jul 6. PMID: 32651031; PMCID: PMC7336933.

[240] Ueda M, Nishiumi S, Nagayasu H, Fukuda I, Yoshida K, Ashida H. Epigallocatechin gallate promotes GLUT4 translocation in skeletal muscle. Biochem Biophys Res Commun. 2008 Dec 5;377(1):286-90. doi: 10.1016/j.bbrc.2008.09.128. Epub 2008 Oct 7. PMID: 18845128.

Pathway 62: PROTECTION AGAINST MITOCHONDRIAL DAMAGE

Mechanisms involved in SARS-CoV-2 hijacking of host mitochondria. Schematic showing the SARS-CoV-2 entry into the host cell utilizing angiotensin-converting enzyme carboxypeptidase 2 (ACE2), a polymorphic protein that regulates mitochondrial function. Upon entry into the cells, viral RNA and proteins localize to mitochondria. Post-infection noncoding RNA may also regulate host proteins (such as USP30) involved in mitochondrial dynamics. SARS-2-CoV-2 appears to hijack host mitochondria to suppress host immunity by regulating mitochondrial dynamics, mitochondrial function, and mtDNA release. Hijacking mitochondria may be one of the essential mechanisms leading to COVID-19.

To escape from the immune response and proliferate, viruses strategically modulate cellular metabolism and alter subcellular organelle architecture and functions. This versatile nature of mitochondria defends host cells from viruses through several mechanisms including cellular apoptosis, ROS signalling,[241] MAVS activation and mitochondrial DNA-dependent immune activation. These events are regulated by mitochondrial dynamics, a process by which mitochondria alter their structure (including their length and connectivity) in response to stress or other cues .Altered bioenergetics and mitochondrial dysfunction, had a reduced basal and maximal respiration, reduced spare respiratory capacity, and decreased proton leak in Covid patients .[242]

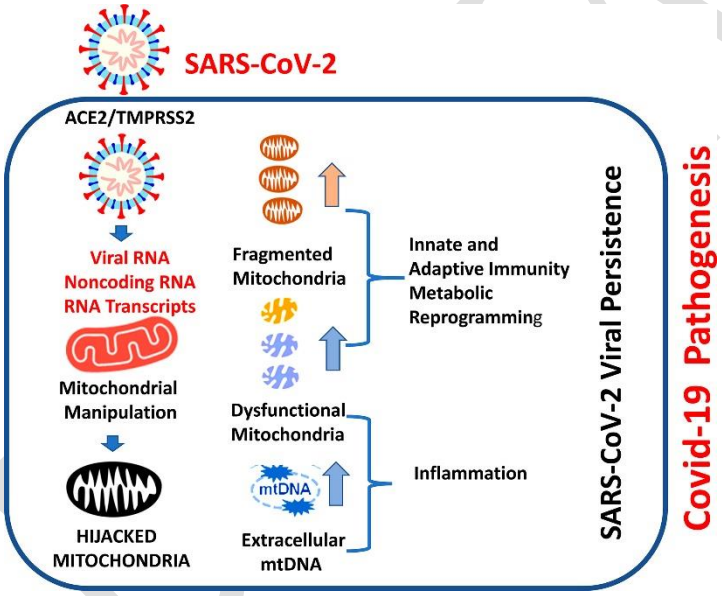

Fig: MITOCHONDRIAL DADAMAGE

Role of Vedicinal:Role of Vedicinal: Mitochondria are the powerhouses of the cell and are largely involved in maintaining cell immunity, homeostasis, and cell survival/death.Phytocompound 1, 2, 3, 5, 6, 7 and 9 impede the mitochondrial damage by Viral RNA which stops inflammation. Epigallocatechin 3-gallate accumulates in mitochondria and displays a selective antiapoptotic effect against inducers of mitochondrial oxidative stress .Luteolin decreases mitochondrial damage-associated inflammation in human .Glycyrrhizin shows protective effects against hypoxia/reoxygenation-induced human coronary artery endothelial cell damage by regulating mitochondria .Quercetin is now recognized as a phytochemical that can modulate pathways associated with mitochondrial biogenesis,

[241] Decoding SARS-CoV-2 hijacking of host mitochondria in COVID-19 pathogenesisKeshav K. Singh, Gyaneshwer Chaubey, Jake Y. Chen, and Prashanth SuravajhalaAmerican Journal of Physiology-Cell Physiology 2020 319:2, C258-C267

[242] Srinivasan S, Guha M, Kashina A, Avadhani NG. Mitochondrial dysfunction and mitochondrial dynamics-The cancer connection. *Biochim Biophys Acta Bioenerg*. 2017;1858(8):602-614. doi:10.1016/j.bbabio.2017.01.004

mitochondrial membrane potential, oxidative respiration and ATP anabolism, intra-mitochondrial redox status, and inhibition of mitochondria-induced apoptosis .Curcumin, Rutin plays critical role in regulating mitochondrial metabolism .[243],[244]

Pathway 63 : PROTECTING AND RESTORING TIGHT JUNCTIONS (INTESTINAL AND BBB)

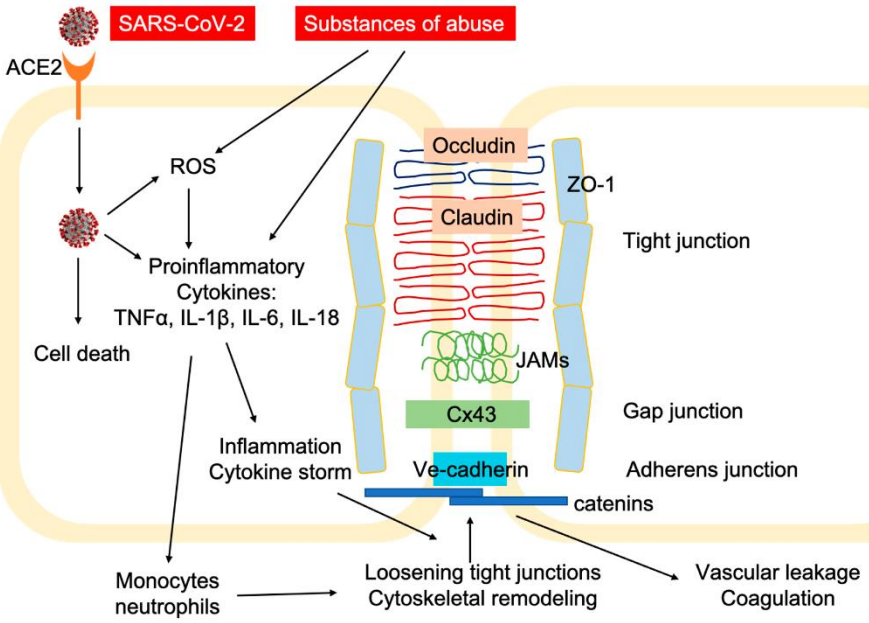

Fig: TIGHT JUNCTIONS (INTESTINAL AND BBB)

The envelope (E) protein a type 1 transmembrane protein that is able to form pentamers by associating with other E proteins to form a membrane pore that is able to transport ions. The pore structure is called viroporin and it is present in many common viruses. The E protein localizes mainly in the ER and Golgi apparatus, where it participates in assembly, budding, and intracellular trafficking of newly formed virions. On the other hand, the E protein PDZ-Binding Motif (PBM) interacts with PDZ domains of host proteins. The interactions of the E protein and lin-7 protein 1 (PALS1), a PDZ-containing protein, disrupts tight junctions in the lungs to reach the alveolar wall and develops into a systemic infection.

This interaction with syntenin caused it to concentrate in the cytoplasm, triggering an overexpression of inflammatory cytokines, which activates an exaggerated immune response, resulting in lung tissue damage, edema accumulation, and leading to acute respiratory distress syndrome. Interaction between the E protein and the Bcl-xL protein caused

[243] Tang Q, Cao Y, Xiong W, Ke X, Zhang J, Xia Y, Liu D. Glycyrrhizic acid exerts protective effects against hypoxia/reoxygenation-induced human coronary artery endothelial cell damage by regulating mitochondria. Exp Ther Med. 2020 Jul;20(1):335-342. doi: 10.3892/etm.2020.8668. Epub 2020 Apr 15. PMID: 32509013; PMCID: PMC7271712.

[244] Asieh Karimani, Mohammad Heidarpour & Amir Moghaddam Jafari (2019) Protective effects of glycyrrhizin on sub-chronic diazinon-induced biochemical, hematological alterations and oxidative stress indices in male Wistar rats, Drug and Chemical Toxicology, 42:3, 300-308, DOI: 10.1080/01480545.2018.1497053

lymphopenia [245]flavonoids have been reported to exhibit promotive and protective effects on intestinal tight junction barrier functions .

Role of Vedicinal: Phytocompound 1, 2, 3, 5, 6, 7, 8 and 9 acts on restoration of tight junction between cells which are disrupted due to cytoskeletal remodelling during CoVs infection. Curcumin prevents leptin-induced tight junction dysfunction in intestinal .Baicalin, Epigallocatechin gallate increasing the expression of tight junction proteins in cells). Hesperidin can improve the expression of tight junction proteins and intestinal permeability, as well as increases the Treg population. Disruption of the tight junctions ZO-1 and occludin was also prevented by luteolin treatment .Quercetin enhances barrier function via transcriptional expression regulation of the TJ protein claudin-4, which represents an important protective effect of this food component against barrier disturbance in intestinal inflammation .Piperine, a functional food alkaloid, exhibits inhibitory potential against TNBS-induced colitis via the inhibition of IκB-α/NF-κB and induces tight junction protein [246].

Pathway 64: SENOLYTICS

Senolytics are a class of drugs that selectively clear senescent cells (SC). Most lethal SC are resistant to apoptosis and have up-regulation of anti-apoptotic pathways which protect SC against their own inflammatory senescence-linked secretory phenotype (SASP), allowing them to survive, despite killing neighbouring cells. Senolytics transiently disable these SCAPs, causing apoptosis of those SC with a tissue-destructive SASP. Because SC take weeks to reaccumulate, senolytics can be administered intermittently – a 'hit-and-run' approach. In preclinical models, senolytics delay, prevent or alleviate frailty, cancers and cardiovascular, neuropsychiatric, liver, kidney, musculoskeletal, lung, eye, haematological, metabolic and skin disorders as well as complications of organ transplantation, radiation and cancer treatment. As anticipated for agents targeting the fundamental ageing mechanisms that are 'root cause' contributors to multiple disorders, potential uses of senolytics are protean, potentially alleviating over 40 conditions in preclinical studies, opening a new route for treating age-related dysfunction and diseases. Early pilot trials of senolytics suggest they decrease senescent cells, reduce inflammation and alleviate frailty in humans. Clinical trials for diabetes, idiopathic pulmonary fibrosis, Alzheimer's disease, COVID-19, osteoarthritis, osteoporosis, eye diseases and bone marrow transplant and childhood cancer survivors are underway or beginning. Until such studies are done, it is too early for senolytics to be used outside of clinical trials. [247]

[245] Prates, I., Barbosa, R.J. The Impact of COVID-19 in Brazil: Labour Market and Social Protection Responses. *Ind. J. Labour Econ.* **63,** 31–35 (2020). https://doi.org/10.1007/s41027-020-00252-3

[246] Lee B, Moon KM, Kim CY. Tight Junction in the Intestinal Epithelium: Its Association with Diseases and Regulation by Phytochemicals. *J Immunol Res*. 2018;2018:2645465. Published 2018 Dec 16. doi:10.1155/2018/2645465

[247] Kirkland JL, Tchkonia T. Senolytic drugs: from discovery to translation. J Intern Med. 2020 Nov;288(5):518-536. doi: 10.1111/joim.13141. Epub 2020 Aug 4. PMID: 32686219; PMCID: PMC7405395.

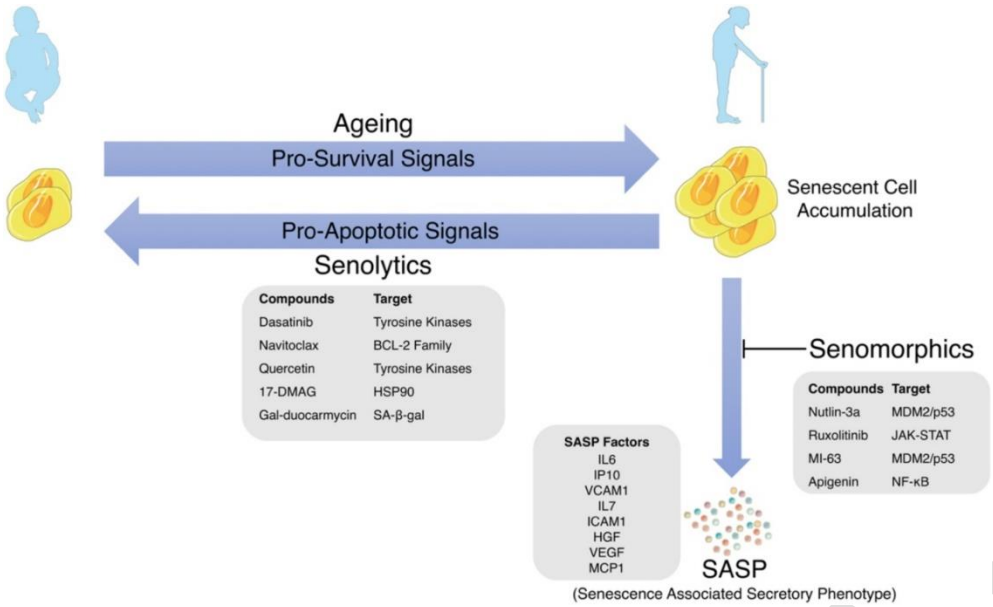

Fig:Senolytics Function

Cellular senescence is one such mechanism, which results in a permanent cell cycle arrest that has been described as a mechanism to limit cancer cell growth. However, recent studies have also suggested a dark side of senescence in which a build-up of senescent cells with age leads to increased inflammation due to a senescence-associated secretory phenotype (SASP). This phenotype that includes many cytokines promotes tumorigenesis and can exhaust the pool of immune cells in the body. Studies clearing senescent cells from mice using the p16-based transgene INK-ATTAC have shown that senescent cells can impact both organismal aging and lifespan. Here we discuss these advances that have resulted in the development of a whole new class of compounds known as senolytics, some of which are currently undergoing clinical trials in humans for treating a variety of age-related pathologies such as osteoarthritis.

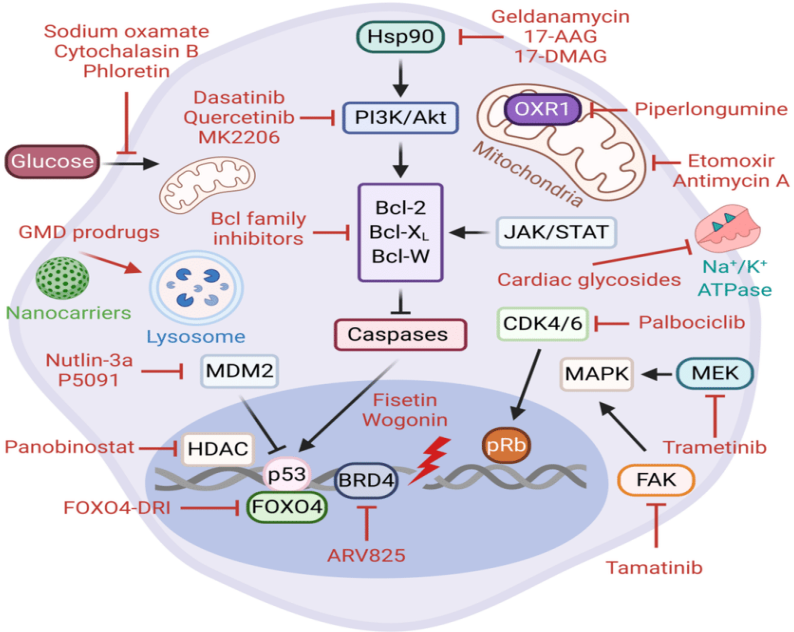

Fig: Senolytics mainly target the Senescent Cell Anti-apoptotic Pathways

To examine the selectivity of the compounds, confluent, non-senescent wild-type MEFs and senescent *Ercc1* $^{-/-}$ MEFs were treated with four different concentrations of each drug for 48 h and their viability measured using a CellTox Green assay. All but two drugs had the same or higher toxicity to non-senescent cells at the concentrations used . Plotting the viability of confluent, non-senescent cells vs. senescent cells after drug treatment clearly shows that only two drugs, geldanamycin and 17-AAG (tanespimycin), were able to reduce the viability of senescent cells specifically at a concentration of 1 μM without significantly affecting the viability of healthy cells . Both of these drugs are N-terminal ansamycin-derived heat shock protein (HSP90) inhibitors. HSP90 is a ubiquitously expressed molecular chaperone, which plays an important role in protein stabilization and degradation. It is upregulated in many cancers, stabilizing otherwise unstable oncogenic drivers such as mutant EGFR, mutant BRAF, wild-type and mutant HER2, as well as certain anti-apoptotic factors. In addition to geldanamycin, a benzoquinone ansamycin antibiotic original discovered in the bacterium *Streptomyces hygroscopicus*, and its first synthetic derivate 17 AAD, an improved, more water soluble geldanamycin-derived HSP90 inhibitor 17-DMAG (alvespimycin) also has been tested in clinical trials. We used 17-DMAG for all subsequent studies as it showed a very promising profile with an almost 10-fold lower EC_{50} values on senescent cells compared to overall cell death. [248],[249]

COVID-19, also known as SARS-CoV-2, is a new emerging zoonotic corona virus of the SARS (Severe Acute Respiratory Syndrome) and the MERS (Middle East Respiratory Syndrome) family. COVID-19 originated in China and spread world-wide, resulting in the pandemic of 2020. For some reason, COVID-19 shows a considerably higher mortality rate in patients with advanced chronological age. This begs the question as to whether there is a functional association between COVID-19 infection and the process of chronological aging. Two host receptors have been proposed for COVID-19. One is CD26 and the other is ACE-2 (angiotensin-converting enzyme 2). Interestingly, both CD26 and the angiotensin system show associations with senescence.

Role of Vedicinals:

Quercetin has been reported to act as a senolytic by selectively removing senescent endothelial cells, and thus it would seem quercetin could revolutionize the field of gerontology. However, given quercetin's narrow therapeutic index reported in work done with human umbilical vein endothelial cells (HUVECs), we hypothesized that quercetin is not innocuous for non-senescent adult human vascular endothelial cells at concentrations that have been reported to be safe for proliferating HUVECs. Furthermore, we investigated quercetin 3-D-galactoside (Q3G; hyperoside), an inactive

[248] Trepel, J., Mollapour, M., Giaccone, G. & Neckers, L. Targeting the dynamic HSP90 complex in cancer. *Nat. Rev. Cancer.* **10**, 537–549 (2010).

[249] Fuhrmann-Stroissnigg, H., Ling, Y.Y., Zhao, J. *et al.* Identification of HSP90 inhibitors as a novel class of senolytics. *Nat Commun* **8,** 422 (2017). https://doi.org/10.1038/s41467-017-00314-z

quercetin derivative that needs to be cleaved by beta-galactosidase overexpressed in senescent cells to release quercetin, as a potential safer senolytic. [250],[251]

Baicalin, a natural flavonoid glycoside, elicited human colon cancer cell senescence by activating RAS/Raf/MEK/ERK signaling via enhanced combination of hypoxia-response gene decidual protein induced by progesterone (DEPP) and RAS [73]. P53 and p16 are crucial molecules involved in senescence induction. P53 activation was required for resveratrol to upregulate C-X-C motif chemokine receptor 2 (CXCR2) to drive cancer cell senescence and protect the cells from apoptosis. [252]

Several senolytic compounds, especially dietary plant metabolites that activate the cytoprotective NRF2 (nuclear factor erythroid derived 2-related factor 2) pathway, which is involved in complex cytoprotective responses, have been shown to target senescent cells. In this study, we have performed a systematic review of in vitro and in vivo effects of selected NRF2-interacting phytocompounds: quercetin, fisetin, hesperidin, epicatechin, metformin and resveratrol on senescent cells and evaluated their prospective utilization in gerotherapeutics.[253]

Epigallocatechin gallate (EGCG), anthocyanidin, resveratrol, phloretin, spermidine, butyrate, and *β*-hydroxybutyrate with regard to their effect on *SIRT3* via *NRF2* and modulation of the proinflammatory senescence-associated secretory phenotype (SASP) in senescence induced 3T3-L1 preadipocytes. [254]

[250] Hwang HV, Tran DT, Rebuffatti MN, Li CS, Knowlton AA. Investigation of quercetin and hyperoside as senolytics in adult human endothelial cells. *PLoS One*. 2018;13(1):e0190374. Published 2018 Jan 9. doi:10.1371/journal.pone.0190374

[251] Harwood M, Danielewska-Nikiel B, Borzelleca JF, Flamm GW, Williams GM, Lines TC. A critical review of the data related to the safety of quercetin and lack of evidence of in vivo toxicity, including lack of genotoxic/carcinogenic properties. *Food and Chemical Toxicology*. 2007;45(11):2179–205. 10.1016/j.fct.2007.05.015. 10.1016/j.fct.2007.05.015 [PubMed] [CrossRef] [CrossRef] [Google Scholar]

[252] Wang, Z.; Ma, L. Baicalin induces cellular senescence in human colon cancer cells via upregulation of DEPP and the activation of Ras/Raf/MEK/ERK signaling. Cell. Death Dis. 2018, 9, 217. [CrossRef] [PubMed]

[253] Salekeen, Rahagir & Habib, Ahsan & Ashraf, Ayesha. (2020). Senolytic Activity of Selected NRF2-Interacting Natural Compounds. Salekeen, Rahagir & Habib, Ahsan & Ashraf, Ayesha. (2020). Senolytic Activity of Selected NRF2-Interacting Natural Compounds

[254] Lilja S, Oldenburg J, Pointner A, Dewald L, Lerch M, Hippe B, Switzeny O, Haslberger A. Epigallocatechin Gallate Effectively Affects Senescence and Anti-SASP via *SIRT3* in 3T3-L1 Preadipocytes in Comparison with Other Bioactive Substances. Oxid Med Cell Longev. 2020 Oct 21;2020:4793125. doi: 10.1155/2020/4793125. PMID: 33149809; PMCID: PMC7603628.

Pathway 65: PROTECTING & TREATMENT OF AUTO-IMMUNE SYSTEM

Autoimmunity, the state in which the immune system reacts against the body's own normal components, producing disease or functional changes.

The human immune system performs a surveillance function, identifying and disposing of antigens—materials such as toxins or infectious microbes that it recognizes as foreign. This surveillance is carried out mostly by the white blood cells called lymphocytes, which recognize foreign antigens and either attack them directly or produce antibodies against them. With a vast diversity of antigen-fighting agents in constant circulation, some are inevitably produced that would react to self antigens—healthy cells or harmless substances of the body that the immune system treats as if they were foreign. Normally, lymphocytes that would trigger immune reactions to the body's own tissues are eliminated before they mature. How this occurs is not completely understood. There is evidence that self-reactive T lymphocytes, or T cells, are killed in the thymus, whereas B lymphocytes, or B cells, that would produce auto antibodies are prevented from maturing after they leave the bone marrow. For reasons that are little understood, the elimination process sometimes fails, producing autoimmune disorders or diseases. [255]

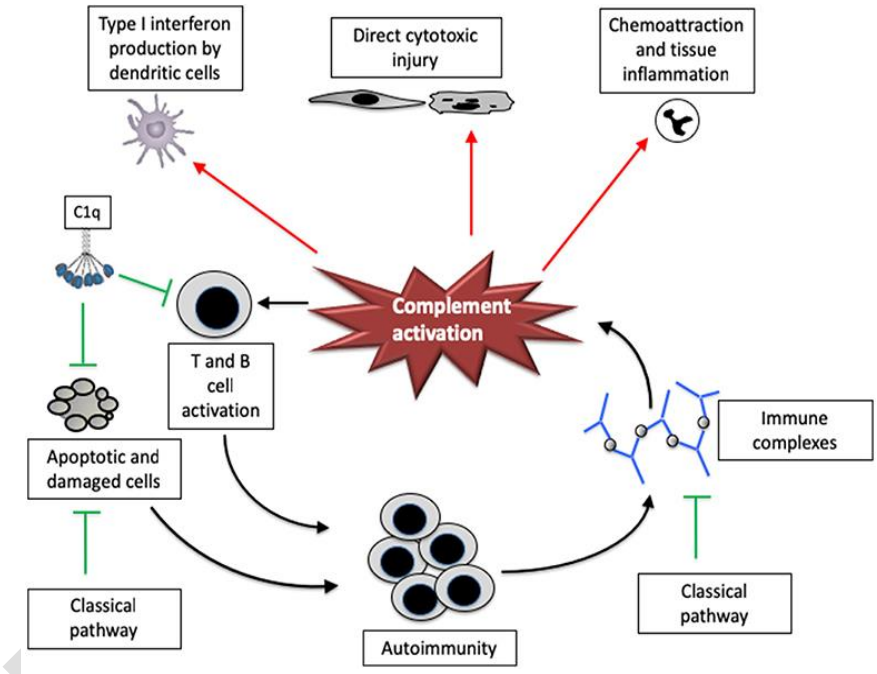

Fig: Autoimmune condition

[255] Britannica, The Editors of Encyclopaedia. "Autoimmunity". *Encyclopedia Britannica*, 20 Nov. 2018, https://www.britannica.com/science/autoimmunity. Accessed 30 May 2021

Insulin-like growth factor binding proteins (IGFBPs) are a group of secreted proteins which serve as transport proteins for insulin-like growth factors (IGFs) with high affinity, regulating the bioavailability and function of IGFs. The IGFBP family consists of six IGFBPs, namely IGFBP1 through IGFBP6, however other proteins with low binding affinity to IGFs were incorrectly named as IGFBP7, IGFBP8, IGFBP9 etc., a consequence of belonging to the IGFBP-related protein (IGFBP-rPs) family . Due to the conserved protein structure and high binding affinity to IGFs, only IGFBP1 through IGFBP6 are considered true IGFBPs. The eponymous function of IGFBPs is achieved through binding to IGFs thus regulating their biological activity; however, in pathological conditions, especially under cancer status, the role of IGFBPs in IGF-independent pathways has prompted increasing attention (3). In autoimmune diseases, IGFBPs are also known to play a role in the development of diseases both through IGF-dependent and IGF-independent pathologies . In this review, we focus on the six true IGFBPs and their roles. [256]

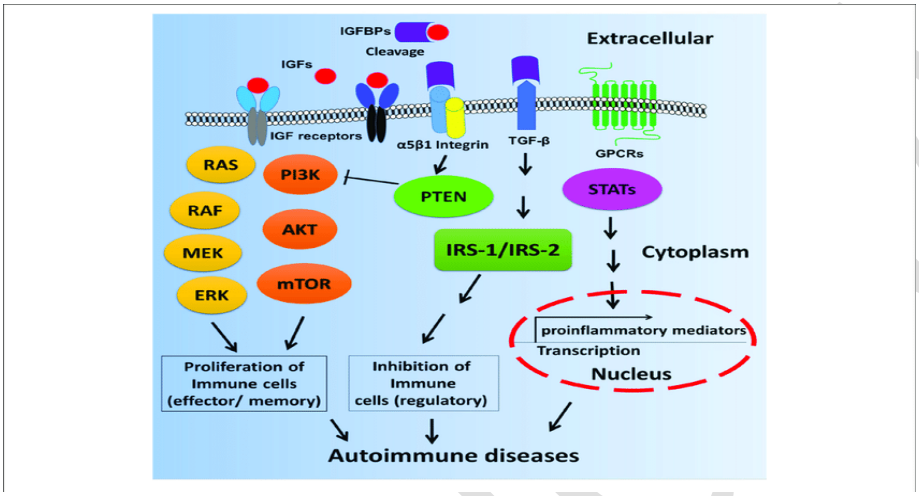

Fig: Pathway involved in autoimmune condition

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of COVID-19, which has affected more than 6 million people worldwide causing more than 400 000 deaths. The disease affects predominantly the upper and lower respiratory tracts causing severe pulmonary disease which often evolves to a multiorgan systemic disease.This is evidenced by thromboembolic lesions of the heart and lungs, pulmonary haemorrhage, muscle weakness, hyperbilirubinaemia and lymphopenia suggesting that COVID-19 affects epithelial barriers, endothelial cells, coagulation, fibrinolysis and the immune system. In patients who are severely ill, innate immune hyperactivity causes a cytokine storm which disturbs microcirculation resulting in shock and acute respiratory distress syndrome. Systemic

[256] Ding, Huihua & Wu, Tianfu. (2018). Insulin-Like Growth Factor Binding Proteins in Autoimmune Diseases. Frontiers in Endocrinology. 9. 10.3389/fendo.2018.00499.

disease perpetuation may be due to the virus itself, infecting cells via ACE2 receptor or, following the cytokine storm, due to autoinflammatory and/or autoimmune mechanisms.[257],[258]

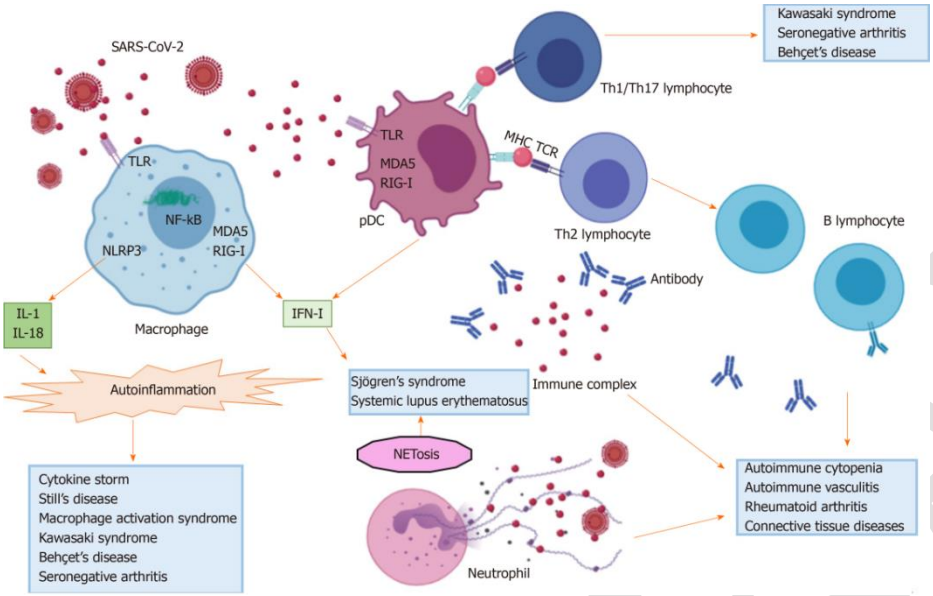

Fig :Autoimmunity as the comet tail of COVID-19 pandemic

[257] Lodigiani C, Iapichino G, Carenzo L, Cecconi M, Ferrazzi P, Sebastian T, Kucher N, Studt JD, Sacco C, Bertuzzi A, Sandri MT, Barco S; Humanitas COVID-19 Task Force. Venous and arterial thromboembolic complications in COVID-19 patients admitted to an academic hospital in Milan, Italy. Thromb Res. 2020 Jul;191:9-14. doi: 10.1016/j.thromres.2020.04.024. Epub 2020 Apr 23. PMID: 32353746; PMCID: PMC7177070.

[258] Vlachoyiannopoulos PG, Magira E, Alexopoulos H*, et al*
Autoantibodies related to systemic autoimmune rheumatic diseases in severely ill patients with COVID-19*Annals of the Rheumatic Diseases* 2020;**79:**1661-1663.

Role of Vedicinals:

Therapeutic effect of baicalin on experimental autoimmune encephalomyelitis is mediated by SOCS3 regulatory pathway.

Baicalin effectively ameliorates clinical Experimental Autoimmune Encephalomyelitis(EAE)

Baicalin Treatment Reduces CNS Inflammation.

Baicalin exerts Therapeutic Effects on EAE by Suppressing Th1 and Th17 Cell Development. [259]

Quercetin exerts inflammation and immune modulating activity in several murine models of autoimmunity. In vivo, animal experiments also support an anti-inflammatory effect. Quercetin ameliorates the inflammatory response induced by carrageenan and a high-fat diet . Quercetin reduced visceral adipose tissue TNF-α and nitric oxide production and downregulated nitric oxide synthase (NOS) expression in obese Zucker rats . In chronic rat adjuvant induced arthritis, quercetin decreased clinical signs of arthritis compared to untreated controls. [260]

Quercetin, a flavonoid class of polyphenolic compound was tested for its beneficial effect to reduce oxidative stress and inflammation in sarcoidosis. A double-blind randomized placebo controlled clinical trial on 18 non-treated sarcoidosis patients was conducted. Quercetin at a dose of 4×500mg was administered within 24h. Quercetin treatment was increased total plasma antioxidant capacity. The oxidative and inflammatory markers (malondialdehyde, TNFα/IL-10 and IL-8/IL-10) were also downregulated in sarcoidosis patients after curcumin treatment. Quercetin suppressed the phosphorylation of Akt by direct binding and inhibition of PI3K in JB6 mouse epidermal cells . Silibinin, applied topically to inflamed murine ears suppressed the expression of pro-inflammatory cytokines and COX-2 by PI3K/Akt inhibition . EGCG inhibited epithelial-mesenchymal transition and inflammation via the PI3K/AKT pathway by upregulating the expression of phosphatase and tensin homolog (PTEN). [261,262]

[259] Zhang, Y., Li, X., Ciric, B. *et al.* Therapeutic effect of baicalin on experimental autoimmune encephalomyelitis is mediated by SOCS3 regulatory pathway. *Sci Rep* **5,** 17407 (2015). https://doi.org/10.1038/srep17407.

[260] Li Y, Yao J, Han C, et al. Quercetin, Inflammation and Immunity. *Nutrients*. 2016;8(3):167. Published 2016 Mar 15. doi:10.3390/nu8030167

[261] Khan H, Sureda A, Belwal T, et al. Polyphenols in the treatment of autoimmune diseases. *Autoimmun Rev*. 2019;18(7):647-657. doi:10.1016/j.autrev.2019.05.001

[262] Busch F, Mobasheri A, Shayan P, Lueders C, Stahlmann R, Shakibaei M. Resveratrol modulates interleukin-1β-induced phosphatidylinositol 3-kinase and nuclear factor κB signaling pathways in human tenocytes. *Journal of Biological Chemistry* 2012;287:38050–63. [PMC free article] [PubMed] [Google Scholar] [Ref list]

Luteolin with or without IFN-β, could be helpful in MS by not only inhibiting PBMC release of cytokines, but also by inhibiting T cells, which we recently showed can be superstimulated by mast cells, an action also inhibited by luteolin . In addition to T cells, recent evidence implicates also TH2 processes typically associated with allergic reactions , which involve mast cells (Fig. 1. In fact, mast cells have been considered as the next target for MS therapy. [263]

Overactivation of neutrophils is associated with excess degranulation, which was obviously seen in this study, as administration of PMA to isolated human peripheral blood neutrophils significantly increased NO and TNF-α productions and MPO activity. Our results showed that pre-incubation of neutrophils with rutin significantly reduced the productions/ activity of these factors. Rutin can effectively inhibit lipopolysaccharide (LPS)-induced iNOs gene expression and subsequent NO production in macrophages . We obtained a similar result in our study as rutin significantly reduced NO from neutrophils in vitro. The mechanism underlying this result in neutrophils may be similar to that in the mentioned studies on macrophages. Rutin has been found to be able to attenuate the LPS-induced increased serum systemic TNF-α level in D-galactosamine-sensitized mice. [264]

Curcumin inhibits these autoimmune diseases by regulating inflammatory cytokines such as IL-1beta, IL-6, IL-12, TNF-alpha and IFN-gamma and associated JAK-STAT, AP-1, and NF-kappaB signaling pathways in immune cells. [265]

EGCG inhibited CD4(+) T cell expansion in response to either polyclonal or antigen specific stimulation. We then determined how EGCG affects naïve CD4(+) T cell differentiation and found that it impeded Th1 and Th17 differentiation and prevented IL-6-induced inhibition on Treg development. Further demonstrated that EGCG inhibited Th1 and Th17 differentiation by downregulating their corresponding transcription factors (STAT1 and T-bet for Th1, and STAT3 and RORγt for Th17). These effects provide further explanation for previous findings that administration of EGCG by gavage to experimental autoimmune encephalomyelitis (EAE) mice, an animal model for human multiple sclerosis (MS), reduced the clinical symptoms, brain pathology, and proliferation and TNF-α production of encephalitogenic T cells. [266]

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[263] Kempuraj D, Tagen M, Iliopoulou BP, Clemons A, Vasiadi M, Boucher W, House M, Wolfberg A, Theoharides TC: Luteolin inhibits myelin basic protein-induced human mast cell activation and mast cell dependent stimulation of Jurkat T cells. Br J Pharmacol. 2008, 155: 1076-1084. 10.1038/bjp.2008.356.

[264] Nikfarjam BA, Adineh M, Hajiali F, Nassiri-Asl M. Treatment with Rutin - A Therapeutic Strategy for Neutrophil-Mediated Inflammatory and Autoimmune Diseases: - Anti-inflammatory Effects of Rutin on Neutrophils. *J Pharmacopuncture*. 2017;20(1):52-56. doi:10.3831/KPI.2017.20.003

[265] Bright JJ. Curcumin and autoimmune disease. Adv Exp Med Biol. 2007;595:425-51. doi: 10.1007/978-0-387-46401-5_19. PMID: 17569223.

[266] Wu D, Wang J, Pae M, Meydani SN. Green tea EGCG, T cells, and T cell-mediated autoimmune diseases. Mol Aspects Med. 2012 Feb;33(1):107-18. doi: 10.1016/j.mam.2011.10.001. Epub 2011 Oct 14. PMID: 22020144.

Piperine has anti-apoptotic and neuroprotective effect in EAE through reducing caspase-3 (apoptosis marker) and enhancing BDNF and NeuN expressing cells. This study strongly indicates that piperine has a beneficial effect on the EAE progression and could be considered as a potential therapeutic target for MS treatment.[267]

Autoimmune encephalomyelitis is a chronic autoimmune disease caused by immune-mediated sterile inflammatory response and demyelination in the central nervous system (CNS). High-mobility group box protein 1 (HMGB1) is a ubiquitous nuclear protein, which can be released from damaged cells and induce proinflammatory responses in autoimmune encephalomyelitis. Glycyrrhizin (GL), a major constituent of licorice root, can inhibit the proinflammatory bioactivities of HMGB1. [268]

[267] Reza Nasrnezhad, Sohrab Halalkhor, Farzin Sadeghi et al. Piperine Improves Experimental Autoimmune Encephalomyelitis (EAE) in Lewis Rats through its Neuroprotective, Anti-inflammatory and Anti-Oxidant Effects, 30 April 2021, PREPRINT (Version 1) available at Research Square [https://doi.org/10.21203/rs.3.rs-461934/v1]

[268] Li J, Shi J, Sun Y, Zheng F. Glycyrrhizin, a Potential Drug for Autoimmune Encephalomyelitis by Inhibiting High-Mobility Group Box 1. DNA Cell Biol. 2018 Dec;37(12):941-946. doi: 10.1089/dna.2018.4444. Epub 2018 Oct 16. PMID: 30325653.

Chapter 4: Treatment of Coinfection

Pathway 66: TREATMENT OF MALARIA CO-INFECTION WITH VEDICINAL 9

Plasmodium is a genus of parasites belonging to the family Plasmodiidae, order Haemosporidia and phylum Apicomplexa. The genus *Plasmodium* is subdivided into 10 subgenera. Malaria parasites in humans are all classified in the subgenera *Plasmodium* and *Laverania*. Four are well-characterized, strict human pathogens (e.g. *P. falciparum, P. vivax, P. ovale, P. malariae*), and one (*P. knowlesi*) is a recently identified human pathogen.[269]

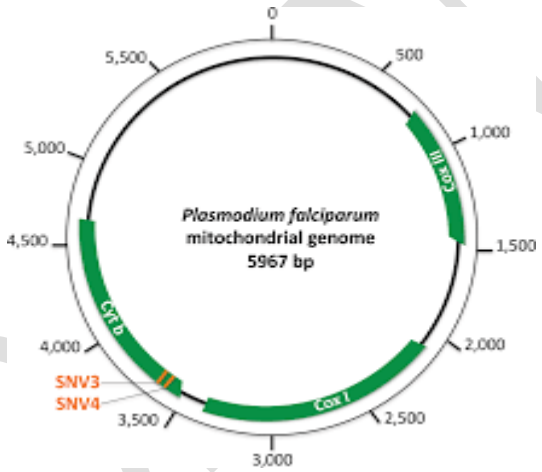

Fig: Genome Structure Of *P. falciparum*.

Malaria is a protozoan infection of the red blood cells, transmitted by the bite of a female anopheles mosquito. Malaria is caused by the protozoa of the genus *Plasmodium*. There are four species that infect humans: *P. vivax, P. ovale, P. malariae*, and *P. falciparum*. While widespread throughout the tropics, malaria has been virtually eliminated from temperate climates. Malaria is characterized by paroxysms of fever, the length of which depends on the

[269] Sato, S. *Plasmodium*—a brief introduction to the parasites causing human malaria and their basic biology. *J Physiol Anthropol* **40,** 1 (2021). https://doi.org/10.1186/s40101-020-00251

particular *Plasmodium* species that is the causative organism. Serious consequences, including death, can result, especially after infection with *P. falciparum.* [270]

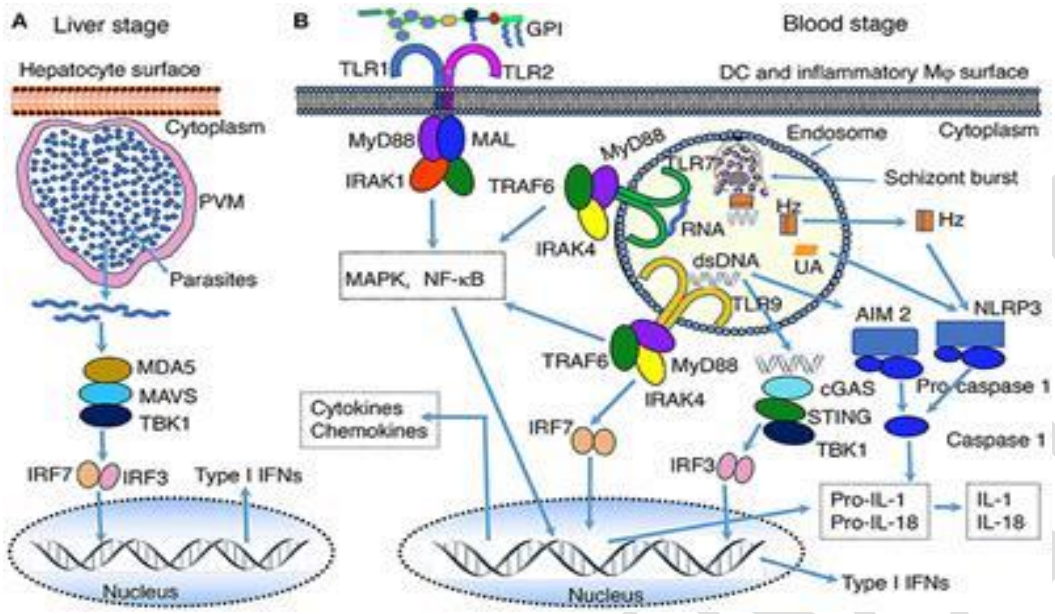

Fig2 : AMP-PRR interaction-induced signaling pathways.

(A) RNA of the liver stage parasites growing inside hepatocytes is recognized by MDA5 leading to the activation of MAVS-TBK1-IRF3/IRF7 signaling and downstream production of type I IFNs.

(B) At the blood stage infection, parasite DNA, RNA and GPI interact with, respectively, TLR9, TLR7, and TLR2, leading to the activation of primarily MAPK and NF-κB signaling pathways and downstream cytokine and chemokine responses. In the cytosol, similar to the liver stage parasite RNA, the blood stage parasite RNA is sensed by MDA5 leading to the activation of MAVS-TBK1-IRF3/IRF7 signaling (see A). However, this signaling seems induce the expression of SOCS1, which downregulate RNA-TLR7-induced type I IFN production (81). Parasite DNA in the cytosol is sensed by cGAS, resulting in the activation of STING-TBK1-IRF3 signaling and type I IFN response. Parasite DNA also activates AIM2 inflammasome, which cleaves pro-caspase 1 to activate caspase 1. Hemozoin (Hz) and uric acid (UA) induce danger signaling, activating NLRP3 inflammasome and the cleavage of pro-caspase 1 to activate caspase 1. Parasites have also been reported to activate NLRP12 inflammasome through unidentified interaction, leading to the cleavage of pro-caspase 1 to activate caspase 1 (44, 82). It appears that microparticles released from IRBCs and heme

[270] D. Channe Gowda* and Xianzhu Wu Department of Biochemistry and Molecular Biology, The Pennsylvania State University College of Medicine, Hershey, PA, United States.Front. Immunol., 19 December 2018 | https://doi.org/10.3389/fimmu.2018.03006

produced during infection activate TLR4 signaling (83, 84). Ligands bind to TLR4 homodimer through the cooperation of accessory proteins CD14 and MD2, leading to MAPK, NF-κB and TRIF-TBK1-IRF3 signaling.[271],[272]

While malaria and COVID-19 can have similar presentation, common symptoms they share include but not limited to: fever, breathing difficulties, tiredness and acute onset headache, which may lead to misdiagnosis of malaria for COVID-19 and vice versa, particularly when clinician relies mainly on symptoms.

Studies have revealed that SARS-CoV-2 uses the angiotensin-converting enzyme 2 (ACE2) receptor to enter the host cells . ACE2 is a type I transmembrane amino-peptidase that is mainly anchored at the apical surface of cells of the gastrointestinal system, heart, kidneys, blood vessels and is highly expressed in the heart and type II alveolar cells of the lungs . In addition to the membrane-bound form, there are soluble forms in the plasma and urine. It was first discovered in 2000 as an ACE homologue and shares approximately 42% homology with angiotensin-converting 1 (ACE1). It is capable of producing the lung-protective Ang-(1–7) from angiotensin II (ANG II) and converting angiotensin I to angiotensin(1–9) . If the ACE2 receptor activity underwent downregulation, ANG II-the substrate for ACE2- will then accumulate. Accumulated ANG II will then increase neutrophils aggregation and enhances vascular permeability. An exacerbation of pulmonary oedema and ARDS will eventually ensue. [273]

[271] Gazzinelli RT, Kalantari P, Fitzgerald KA, Golenbock DT. Innate sensing of malaria parasites. *Nat Rev Immunol.* (2014) 14:744–57. doi: 10.1038/nri3742 PubMed Abstract | CrossRef Full Text | Google Scholar

[272] Olivier M, Van Den Ham K, Shio MT, Kassa FA, Fougeray S. Malarial pigment hemozoin and the innate inflammatory response. *Front Immunol.* (2014) 5:25. doi: 10.3389/fimmu.2014.00025PubMed Abstract | CrossRef Full Text | Google Scholar

[273] Zhang H, Baker A. Recombinant human ACE2: acing out angiotensin II in ARDS therapy. Crit Care. 2017;21:305

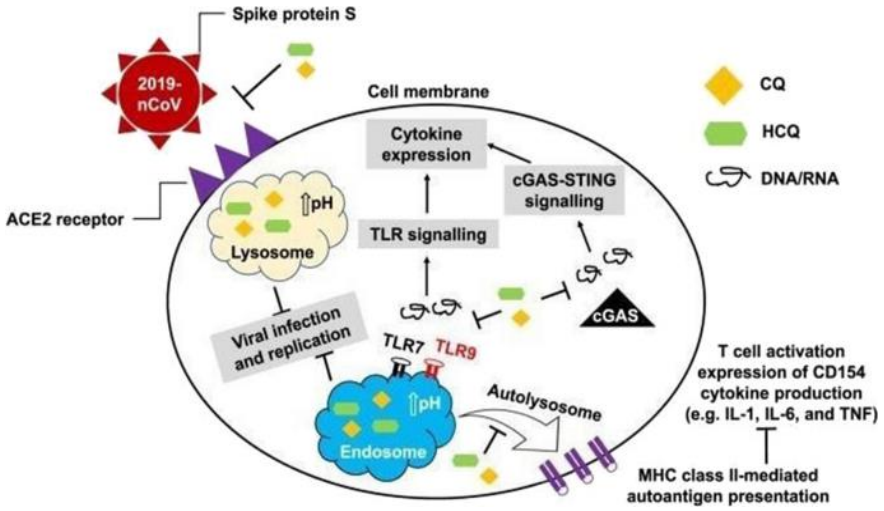

Fig:Malaria and Sars cov 2 Host entry and infection Similarities

Reports that ANG II impairs *Plasmodium* development were initially described in the sexual cycle of *Plasmodium gallinaceum*, an avian malaria parasite. ANG II decreases the buildup of sporozoites in mosquitoes' salivary glands by directly disturbing the parasite membrane . Other studies have illustrated a clear protective effect of Ang II in malaria. The ACE1 enzyme is distinguished by a genetic deletion/insertion polymorphism in intron 16. The presence of this (D/I) polymorphism is linked to changes in the concentration of both circulating and tissue-bound forms of ACE. When the presence of D allele is dominating, this is associated with reduced expression of ACE2 receptor. In a genetic association study, it has been shown that the presence of D-allele of ACEI/D polymorphism, which enhances Angiotensin II production, is associated with a mild pattern of malaria. The reduced expression of ACE2 receptor in populations with this polymorphism may play a protective role against COVID-19. [274]

Role of Vedicinal:

The influence of the polyphenolic flavonoid Baicalein on eryptosis, the suicidal erythrocyte death characterized by cell shrinkage and phosphatidylserine translocation to the cell surface.

In a first step, cell volume was estimated from forward scatter determined in flow cytometry following an incubation of human erythrocytes for 48 h in Ringer solution without or with Baicalein (5–50 µM). As shown in , Baicalein treatment was followed by a decrease of average erythrocyte forward scatter reflecting cell shrinkage, an effect reaching statistical significance at 10 µM Baicalein concentration. The histogram reveals that Baicalein increases forward scatter in a

[274] Silva LS, Silva-Filho JL, Caruso-Neves C, Pinheiro AAS. New concepts in malaria pathogenesis: the role of the renin-angiotensin system. Front Cell Infect Microbiol. 2016;5:103.

subpopulation of erythrocytes. the polyphenolic flavonoid Baicalein stimulates Ca^{2+} entry and ceramide formation thus leading to subsequent erythrocyte shrinkage and erythrocyte cell membrane scrambling. Accordingly, Baicalein stimulates eryptosis, the suicidal erythrocyte death. The concentrations required for those effects are 10–50 µM. [275]

A number of reports have demonstrated growth inhibitory effects of flavonoids, particularly of the flavonol quercetin and of the flavone luteolin, on the protozoan parasite genera *Toxoplasma* , *Trypanosoma* and *Leishmania* . The majority of studies involving flavonoids and malaria describe the antiplasmodial activity-guided fractionation of plants (including species used in traditional medicine). Flavonoids (usually along with other compounds) have been identified in the antiplasmodial fractions of many plants, and in some cases have been shown to possess antiplasmodial activity when isolated.[276]

IC_{50} values of the rutin and swertiamarin via *in vitro* study revealed (9.50 ± 0.29) µg/mL and (8.17 ± 0.17) µg/mL respectively. Whereas, the combination in 1:1 ratio [IC_{50} of (5.51 ± 0.18) µg/mL] showed better antiplasmodial activity against *Plasmodium falciparum. In vivo* results showed that rutin and swertiamarin had chemosuppressant effects in a dose-dependent manner, whereas, combination in 1:1 ratio possessed potential antimalarial activity similar to chloroquine phosphate.

Hesperidin Inhibits Inflammatory Response Induced by *Aeromonas hydrophila* Infection and Alters $CD4^+/CD8^+$ T Cell Ratio . [277]

Among its antiprotozoan activities, curcumin was potent against both chloroquine-sensitive and -resistant *Plasmodium falciparum* strains. Consistent with findings in mammalian cell lines, curcumin's prooxidant activity promoted the production in *P. falciparum* of reactive oxygen species (ROS), whose cytotoxic effect could be antagonized by coincubation with antioxidants and ROS scavengers. Curcumin treatment also resulted in damage of both mitochondrial and nuclear DNA, probably due to the elevation of intracellular ROS. Furthermore, we have demonstrated that curcumin inhibited the histone acetyltransferase (HAT) activity of the recombinant *P. falciparum* general control nonderepressed 5 (PfGCN5) in vitro and reduced nuclear HAT activity of the parasite in culture. [278]

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[275] Bissinger R, Malik A, Honisch S, Warsi J, Jilani K, Lang F. In vitro sensitization of erythrocytes to programmed cell death following baicalein treatment. *Toxins (Basel)*. 2014;6(9):2771-2786. Published 2014 Sep 18. doi:10.3390/toxins6092771

[276] Yenesew A, Induli M, Derese S, Midiwo JO, Heydenreich M, Peter MG, Akala H, Wangui J, Liyala P, Waters NC: Anti-plasmodial flavonoids from the stem bark of *Erythrina abyssinica*. Phytochemistry. 2004, 65 (22): 3029-3032. 10.1016/j.phytochem.2004.08.050.

[277] *Abdelaziz S. A. Abuelsaad, Gamal Allam, Adnan A. A. Al-Solumani, "Hesperidin Inhibits Inflammatory Response Induced by Aeromonas hydrophila Infection and Alters CD4+/CD8+ T Cell Ratio", Mediators of Inflammation, vol. 2014, Article ID 393217, 11 pages, 2014.* https://doi.org/10.1155/2014/393217

[278] Cui L, Miao J, Cui L. Cytotoxic effect of curcumin on malaria parasite Plasmodium falciparum: inhibition of histone acetylation and generation of reactive oxygen species. *Antimicrob Agents Chemother*. 2007;51(2):488-494. doi:10.1128/AAC.01238-06

Epigallocatechin-3-gallate (EGCG) and epicatechin gallate (ECG), strongly inhibit Plasmodium falciparum growth in vitro. Both these catechins are found to potentiate the antimalarial effects of artemisinin without interfering with the folate pathway. [279]

The fruit of *Piper chaba* Hunt. was demonstrated to exhibit promising antimalarial activity against the asexual stage of 3D7 (chloroquine-sensitive) and K1 (chloroquine-resistant) *P. falciparum* clones. The aim of the present study was to further investigate the antimalarial activity of piperine, the major isolated constituent of *Piper chaba* Hunt. fruits against both *P. falciparum* clones. The antimalarial activity was determined using SYBR green-I-based assay and morphological change was observed under the light microscope with Giemsa staining. The median IC_{50} (concentration that inhibits parasite growth by 50%) values of piperine against 3D7 and K1 *P. falciparum* were 111.5 and 59 μM, respectively. A marked change in parasite morphology was observed within 48 hours of piperine exposure. Results of real-time PCR showed no effect of piperine on modulating the expression of the three genes associated with antimalarial drug resistance in *P. falciparum*, *i.e*., *pfcrt*, *pfmdr1*, and *pfmrp1*. Piperine could be a promising candidate for further development as an antimalarial drug based on its antimalarial potency and low risk of resistance development. [280]

Glycyrrhizaglabra were chemically investigated, which resulted in the isolation and characterization of 18β-glycyrrhetinic acid (GA) as a major constituent. The in vitro studies against P. falciparum showed significant (IC50 1.69µg/ml) anti-malarial potential for GA. Similarly, the molecular docking studies showed adequate docking (LibDock) score of 71.18 for GA and 131.15 for standard anti-malarial drug chloroquine. [281]

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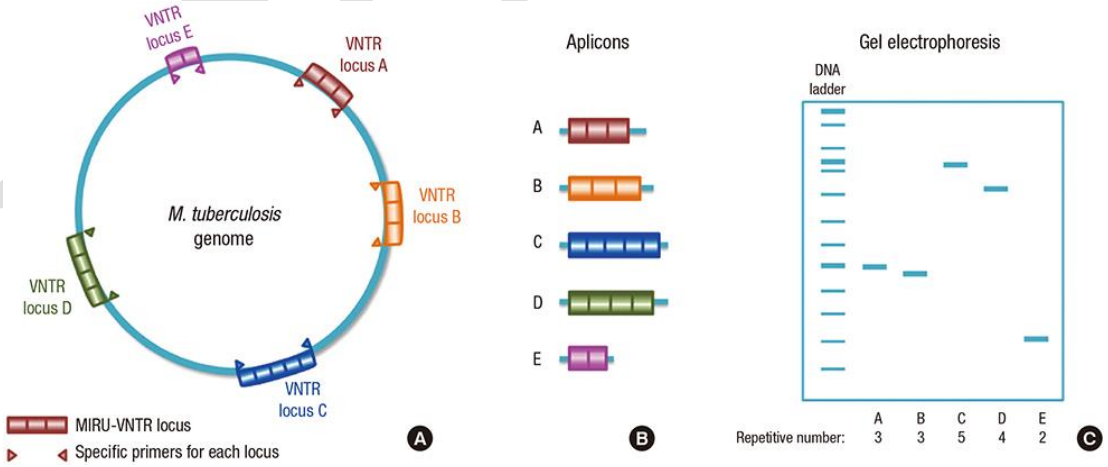
[279] Sannella AR, Messori L, Casini A, Francesco Vincieri F, Bilia AR, Majori G, Severini C. Antimalarial properties of green tea. Biochem Biophys Res Commun. 2007 Feb 2;353(1):177-81. doi: 10.1016/j.bbrc.2006.12.005. Epub 2006 Dec 11. PMID: 17174271

[280] Thiengsusuk A, Muhamad P, Chaijaroenkul W, Na-Bangchang K. Antimalarial Activity of Piperine. J Trop Med. 2018 Dec 6;2018:9486905. doi: 10.1155/2018/9486905. PMID: 30631371; PMCID: PMC6304611.

[281] Kalani, Komal & Agarwal, Jyoti & Alam, Sarfaraz & Khan, Feroz & Pal, Anirban & Srivastava, Santosh. (2013). In Silico and In Vivo Anti-Malarial Studies of 18β Glycyrrhetinic Acid from Glycyrrhiza glabra. PloS one. 8. e74761. 10.1371/journal.pone.0074761.

Pathway 67 : TREATMENT OF MYCOBACTERIUM TUBERCULOSIS CO-INFECTION

Mycobacterium tuberculosis is a human pathogen that has had a staggering global impact. Its origins are ancient. Spinal deformities typical of those resulting from M. tuberculosis disease have been found in human remains as far apart as Peru and Egypt and dating from at least 5000 BCE . The emergence of M. tuberculosis as a human pathogen is not well understood, but it has been plausibly suggested that the domestication of cattle facilitated close contact to humans, resulting in transmission with eventual evolution of M. bovis, the bovine tuberculosis strain. [282]



[282] Elizabeth A. Talbot, Brittany J. Raffa, in Molecular Medical Microbiology (Second Edition),2015

Fig : **Genome structure of M.Tuberculosis**

M. tuberculosis is an intracellular pathogen transmitted via inhalation of aerosolized, bacteria-containing droplets. Innate immune cells in the lungs, primarily macrophages, dendritic cells, monocytes, and neutrophils, readily phagocytose *M. tuberculosis* and are the earliest defenders against the pathogen. Transformation of bacteria-containing phagosomes into acidified, antimicrobial compartments is a central tenet of defense against *M. tuberculosis*. In this regard, the production of IFN-γ, which can activate infected myeloid cells and inhibit bacterial replication, is a well-known antimycobacterial contribution by adaptive immune cells such as CD4 and CD8 T-cells. Despite pressures from host immunity, *M. tuberculosis* is able to persist in the host. *M. tuberculosis* infection results in hallmark lesions called granulomas, which are initially aggregates of infected and uninfected myeloid cells circumscribed by a lymphocytic cuff. The granuloma is thought to prevent bacterial dissemination to extrapulmonary sites but can also become niches for long-term bacterial persistence. [283]

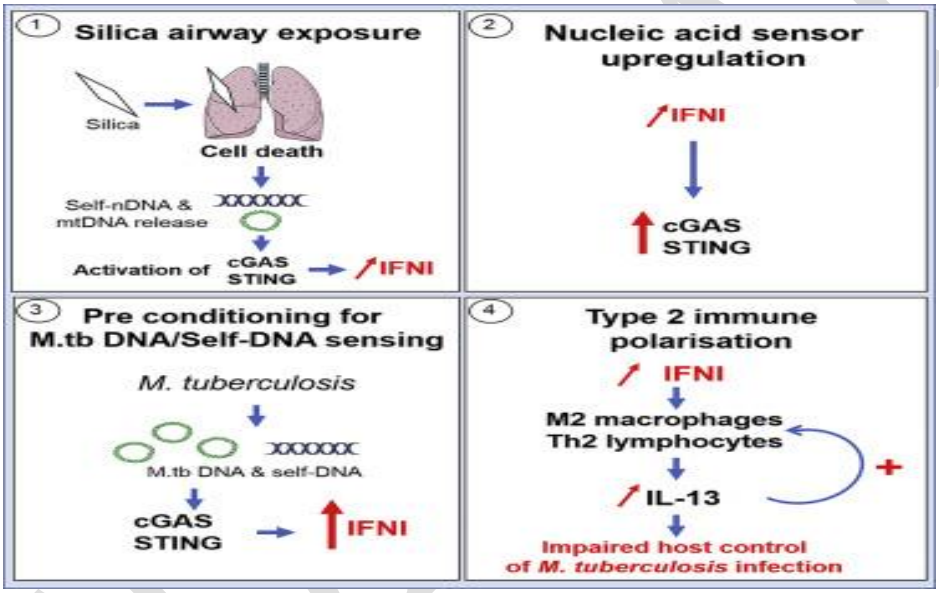


Fig:M.Tuberculosis Infection Cycle

[283] Sia JK, Rengarajan J. Immunology of *Mycobacterium tuberculosis* Infections. *Microbiol Spectr*. 2019;7(4):10.1128/microbiolspec.GPP3-0022-2018. doi:10.1128/microbiolspec.GPP3-0022-2018

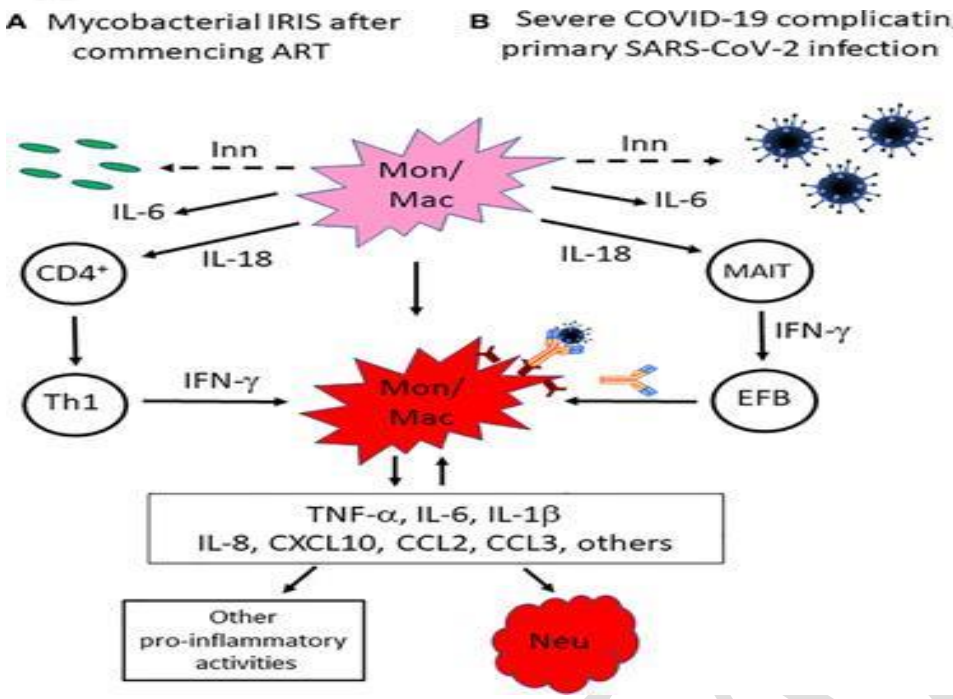


Fig :SARS COV 2 and M Tuberculosis Common Infection Pathway

TB in SARS patients has been reported in several studies from TB endemic countries such as Singapore, China, or Taiwan , all with known TB patients that acquired SARS and in individuals that developed TB after recovery from SARS . The transient immunosuppression characterized both conditions , a reason for poorer IgG antibody response and a delayed viral clearance in coinfected SARS patients . Also, the use of corticoid therapy in SARS added even more on immunosuppression. [284]

When dealing with a possible SARS patient from an endemic TB region, one should never forget TB as a coexisting pathology. In April 2003, a SARS-related hospital screening from Taipei (Taiwan) resulted in discovering 60 TB cases among healthcare workers . Moreover, during the SARS-CoV-1 epidemic from Singapore, SARS cases were reported developing active PTB short after recovering from SARS , data compatible with previous studies on mice regarding the suppression of cellular immunity after a viral infection . There is also data on MERS-CoV augmenting TB by the added immunosuppression and reinforcing the need to evaluate a suspected patient. Since the SARS-CoV-2 is a newly discovered pathogen (first infection being reported in December 2019) , little data about the coinfection with MTB could be found (especially considering the long incubation period of MTB from exposure to developing the disease, often with a slow onset) . Still, the existent studies showed that TB status might play a role in the development of severe acute respiratory syndrome in SARS-CoV-2 coinfection, considering the cases described in China and India . A recent meta-analysis concluded that patients with TB are not more likely to get COVID-19, but TB is associated with a 2.1-

[284] Radu Crisan-Dabija, Cristina Grigorescu, Cristina-Alice Pavel, Bogdan Artene, Iolanda Valentina Popa, Andrei Cernomaz, Alexandru Burlacu, "Tuberculosis and COVID-19: Lessons from the Past Viral Outbreaks and Possible Future Outcomes", Canadian Respiratory Journal, vol. 2020, ArticleID 1401053, 10 pages, 2020. https://doi.org/10.1155/2020/1401053

fold increased risk of severe COVID-19 disease, although the statistical difference was not significant. Moreover, no increased risk for mortality in coincident COVID-19 and TB was found. [285], [286]

Role Of Vedicinal :

Herbal medicine, baicalin, could induce autophagy in macrophage cell line Raw264.7 and caused increased killing of intracellular Mtb. Further, baicalin inhibited Mtb-induced NLRP3 inflammasome activation and subsequent inflammasome-derived IL-1β. To investigate the molecular mechanisms of baicalin, the signaling pathways associated with autophagy were examined. Results indicated that baicalin decreased the levels of phosphorylated protein kinase B (p-Akt) and phosphorylated mammalian target of rapamycin (p-mTOR) at Ser473 and Ser2448, respectively, but did not alter the phosphorylation of p38, JNK, or ERK both in Raw264.7 and primary peritoneal macrophages. Moreover, baicalin exerted an obvious inhibitory effect on nuclear factor-kappa B (NF-κB) activity. [287]

In searching for a biologically safe alternative Kv1.3 inhibitor we explored 3,4,5,7-tetrahydroxyflavone, also known as luteolin, a plant-based flavonoid that inhibits Kv1.3 . Luteolin has recently been employed as a food supplement and is considered safe for human use. It is a flavonoid found in many fruits, vegetables, and medicinal plants such as *Reseda luteola* L., *Achillea millefolium* L. and many others. Luteolin-rich herbal extracts have been used for a long time as traditional herbal remedies . We observed that luteolin had mild bactericidal activity in vitro , as reported . Moreover, we also found that luteolin treatment significantly activated macrophages, as evidenced by increased expression of co-stimulatory molecules and improved bactericidal activity (S2A & S2B). Taken together, these findings suggested that luteolin might have potent immunomodulatory effects, which along with selective enrichment of the T_{CM} pool may be highly beneficial in combating TB. [288]

Hesperidin methyl chalcone alleviates significantly attenuated the granulation in adjacent vertebral bone tissues. The expression of p65, IL-4, IL-10, and MCP-1 was reduced. The NF-κB pathway was suppressed, in which the phosphorylation of IκBα, IKKα/β, and p65 was inhibited whereas the relative level of IκBα was increased. HMC could

[285] S. Yasri and V. Wiwanitkit, "Tuberculosis and novel Wuhan coronavirus infection: pathological interrelationship," *Indian Journal of Tuberculosis*, vol. 67, no. 2, p. 264, 2020.View at: Publisher Site | Google Scholar

[286] Y. Gao, M. Liu, Y. Chen, S. Shi, J. Geng, and J. Tian, "Association between tuberculosis and COVID-19 severity and mortality: a rapid systematic review and meta-analysis," *Journal of Medical Virology*, 2020.View at: Publisher Site | Google Scholar

[287] Zhang, Qingwen & Sun, Jinxia & Wang, Yuli & He, Weigang & Wang, Lixin & Zheng, Yuejuan & Wu, Jing & Zhang, Ying & Jiang, Xin. (2017). Antimycobacterial and Anti-inflammatory Mechanisms of Baicalin via Induced Autophagy in Macrophages Infected with Mycobacterium tuberculosis. Frontiers in Microbiology. 8. 2142. 10.3389/fmicb.2017.02142.

[288] Dhiraj Kumar Singh,Ved Prakash Dwivedi,Shashi Prakash Singh,Anjna Kumari,Saurabh Kumar Sharma,Anand Ranganathan,Luc Van Kaer,Gobardhan Das Published: September 21, 2020 https://doi.org/10.1371/journal.ppat.1008887

serve as a therapeutic option to effectively inhibit granulomas formation through downregulation of MCP-1, IL-4, IL-10, and NF-κB in the treatment of ST. [289]

Curcumin appeared to reduce growth of M. tuberculosis by promoting a form of cell death known apoptosis among macrophages infected by tuberculosis. This cell death presumably kills the tuberculosis organisms inside the cells as well. Curcumin enhanced the clearance of MTB in differentiated THP-1 human monocytes and in primary human alveolar macrophages. It has been found that curcumin was an inducer of caspase-3-dependent apoptosis and autophagy. Curcumin mediated these anti-MTB cellular functions, in part, via inhibition of nuclear factor-kappa B (NFκB) activation.Curcumin protects against MTB infection in human macrophages. The host-protective role of curcumin against MTB in macrophages needs confirmation in an animal model; if validated, the immunomodulatory anti-TB effects of curcumin would be less prone to drug resistance development. [290]

Epigallocatechin-3-gallate had the inherent capacity to down-regulate TACO gene transcription within human macrophages through its ability to inhibit Sp1 transcription factor. We also found out that TACO gene promoter does contain Sp1 binding sequence using bioinformatics tools. The down-regulation of TACO gene expression by epigallocatechin-3-gallate was accompanied by inhibition of mycobacterium survival within macrophages as assessed through flow cytometry and colony counts. Based on these results, we propose that epigallocatechin-3-gallate may be of importance in the prevention of tuberculosis infection. [291]

The available scientific research on bioenhancers has shown to produce significant enhancing effect on bioavailability when coadministered or pretreated with many drugs and nutraceuticals. These natural compounds include piperine, *Zingiber officinale*, niaziridin, glycyrrhizin, *Cuminum cyminum*, *Carum carvi*, allicin, lysergol, *Aloe vera*, *Stevia rebaudiana*, curcumin, sinomenine, genistein, *Ammannia multiflora*, capsaicin, quercetin, naringin, capmul and cow urine distillate. They reduce the dose, shorten treatment, and thus reduce drug-resistance and drug toxicity or adverse reactions. Due to dose economy, treatment is cost-effective. Bioenhancers are also found to decrease or having no effect or little effect on the bioavailability of some drugs.[292]

[289] Zhao Y, Jiao Y, Wang L. Hesperidin methyl chalcone alleviates spinal tuberculosis in New Zealand white rabbits by suppressing immune responses. J Spinal Cord Med. 2020 Jul;43(4):532-539. doi: 10.1080/10790268.2018.1507805. Epub 2018 Aug 20. PMID: 30124375; PMCID: PMC7480517.

[290] Bai, Xiyuan & Oberley-Deegan, Rebecca & Bai, An & Ovrutsky, Alida & Kinney, William & Weaver, Michael & Zhang, Gong & Honda, Jennifer & Chan, Edward. (2016). Curcumin enhances human macrophage control of Mycobacterium tuberculosis infection. Respirology. 21. n/a-n/a. 10.1111/resp.12762.

[291] Anand, Paras & Kaul, Deepak & Sharma, Meera. (2006). Green tea polyphenol inhibits Mycobacterium tuberculosis survival within human macrophages. The international journal of biochemistry & cell biology. 38. 600-9. 10.1016/j.biocel.2005.10.021.

[292] Ghanshyam B. Dudhatra, Shailesh K. Mody, Madhavi M. Awale, Hitesh B. Patel, Chirag M. Modi, Avinash Kumar, Divyesh R. Kamani, Bhavesh N. Chauhan, "A Comprehensive Review on Pharmacotherapeutics of Herbal Bioenhancers", *The Scientific World Journal*, vol. 2012, ArticleID 637953, 33 pages, 2012. https://doi.org/10.1100/2012/637953

Pathway 68: TREATMENT OF DENGUE CO-INFECTION

Dengue viral infections are one of the most important mosquito borne diseases in the world. They may be asymptomatic or may give rise to undifferentiated fever, dengue fever, dengue haemorrhagic fever (DHF), or dengue shock syndrome. Annually, 100 million cases of dengue fever and half a million cases of DHF occur worldwide. Ninety percent of DHF subjects are children less than 15 years of age. At present, dengue is endemic in 112 countries in the world. No vaccine is available for preventing this disease. Early recognition and prompt initiation of appropriate treatment are vital if disease related morbidity and mortality are to be limited. This review outlines aspects of the epidemiology of dengue infections, the dengue virus and its mosquito vector, clinical features and pathogenesis of dengue infections, and the management and control of these infections. [293]

The four DENV serotypes can cause a wide range of diseases in humans even though DENV infections may also be asymptomatic. The diseases range in severity from undifferentiated acute febrile illness, classical dengue fever (DF), to the life-threatening conditions DHF/DSS (5). Dengue illness was previously categorized on a I–IV grade scale, but a simplified categorization for dengue case classification has been proposed by WHO's Special Program for Research and

[293] Malavige GN, Fernando S, Fernando DJ, Seneviratne SL. Dengue viral infections. *Postgrad Med J.* 2004;80(948):588-601. doi:10.1136/pgmj.2004.019638

Training in Tropical Diseases (TDR) in 2009 where DHF and DSS cases are grouped together as 'severe dengue' (group C) to avoid false-negative DHF/DSS diagnosis. [294]

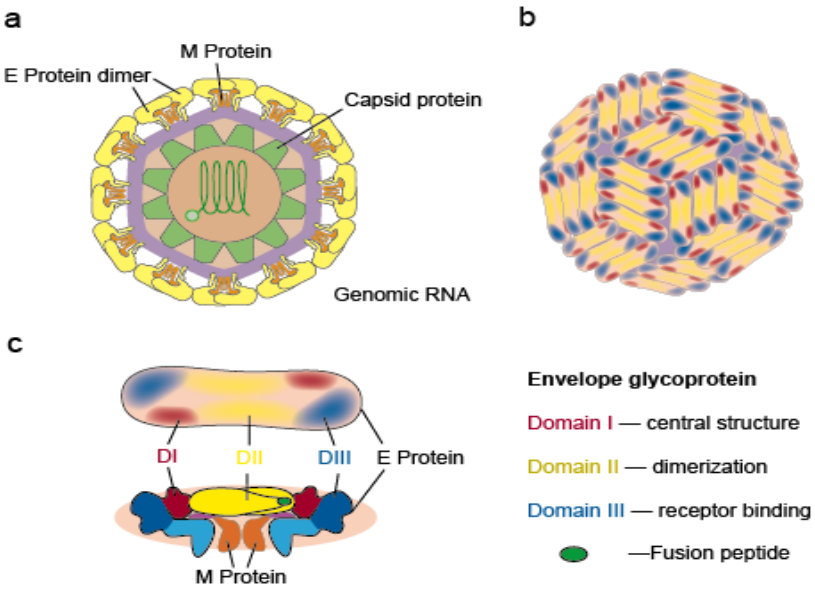


Fig : Dengue Virus Genome

Co-infection and co-occurrence of Covid-19 and dengue have introduced a significant burden on healthcare systems in dengue-endemic regions. The complexity of diverse disease severities, prolonged infectious periods, and shared clinical manifestations and pathogenesis have made their diagnosis, treatment, and resource allocation challenging, particularly in developing countries in Asia with high prevalence of dengue and other airbornviruses. [295]

Covid-19 and dengue exhibit some pathophysiological similarities, such as capillary leakage, thrombocytopenia, and coagulopathy . Plasma leakage is a crucial factor for dengue pathophysiology and is primarily mediated by the host immunological response. Several immuno-mediators, including pro-inflammatory cytokines such as tumor necrosis factor (TNF), interleukin-6 (IL-6), interferon gamma (IFN-γ), and chemokines such as macrophage migration inhibitory factor (MIF), are associated with plasma leakage. Altered platelet function[53] and increased C-reactive protein (CRP) levels are also responsible for plasma leakage. Covid-19 is characterized by over-activation of effector T-cell function and increased inflammatory cytokine production, especially IL-6, often leading to a cytokine storm. IL-6, along with other inflammatory mediators, such as IL-1, TNF, and IFN-γ, contribute not only to plasma leakage but also to other

[294] Gubler DJ. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. *Trends Microbiol.* 2002;10:100–3. [PubMed] [Google Scholar]

[295] Saavedra-Velasco M, Chiara-Chilet C, Pichardo-Rodriguez R, Grandez-Urbina A, Inga-Berrospi F. Coinfection between dengue and covid-19: need for approach in endemic zones. *Revista de la Facultad de Ciencias Medicas (Cordoba, Argentina)*. 2020; **77**: 52- 54.

vascular disorders, including vascular permeability and disseminated intravascular coagulation (DIC). In dengue, plasma leakage is associated with the interaction between nonstructural protein 1 (NS1)-specific antibodies and proteins expressed on endothelial cell surfaces, that may pave the way for the elevated rate of viral replication and inflammatory cytokine secretion

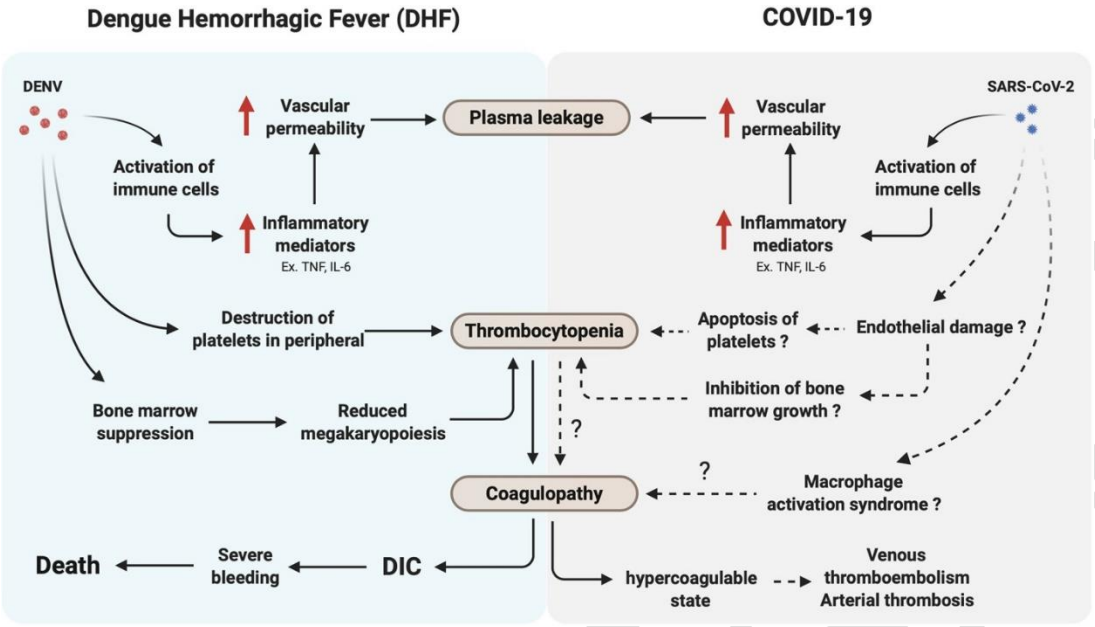


Fig : **Pathophysiological similarities between DHF and Covid-19**

Role of Vedicinal :

Anti-DENV properties of baicalin *in vitro* and described the inhibitory potentials of baicalin at different steps of DENV-2 (NGC strain) replication. Our in vitro antiviral experiments showed that baicalin inhibited virus replication at IC_{50} = 13.5 ± 0.08 µg/ml with SI = 21.5 following virus internalization by Vero cells. Baicalin exhibited virucidal activity against DENV-2 extracellular particles at IC_{50} = 8.74 ± 0.08 µg/ml and showed anti-adsorption effect with IC_{50} = 18.07 ± 0.2 µg/ml. Our findings showed that baicalin as the main metabolite of baicalein exerting *in vitro* anti-DENV activity. Further investigations on baicalein and baicalin to deduce its antiviral therapeutic effects are warranted As per depected by [296]

In a similar context, leaf methanol extracts of *Spondias mombin* and *Spondias tuberosa* showed promising inhibitory capacity against DENV-2 replication at concentrations of 3.31 and 17.98 µg/mL, respectively, believed to be due to the

[296] Moghaddam, E., Teoh, BT., Sam, SS. *et al.* Baicalin, a metabolite of baicalein with antiviral activity against dengue virus. *Sci Rep* **4,** 5452 (2014). https://doi.org/10.1038/srep05452

presence of rutin, quercetin, and ellagic acid in both plants . Petroleum ether extract of *Alternanthera philoxeroides* was also reported to strongly inhibit DENV with a median effective concentration (EC_{50}) value of 47.43 µg/mL [297]

Luteolin was found to reduce infectious virus particle formation, but not viral RNA synthesis, in Huh-7 cells. During the virus life cycle, the host protease furin cleaves the pr moiety from prM protein of immature virus particles in the trans-Golgi network to produce mature virions. Analysis of virus particles from luteolin-treated cells revealed that prM was not cleaved efficiently. Biochemical interrogation of human furin showed that luteolin inhibited the enzyme activity in an uncompetitive manner, with Ki value of 58.6 uM, suggesting that treatment may restrict the virion maturation process. Luteolin also exhibited in vivo antiviral activity in mice infected with DENV, causing reduced viremia. Given the mode of action of luteolin and its widespread source, it is possible that it can be tested in combination with other dengue virus inhibitors. [298]

In this study, we showed that the reportedly highly biologically active green-tea component epigallocatechin gallate (EGCG) inhibited dengue virus infection regardless of infecting serotype, but no or minimal inhibition was observed with other flaviviruses, including Japanese encephalitis virus, yellow fever virus, and Zika virus. EGCG exerted its antiviral effect mainly at the early stage of infection, probably by interacting directly with virions to prevent virus infection. [299]

In a study, the structure-antiviral activity relationships of Glycyrrhizic acid (GL) derivatives was evaluated by the inhibitory assays on the cytopathic effect (CPE) and viral infectivity of DENV type 2 (DENV2) in *Vero* E6 cells. GL (96% purity) had a low cytotoxicity to Vero E6 cells, inhibited DENV2-induced CPE, and reduced the DENV-2 infectivity with the IC50 of 8.1 µM. Conjugation of GL with amino acids or their methyl esters and the introduction of aromatic acylhydrazide residues into the carbohydrate part strongly influenced on the antiviral activity. Among compounds tested GL conjugates with isoleucine **13** and 11-aminoundecanoic acid **17** were found as potent anti-DENV2 inhibitors (IC50 1.2–1.3 µM). [300]

[297] Jiang, W.L.; Luo, X.L.; Kuang, S.J. Effects of *Alternanthera philoxeroides* Griseb against dengue virus *in vitro*. *Di 1 jun yi da xue xue bao= Acad. J. First Med. Coll. PLA* **2005**, *25*, 454–456. [Google Scholar]

[298] Peng, Minhua & Watanabe, Satoru & Chan, Kitti & He, Qiuyan & Zhao, Ya & Zhang, Zhongde & Lai, Xiaoping & Luo, Dahai & Vasudevan, Subhash & Li, Geng. (2017). Luteolin restricts dengue virus replication through inhibition of the proprotein convertase furin. Antiviral research. 143. 10.1016/j.antiviral.2017.03.026.

[299] Raekiansyah, Muhareva & Buerano, Corazon & Luz, Mark & Morita, Kouichi. (2018). Inhibitory effect of the green tea molecule EGCG against dengue virus infection. Archives of Virology. 163. 10.1007/s00705-018-3769-y

[300] Baltina LA, Tasi YT, Huang SH, et al. Glycyrrhizic acid derivatives as Dengue virus inhibitors. *Bioorg Med Chem Lett*. 2019;29(20):126645. doi:10.1016/j.bmcl.2019.126645

Pathway 69 : TREATMENT OF INFLUENZA CO-INFECTION

Influenza viruses are members of the family Orthomyxoviridae. This family represents enveloped viruses the genome of which consists of segmented negative-sense single-strand RNA segments. There are four genera of this family: types A, B, C and Thogotovirus, of which, however, only genera A and B are clinically relevant for humans. The eight genome segments of influenza A and B viruses are loosely encapsidated by the nucleoprotein. The polymerase complexes consisting of the three polymerase proteins PB1, PB2, and PA are located at the ends of the nucleocapsids. [301]

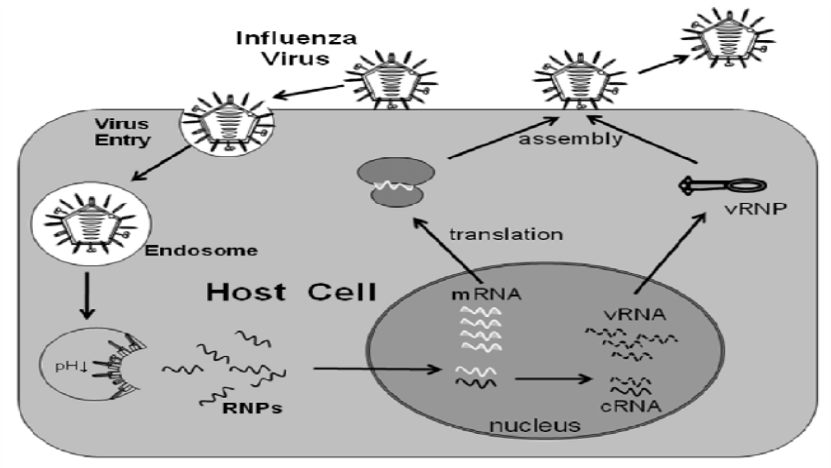


Fig :Influenza Virus

[301] Arbeitskreis Blut, Untergruppe «Bewertung Blutassoziierter Krankheitserreger». Influenza Virus. *Transfus Med Hemother*. 2009;36(1):32-39. doi:10.1159/000197314

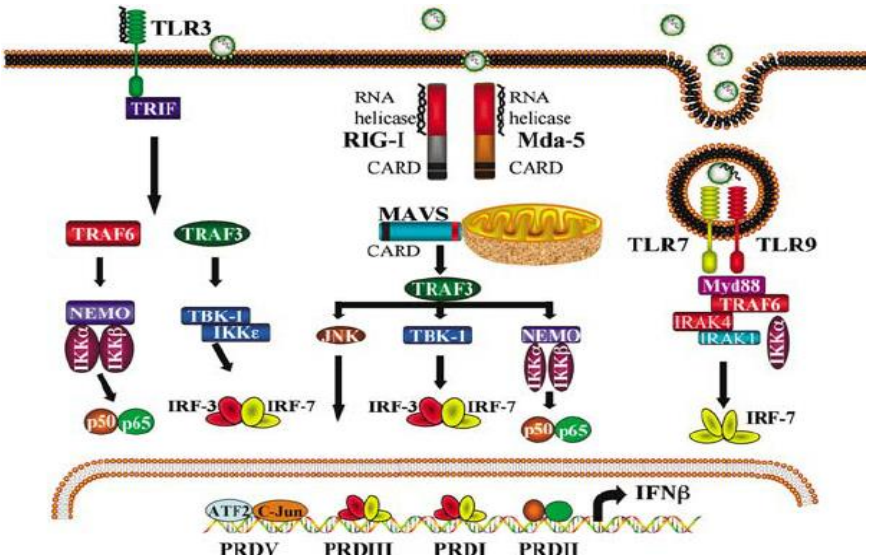


Fig : **Influenza Virus cell signaling pathway**

With respect to cytokine induction, some of the most important signal transduction pathways activated by viruses are shown in Figure . Interferon (IFN) regulatory factor 3 (IRF-3) and IRF-7 are recently discovered virus-activated transcription factors that have been ascribed an important role in IFN-α/β expression . These transcription factors become activated by serine/threonine phosphorylation (see below). The mitogen-activated protein (MAP) kinases p38 and Jun N-terminal kinase (JNK) are also activated in response to many viruses. Following activation, the serine/threonine kinases phosphorylate their downstream targets, notably activating transcription factor 2 (ATF-2) and Jun, thus promoting their *trans*-activating potential. [302]

Influenza virus as an enveloped virus is relatively vulnerable to damaging environmental impacts. Depending on environmental conditions (e.g. humidity and temperature), however, it can survive up to several hours and in water at low temperatures (e.g. <20 °C) also considerably longer (up to several months). [303]

[302] Mogensen TH, Paludan SR. Molecular pathways in virus-induced cytokine production. *Microbiol Mol Biol Rev*. 2001;65(1):131-150. doi:10.1128/MMBR.65.1.131-150.2001

[303] Wright PF, Webster RG. Orthomyxoviruses. In: Fields BN, Knipe DM, Howley PM, et al., editors. *Fields Virology*. 4th ed. Philadelphia: Lippincott Williams and Wilkins; 2001. p. 1533

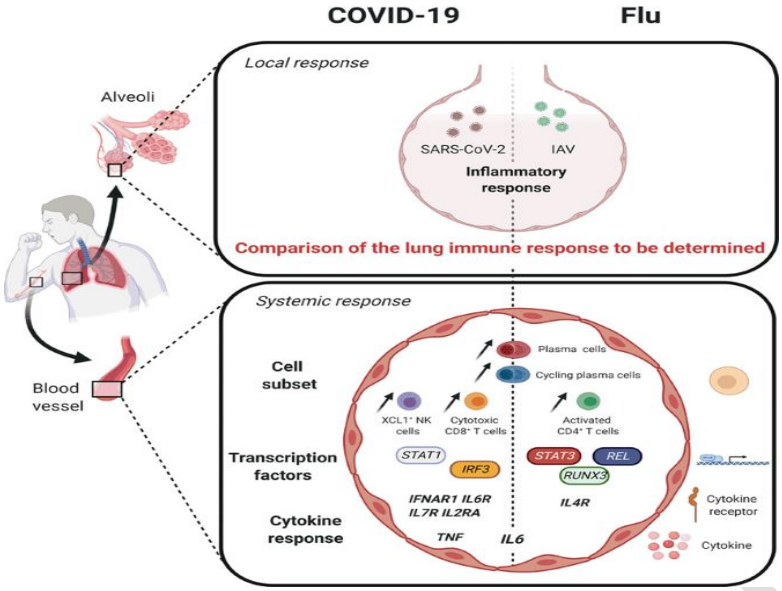


Fig : **Covid 19 and Influenza like Viruses infection cycle (common)**

Influenza viruses cause annual epidemics and occasional pandemics of respiratory tract infections that produce a wide spectrum of clinical disease severity in humans. The novel betacoronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in December 2019 and has since caused a pandemic. Both viral and host factors determine the extent and severity of virus-induced lung damage. The host's response to viral infection is necessary for viral clearance but may be deleterious and contribute to severe disease phenotypes. Similarly, tissue repair mechanisms are required for recovery from infection across the spectrum of disease severity; however, dysregulated repair responses may lead to chronic lung dysfunction. [304]

In a study the proportion of some B cell subsets (plasma and cycling plasma cells) was greater in all patients with viral infection than in healthy controls, suggesting an active mechanism of producing protective antibodies (as exemplified by the upregulation of signature genes such as *PRDM1* and *IRF4* in the B cells). This indicates that on admission, patients have already started to mount a virus-specific immune response. Compared to IAV-infected patients, the counts of XCL1$^+$ NK cells and CD8$^+$ T cells were significantly increased in patients with COVID-19. Conversely, IAV-infected patients had an increased count of activated CD4$^+$ T cells. Regarding the molecular components of the immune response, the expression of proinflammatory cytokines (*TNF*) and cytokine receptors (*IL6R*, *IL2RA,* and *IL7R*) was higher in multiple cell types in patients with COVID-19 than in IAV-infected patients. [305]

Role of Vedicinals :

Baicalin inhibits influenza virus A replication via activation of type I IFN signaling by reducing miR-146a

[304] Hause, B. M. et al. Characterization of a novel influenza virus in cattle and swine: proposal for a new genus in the *Orthomyxoviridae* family. *mBio* **5**, e00031–14 (2014).

[305] Paget, C., Trottein, F. COVID-19 and flu: conserved or specific immune signature?. *Cell Mol Immunol* **18,** 245–246 (2021). https://doi.org/10.1038/s41423-020-00595-3

(Li R and Li R: Baicalin inhibits influenza virus A replication via activation of type I IFN signaling by reducing miR-146a. Mol Med Rep 20: 5041-5049, 2019)

In a study Scientist found, Quercetin possessed anti-influenza activity. The subsequent mechanism study indicated Quercetin showed inhibitory effect during virus entry. Then we found Quercetin interacted with influenza hemagglutinin protein and then inhibited viral-cell fusion. The study showed Quercetin may be developed as a future therapeutic option for the therapy and prophylaxis of IAV infection. [306]

Luteolin has many beneficial properties, including antioxidant, anti-inflammatory, anticancer, anti-diabetic, and cardio-protective effects and widely used in the development of different traditional medicines for the treatment of diseases. It is also well known to have good effects on anti-angiogenesis, anti-metastasis, anti-inflammation, and estrogenic regulation and can regulate many signal pathways. Besides, luteolin is considered to have potential clinical value for cancer prevention and therapies. Luteolin can obstruct the later stages of the DENV viral life cycle in infected cells by inhibiting the host proprotein convertase furin. Luteolin also exhibits inhibitory effects on Epstein-Barr Virus, Japanese encephalitis virus, HIV-1, Hepatitis B virus, Hepatitis C virus, enterovirus 71, coxsackievirus A16, and chikungunya virus. [307]

sulfated rutin was shown to block the entry of *human immunodeficiency viruse (HIV)-1* without interactions with the host cell membrane. Cell fusion and entry assays revealed that sulfated rutin could drastically inhibit *HIV-1* infection when cells were treated during the early adsorption phase. The probable mechanism proposed by investigators was the inhibition of *HIV* glycoprotein-mediated cell-cell fusion step . In the same study, it was identified that sulfated rutin could also inhibit *HSV*, the mechanism of inhibition is still unknown. [308]

Hesperidin showed efficacy in improving H1N1-induced impairment of pulmonary function in a dose-dependent manner. Local numbers of immune cells and concentrations of cytokines were significantly limited by hesperidin. However, we found that hesperidin neither inhibited virus replication, nor rescued infected pulmonary microvascular endothelial cells. Rather, we observed that hesperidin reduced pro-inflammatory cytokine production by suppressing mitogen-activated protein kinase (MAPK) signalling pathways. [309]

[306] Wu W, Li R, Li X, et al. Quercetin as an Antiviral Agent Inhibits Influenza A Virus (IAV) Entry. *Viruses*. 2015;8(1):6. Published 2015 Dec 25. doi:10.3390/v8010006

[307] Wang L, Song J, Liu A, Xiao B, Li S, Wen Z, Lu Y, Du G. Research Progress of the Antiviral Bioactivities of Natural Flavonoids. Nat Prod Bioprospect. 2020 Oct;10(5):271-283. doi: 10.1007/s13659-020-00257-x. Epub 2020 Sep 18. PMID: 32948973; PMCID: PMC7500501.

[308] Lalani, S.; Poh, C.L. Flavonoids as Antiviral Agents for *Enterovirus A71* (*EV-A71*). *Viruses* **2020**, *12*, 184. https://doi.org/10.3390/v12020184

[309] Ding Z, Sun G, Zhu Z. Hesperidin attenuates influenza A virus (H1N1) induced lung injury in rats through its anti-inflammatory effect. Antiviral Therapy. 2018 ;23(7):611-615. DOI: 10.3851/imp3235

Results from the plaque reduction test and HI test clearly show that curcumin interrupts virus-cell attachment, which leads to inhibition of influenza virus propagation, although it is not known yet whether curcumin directly interacts with the viral HA protein or with other viral surface components. With an established safety profile and high SI value of 92.5, curcumin has promising potential
for using as an anti-influenza drug. [310]

(-)Epigallocatechin gallate (EGCg) and theaflavin digallate (TF3) (1-10 microM) inhibited the infectivity of both influenza A virus and influenza B virus in Madin-Darby canine kidney (MDCK) cells in vitro. Study by electron microscope revealed that EGCg and TF3 (1 mM) agglutinated influenza viruses as well as did antibody, and that they prevented the viruses from adsorbing to MDCK cells. EGCg and TF3 more weakly inhibited adsorption of the viruses to MDCK cells. EGCg and TF3 (1-16 microM) also inhibited haemagglutination by influenza viruses. These findings suggest that tea polyphenols bind to the haemagglutinin of influenza virus, inhibit its adsorption to MDCK cells, and thus block its infectivity. [311]

Glycyrrhizin (GL), the main active component of licorice roots, protects cells from infection with influenza A virus (IAV). We found that GL treatment leads to a clear reduction in the number of IAV-infected human lung cells as well as a reduction in the CCID50 titer by 90%. The antiviral effect, however, was limited to one or two virus replication cycles. Analysis of different GL treatment protocols suggested that the antiviral effect of GL was limited to an early step in the virus replication cycle. A direct inhibitory action of GL on IAV particles could be excluded and GL did not interact with virus receptor binding either. The antiviral effect of GL was abolished by treatment 1h after virus infection, whereas pre-treatment and treatment during and after virus adsorption led to a reduction in the cytopathic effect, reduced viral RNA within the cells and in the cell supernatants, and reduced viral hemagglutination titers. Detailed virus uptake analyses unambiguously demonstrated reduced virus uptake in various GL-treated cells. These observations lead to the conclusion, that the antiviral activity of GL is mediated by an interaction with the cell membrane which most likely results in reduced endocytotic activity and hence reduced virus uptake. [312]

[310] Chen, Da-Yuan & Shien, Jui-Hung & Tiley, Laurence & Chiou, Shyan-Song & Wang, Sheng-Yang & Chang, Tien-Jye & Lee, Ya-Jane & Chan, Kin-Wei & Hsu, Wei-Li. (2010). Curcumin inhibits influenza virus infection and haemagglutination activity. Food Chemistry. 119. 1346-1351. 10.1016/j.foodchem.2009.09.011.

[311] Nakayama M, Suzuki K, Toda M, Okubo S, Hara Y, Shimamura T. Inhibition of the infectivity of influenza virus by tea polyphenols. Antiviral Res. 1993 Aug;21(4):289-99. doi: 10.1016/0166-3542(93)90008-7. PMID: 8215301.

[312] Wolkerstorfer A, Kurz H, Bachhofner N, Szolar OH. Glycyrrhizin inhibits influenza A virus uptake into the cell. Antiviral Res. 2009 Aug;83(2):171-8. doi: 10.1016/j.antiviral.2009.04.012. Epub 2009 May 3. PMID: 19416738; PMCID: PMC7126985.

Pathway 70 : RETROVIRUS & REVERSE TRANSCRIPTASE CO-INFECTION

In retroviruses and LTR retrotransposons, reverse transcription is the conversion of a single-stranded RNA (ssRNA) copy of the genome into a double-stranded DNA (dsDNA). To avoid the loss of genetic information, the dsDNA copy is longer, on both ends, than the ssRNA from which it is derived .Although genomic RNA has both a 5′ cap and a poly(A) tail, the ends of the genomic RNA that are transcribed from DNA have short duplications, called R. After reverse transcription, the DNA copy is flanked by longer duplications called the long terminal repeats, or LTRs. [313]

[313] Hughes SH. Reverse Transcription of Retroviruses and LTR Retrotransposons. *Microbiol Spectr*. 2015;3(2):MDNA3-2014. doi:10.1128/microbiolspec.MDNA3-0027-2014

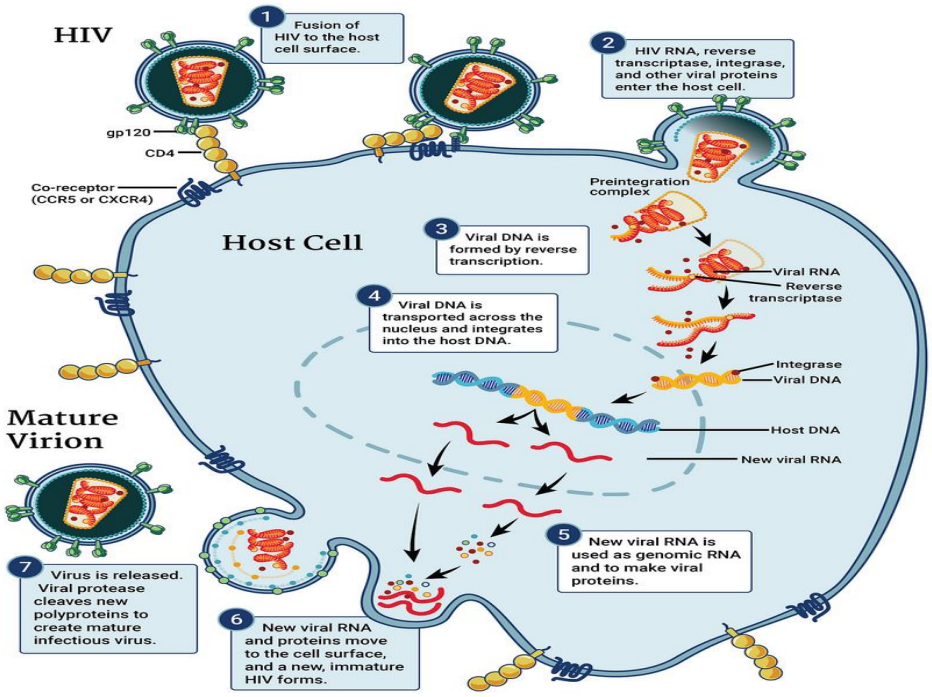
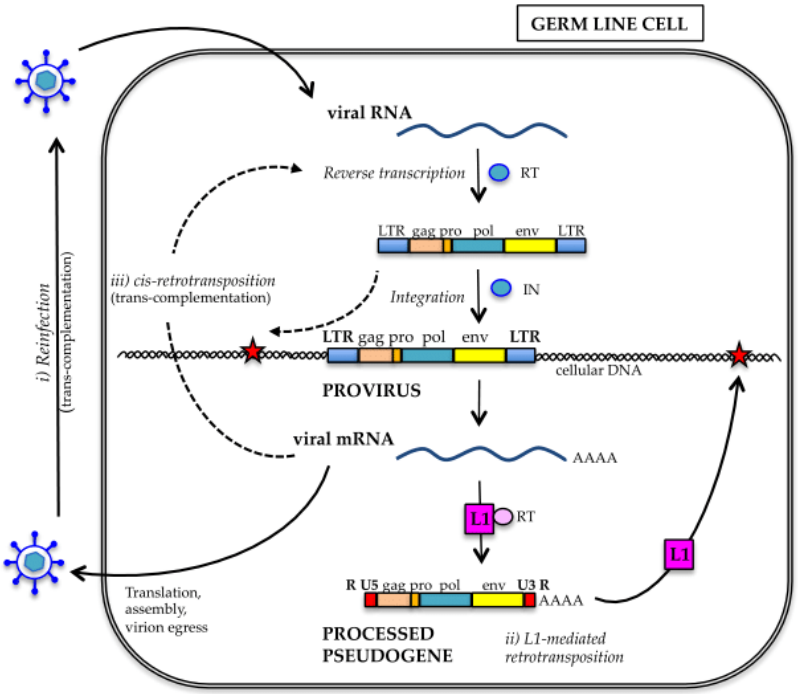


Fig: RETROVIRUS & REVERSE TRANSCRIPTASE

SARS-CoV-2 RNAs can be reverse-transcribed and integrated into the human genome and that transcription of the integrated sequences might account for PCR-positive tests. In support of this hypothesis, we found chimeric transcripts consisting of viral fused to cellular sequences in published data sets of SARS-CoV-2 infected cultured cells and primary cells of patients, consistent with the transcription of viral sequences integrated into the genome. To experimentally corroborate the possibility of viral retro-integration, we describe evidence that SARS-CoV-2 RNAs can be reverse transcribed in human cells by reverse transcriptase (RT) from LINE-1 elements or by HIV-1 RT, and that these DNA sequences can be integrated into the cell genome and subsequently be transcribed. Human endogenous LINE-1 expression was induced upon SARS-CoV-2 infection or by cytokine exposure in cultured cells, suggesting a molecular mechanism for SARS-CoV-2 retro-integration in patients. This novel feature of SARS-CoV-2 infection may explain why patients can continue to produce viral RNA after recovery and suggests a new aspect of RNA virus replication. [314]

[314] Liguo Zhang, Alexsia Richards, M. Inmaculada Barrasa, Stephen H. Hughes, Richard A. Young, Rudolf Jaenischroceedings of the National Academy of Sciences May 2021, 118 (21) e2105968118; DOI: 10.1073/pnas.2105968118



Chr	HERV-W*	Chr	HERV-W*
1	16 (4, 10)	13	6 (2, 3)
2	23 (6, 16)	14	6 (3, 3)
3	22 (4, 16)	15	3 (0, 3)
4	19 (8, 10)	16	0
5	9 (5, 3)	17	4 (1, 3)
6	18 (4, 12)	18	4 (1, 3)
7	12 (7, 5)	19	6 (2, 4)
8	9 (1, 8)	20	2 (0, 2)
9	7 (1, 5)	21	3 (2, 1)
10	7 (2, 5)	22	1 (0, 1)
11	9 (4, 5)	X	12 (1, 10)
12	13 (5, 7)	Y	2 (2, 0)

** Total number of HERV-W insertions.* Numbers into round brackets specify the amount of proviruses and pseudogenes, respectively, with respect to the total. The rest of the sequences can not be classified due to the absence of LTRs distinctive signatures (data from Grandi et al. 2016)

Role Of Vedicinals

Our proposed formulation contains 7 compounds namely compounds Baicalin,Quercetin ,Luteolin,Rutin,Hespersin,Curcumin,Glycyrrhizic shows significant inhibitory effect on Retrovirus and Reverse Transcriptase

Baicalein significantly reduced the levels of human cytomegalovirus (HCMV) early and late proteins, as well as viral DNA synthesis, although it had no effect on viral polymerase activity . Baicalein impaired avian influenza H5N1 virus replication in both human lung epithelial cells and monocyte-derived macrophages by interfering

with neuraminidase activity . Other studies showed that oral administration of baicalein to BALB/c mice infected with influenza H1N1 virus decreased the lung virus titer and increased the mean time to death . Similar effects were recorded on mice infected with Sendai virus . These inhibitory effects *in vivo* were mediated by serum baicalin, a metabolite of baicalein which has a glucose residue.

Baicalin alone exerts its anti-influenza activity by modulating the function of NS1 protein, which down-regulates IFN induction. [315]

Four flavonoids, 5,6,7-trihydroxyflavone (baicalein), 3,3‘,4,5,7-pentahydroxyflavone (quercetin), 3,3’,4,5,6,7-hexahydroxyflavone (quercetagetin) and 3,3’,4’,5,5’,7-hexahydroxyflavone (myricetin), were found to be potent inhibitors of reverse transcriptases from Rauscher murine leukemia virus (RLV) and human immunodeficiency virus (HIV). [316]

Epigallocatechin gallate (EGCG), the most abundant catechin in green tea, has been reported to inhibit HIV-1 replication prior to its integration into host DNA via various proposed mechanisms; however, the specific main target(s) of EGCG remain unclear. In this study, we investigated a number of these proposed detailed mechanism(s) using a cell-based model.

HIV-1inhibition by luteolin was independent of viral entry, as shown by the fact that wild-type and VSV–pseudotyped HIV-1 infections were similarly inhibited. Luteolin was unable to inhibit viral reverse transcription. Luteolin had antiviral activity in a latent HIV-1 reactivation model and effectively ablated both clade-B- and -C -Tat-driven LTR transactivation in reporter assays but had no effect on Tat expression and its sub-cellular localization. We conclude that luteolin confers anti–HIV-1 activity at the Tat functional level. Given its biosafety profile and ability to cross the blood-brain barrier, luteolin may serve as a base flavonoid to develop potent anti–HIV-1 derivatives to complement cART. [317]

Glycyrrhizin (GL), one of the plant extracts, was investigated for its antiviral action on the human immunodeficiency virus [HIV (HTLV-III/LAV)] in vitro, using cytopathic effect and plaque forming assay system in MT-4 cells (a HTLV-I-carrying cell line). [318]

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[315] Nayak MK, Agrawal AS, Bose S, Naskar S, Bhowmick R, Chakrabarti S, Sarkar S, Chawla-Sarkar M (2014) Antiviral activity of baicalin against influenza virus H1N1-pdm09 is due to modulation of NS1-mediated cellular innate immune responses. J Antimicrob Chemother 69(5):1298–1310. doi:10.1093/jac/dkt534

[316] Katsuhiko ONO’, Hideo NAKANE ’, Masanori FUKUSHIMA’, Jean-Claude CHERMANN3 and Franqoise RARRE-SINOUSS14 Eur. J. Riochem. 190,469-476 (2990) FEBS 1990

[317] Mehla R, Bivalkar-Mehla S, Chauhan A. A flavonoid, luteolin, cripples HIV-1 by abrogation of tat function. *PLoS One*. 2011;6(11):e27915. doi:10.1371/journal.pone.0027915

[318] Ito M, Nakashima H, Baba M, Pauwels R, De Clercq E, Shigeta S, Yamamoto N. Inhibitory effect of glycyrrhizin on the in vitro infectivity and cytopathic activity of the human immunodeficiency virus [HIV (HTLV-III/LAV)]. Antiviral Res. 1987 Mar;7(3):127-37. doi: 10.1016/0166-3542(87)90001-5. PMID: 3475037.

Hesperidin interacted with HBV Pol by forming seven hydrogen bonds with Glu39, Ser40, Ser85 and Ser117, and one carbon hydrogen bond with His156 . It also formed two Pi-Pi stacked hydrophobic interactions with Trp3, one alkyl hydrophobic interaction with Leu147 and one Pi-alkyl hydrophobic interaction with Ala86. Other residues that surrounded hesperidin were Glu1, Asp2, Gly4, Pro5, Arg15, Ala86, Asn118, Ser119, Arg120, Leu147, Arg153, His156, Leu157 and Tyr158. The Gibb's free energy of hesperidin-HBV Pol interaction was predicted to be −9.3 kcal/mol, corresponding to a binding constant of 6.6×10^6/mol. [319]

Pathway 71 : TREATMENT OF HIV CO-INFECTION

In the last 50 years we have experienced two big pandemics, the HIV pandemic and the pandemic caused by SARS-CoV-2.Both pandemics are caused by RNA viruses and have reached us from animals. These two viruses are different in the transmission mode and in the symptoms they generate. However, they have important similarities: the fear in the population, increase in proinflammatory cytokines that generate intestinal microbiota

[319] Mohammad K.ParvezMd.Tabish RehmanPerwezAlamMohammed S.Al-DosariSaleh I.AlqasoumiMohammed F.Alajmi, Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia https://doi.org/10.1016/j.jsps.2018.12.008

modifications or NETosis production by polymorphonuclear neutrophils, among others. They have been implicated in the clinical, prognostic and therapeutic attitudes. [320]

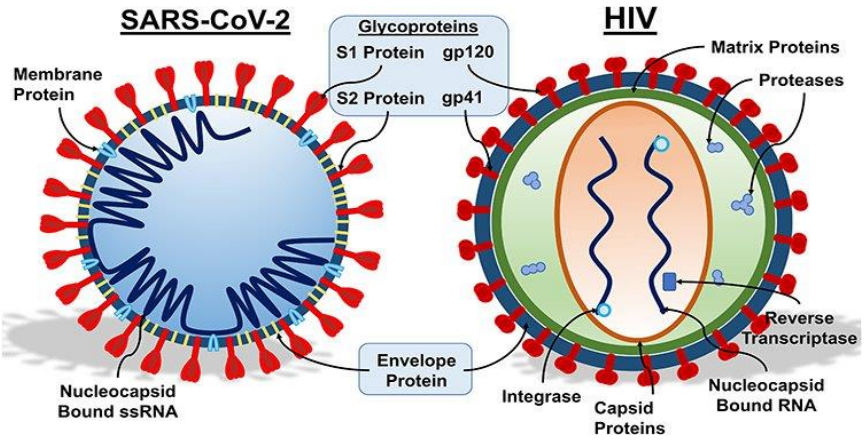

Fig : Part of Common Genome Structure

Since its first appearance in Wuhan, China, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly spread throughout the world and has become a global pandemic. Several medical comorbidities have been identified as risk factors for coronavirus disease 2019 (COVID-19). However, it remains unclear whether people living with human immunodefeciency virus (PLWH) are at an increased risk of COVID-19 and severe disease manifestation, with controversial suggestion that HIV-infected individuals could be protected from severe COVID-19 by means of antiretroviral therapy or HIV-related immunosuppression. Several cases of coinfection with HIV and SARS-CoV-2 have been reported from different parts of the globe. [321]

Patients with human immunodeficiency virus (HIV) infection may be at an increased risk for morbidity and mortality from the coronavirus disease 2019 (COVID-19). We present the clinical outcomes of HIV patients hospitalized for COVID-19 in a matched comparison with historical controls. [322]

[320] Illanes-Álvarez F, Márquez-Ruiz D, Márquez-Coello M, Cuesta-Sancho S, Girón-González JA. Similarities and differences between HIV and SARS-CoV-2. *Int J Med Sci*. 2021;18(3):846-851. Published 2021 Jan 1. doi:10.7150/ijms.50133

[321] Kanwugu ON, Adadi P. HIV/SARS-CoV-2 coinfection: A global perspective. J Med Virol. 2021 Feb;93(2):726-732. doi: 10.1002/jmv.26321. Epub 2020 Jul 28. PMID: 32692406; PMCID: PMC7404432.

[322] Nagarakanti SR, Okoh AK, Grinberg S, Bishburg E. Clinical outcomes of patients with COVID-19 and HIV coinfection. J Med Virol. 2021 Mar;93(3):1687-1693. doi: 10.1002/jmv.26533. Epub 2020 Oct 14. PMID: 32949148; PMCID: PMC7537324.

Role of Vedicinals Constituents : Our proposed formulation contains 4 compounds namely compounds 1,2,3,6 & 7 are inhibitors t blocks the release of pro-inflammatory cytokines like Interleukine 6 (IL-6) and Tumor Necrosis Factor-α (TNF-α). Quercetin is well-known to act against the entry of the virus in the host cell. For example, Hemagglutinin and neuraminidase are envelope glycoproteins responsible for entry of the Influenza virus. This glycoprotein helps in attachment and membrane fusion of the virus to the host cell. The process of membrane fusion further facilitates the release of the viral ribonucleic proteins into the cytosol. [323]

Glycyrrhizic acid from Glycyrrhiza glabra demonstrated inhibition on the replication of SARS-CoV in vivo and also increased interferon-γ production. Kaposi's sarcoma-associated viral elimination via apoptosis and anti-viral effects are also being described against DNA and RNA viruses, like Hepatitis A virus, Hepatitis B virus, Coronavirus, Influenza virus, HIV-1 etc. [324]

Flavones with lesser favorable therapeutic index (2.1 to 2.4) are represented by the compounds baicalin and baicalein. Baicalin isolated from Scutellaria radix was found to suppress p24 antigen production with an IC50 value of 0.5g/ml (1.12M) using a HIV-1 clinical isolate. [325]

Luteolin itself was recently found to inhibit HIV-1 infection in reporter cells and primary human lymphocytes and displayed antiviral activity in a latent HIV-1 reactivation model and abolished effectively HIV-1 Tat-driven long-terminal repeat (LTR) promoter transactivation at a concentration of 10M possibly by interfering with pTEF-b binding with LTR or by abolishing Tat binding altogether. Luteolin may also prevent NFB-activation or inhibit host factors involved in transcription. [326]

The catechins that contain a galloyl moiety GC, ECG, GCG and EGCG were found to inhibit HIV-1 IN with high efficacy displaying IC50 values of 0.56M, 3.02M, 2.4M and 0.96M, respectively, the former and the latter inhibiting HIV-1 IN in more or less the same order of magnitude as raltegravir, the FDA-approved HIV-1 IN

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Mechanisms / pathways action points of our compounds/molecules (* picture of virus used from internet only for marking action points)

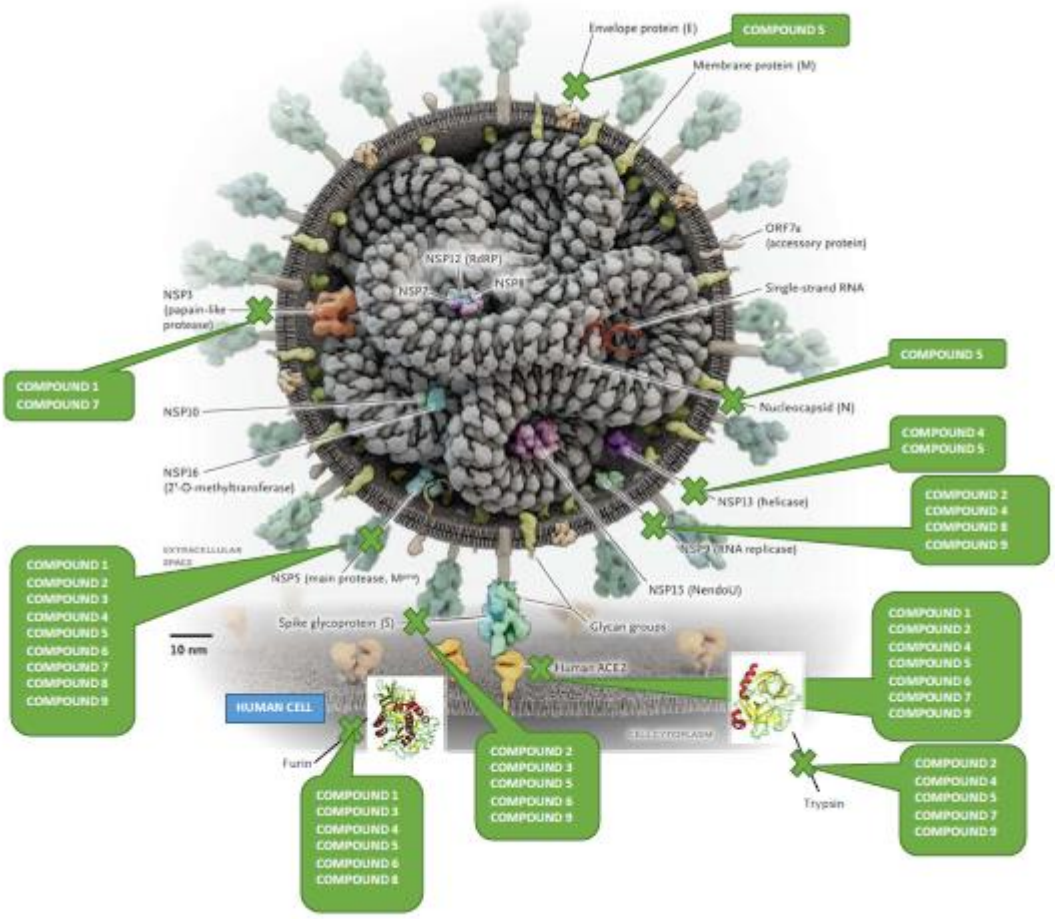

Fig:Compound Efficacy and Intercation Scheme

Following table summarizes the activity matrix for our formulation –

VEDICINALS®9 The Composition *for management of* **COVID-19** *& Preventing* **LONG COVID**

##	Drug Target Pathways	ACTIVE Ingredients								
		1	2	3	4	5	6	7	8	9
	STRUCTURAL PROTEINS OF SARS_CoV_2									
1	3C-LIKE PROTEASE (3CL-pro) 6LU7 INHIBITORS	✸	✸	✸	✸	✸	✸	✸	✸	✸
2	SPIKE GLYCOPROTEIN 6LYEST INHIBITORS		✸	✸		✸	✸			✸
3	ENVELOPE GYLCOPROTIEN INHIBITORS					✸				
4	NUCLEOCAPSID PROTEIN INHIBITORS					✸				
5	PAPAIN-LIKE PROTEASE (PL-pro) INHIBITORS	✸						✸		
6	RNA-DEPENDANT RNA POLYMERASE (RdRp) INHIBITORS		✸		✸				✸	✸
7	HELICASE INHIBITORS				✸	✸				
8	RBD-ACE 2 6VW1 INHIBITORS	✸	✸		✸	✸	✸	✸		✸
9	RECENT MUTATIONS & RECOMBINANT INHIBITORS	✸	✸	✸	✸	✸	✸	✸	✸	✸
	HOST RECEPTORS / CELLS / ENZYMES									
10	ZINC IONOPHORES		✸					✸		
11	DHODH INHIBITORS	✸	✸	✸			✸		✸	✸
12	ENDOCYTOSIS INHIBITORS	✸	✸		✸		✸	✸		✸
13	HEME OXYGENASE (HO-1) ACTIVATORS	✸	✸	✸	✸	✸	✸	✸	✸	✸
14	TMPRSS2 INHIBITORS	✸						✸		✸
15	FURIN INHIBITORS	✸		✸	✸	✸	✸		✸	
16	TRYPSIN INHIBITORS		✸		✸	✸		✸		✸
17	CATHEPSIN-L INHIBITORS		✸							
18	APN & CD 13 INHIBITORS						✸			
19	DPP4 INHIBITORS		✸		✸		✸	✸		
20	CALPAIN INHIBITORS		✸							
21	CASEIN KINASE-2 (CK2) INHIBITORS	✸	✸	✸				✸		✸
22	EMMPRIN CD 147 INHIBITORS	✸					✸	✸	✸	
23	GRP 78 INHIBITORS	✸	✸	✸	✸		✸	✸	✸	
24	GP 41 FUSION INHIBITORS						✸	✸		
25	ANGIOTENSIN II INHIBITORS	✸	✸	✸		✸	✸	✸		✸
26	PALS 1 INHIBITORS	✸	✸	✸		✸	✸	✸	✸	✸
27	P-SELECTIN INHIBITORS	✸	✸					✸		
28	HEPARAN SULFATE BINDING INHIBITORS							✸		
29	CCR5 INHIBITORS	✸	✸	✸				✸		
30	MYOSIN II & FILOPODIA ADHESION INHIBITORS	✸		✸				✸		
31	TYROSINE KINASE INHIBITORS	✸	✸	✸			✸	✸	✸	
32	PPAR GAMMA ACTIVATORS		✸		✸	✸	✸		✸	
33	SYNCYTIUM / SYNCYTIA FORMATION INHIBITORS	✸	✸	✸						
34	GLYCOLYSIS & GLUTAMINOLYSIS INHIBITORS		✸	✸			✸	✸	✸	
	MANAGEMENT OF COVID 19 & PREVENTION OF LONG COVID									
35	INTERLEUKIN - 6 INHIBITORS	✸	✸	✸	✸	✸	✸	✸	✸	✸
36	MACROPHAGE POLARISATION & CCL2 REGULATORS	✸	✸	✸			✸	✸		✸
37	PRO-INFLAMMATORY CYTOKINE SUPPRESSORS	✸	✸	✸	✸	✸	✸	✸	✸	✸
38	TNF ALPHA SUPPRESSORS	✸	✸	✸	✸		✸	✸	✸	✸
39	MAST CELLS STABILIZERS	✸	✸	✸	✸	✸	✸	✸	✸	✸
40	T CELLS STABILIZERS	✸	✸	✸	✸	✸	✸	✸	✸	✸
41	Nrf2 ACTIVATORS	✸	✸	✸	✸	✸	✸	✸	✸	
42	NLRP-3 & CASPASE-1 INHIBITORS	✸	✸	✸		✸	✸	✸	✸	✸
43	STAT 3 PHOSPHORYLATION SUPPRESSORS	✸	✸	✸			✸	✸	✸	✸
44	ALPHA ANTI TRYPSIN ACTIVATORS		✸				✸	✸		
45	C REACTIVE PROTEIN SUPPRESSORS	✸	✸		✸		✸	✸		✸
46	CREATININE KINEASE INHIBITORS	✸	✸			✸	✸	✸		✸
47	RAISING ANTIBODY LEVELS	✸	✸	✸	✸	✸	✸	✸	✸	✸
48	PROTECTING LUNG TISSUES	✸	✸		✸	✸	✸	✸	✸	✸
49	PROTECTING NEURONAL TISSUES	✸	✸	✸	✸	✸	✸	✸	✸	✸
50	PROTECTING KIDNEY TISSUES	✸	✸	✸	✸	✸	✸	✸	✸	✸
51	PROTECTING CARDIO-VASCULAR SYSTEM	✸	✸	✸	✸	✸	✸	✸	✸	✸
52	PROTECTING MYOCARDIAL TISSUES	✸	✸	✸	✸	✸	✸	✸	✸	
53	BDNF & REMYELINATION AGONISTS, MYELIN SHEET PROTECTORS	✸	✸	✸		✸	✸	✸	✸	✸
54	PROTECTING AGAINST THROMBOSIS	✸	✸	✸	✸	✸	✸	✸	✸	✸
55	PROTECTING PANCREATIC BETA CELLS	✸		✸		✸	✸	✸		
56	PROTECTING LIVER TISSUES	✸	✸	✸			✸	✸		
57	TREATMENT OF BACTERIAL CO-INFECTION	✸	✸	✸	✸		✸	✸	✸	✸
58	MODULATING GUT BACTERIA & GUT BRAIN AXIS	✸	✸		✸	✸	✸	✸	✸	✸
59	TREATMENT OF INTESTINAL INFLAMMATION	✸	✸	✸	✸			✸		
60	TREATMENT OF ENCEPHALOMYELITIS	✸	✸	✸				✸		✸
61	MANAGING HYPERGLYCEMIA	✸	✸	✸				✸		
62	PROTECTING AGAINST MITOCHONDRIAL DAMAGE	✸	✸	✸		✸	✸	✸		✸
63	PROTECTING & RESTORING TIGHT JUNCTIONS (INTESTINAL & BBB)	✸	✸	✸		✸	✸	✸	✸	✸
64	SENOLYTICS	✸	✸	✸		✸		✸		
65	PROTECTING & TREATMENT OF AUTO-IMMUNE CONDITIONS	✸	✸	✸	✸	✸	✸	✸	✸	✸
	TREATMENT OF CO-INFECTIONS									
66	TREATMENT OF MALARIA CO-INFECTION	✸	✸	✸	✸	✸	✸	✸	✸	✸
67	TREATMENT OF TUBERCULOSIS CO-INFECTION	✸		✸		✸	✸	✸	✸	✸
68	TREATMENT OF DENGUE CO-INFECTION	✸	✸	✸	✸			✸		✸
69	TREATMENT OF INFLUENZA CO-INFECTION	✸	✸	✸	✸	✸	✸	✸		✸
70	RETROVIRUS & REVERSE TRANSCRIPTASE INHIBITORS	✸	✸	✸	✸	✸	✸			✸
71	TREATMENT OF HIV CO-INFECTION	✸	✸	✸			✸	✸		

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4. The study:

4.1) Review of literature:

Proper review of literature has been carried out for the selection of phytoconstituents along with their source plants. Initially, total 450 phytoconstituents were selected on the basis of their activities, availability, sufficient plant source, etc. All these phytochemical were investigated for Insilco studies.

4.2) In-silco studies:

Proteins/Macromolecules

The crystal structure of SARS-CoV2 receptor binding domain with human antibody CR3022 (PDB ID: 6W41 was obtained from Protein data bank (https://www.rcsb.org/structure/6W41). The total Structure Weight was 74.43 kDa with three unique protein chains, H chain (CR3022 Fab heavy chain, sequence length-222), L chain (CR3022 Fab light chain, sequence length -221), and C chain (Spike protein S1, sequence length-231).

Ligand and Drug Scan

PubChem is a chemical substance and biological activities repository consisting of three databases, including substance, compound, and bioassay databases. The 3-dimensional (3D) structurewas obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/), in .sdf format. Several ligands for which the active compound can be found in herbal medicine were downloaded fromPubChem. Standard drugs and list of phytochemicals with their source and ID.

Drug-like properties were calculated using Lipinski's rule of five, which proposes that molecules with poor permeation and oral absorption have molecular weights >900, C logP> 5, more than 5 hydrogen-bond donors, and more than 10 acceptor groups (Lipinski et al., 2012). Adherence with Lipinski's rule of five as calculated using SWISSADME prediction (http://www.swissadme.ch/).

Determination of Active Sites

The amino acids in the active site of a protein were determined using the Computed Atlas for Surface Topography of Proteins (CASTp) (http://sts.bioe.uic.edu/castp/index.html?201l) and Biovia Discovery Studio 4.5. The determination of the amino acids in the active site was used to analyse the Grid box and docking evaluation results. Discovery Studio is offline life sciences software that provides tools for protein, ligand, and pharmacophore modelling (Xavier et al.,2015).

Molecular Docking

Ligand optimisation was performed by Avogadro version 1.2, with Force Field type MMFF94, and saved in. mol2 format. Autodock version 4.2 used for protein optimisation, by removing water and other atoms, and then adding a polar hydrogen group. Autodock Vina was supported by Autodock tools, MGL tools, and Rasmol. Autogrid then determined the native ligand position on the binding site by arranging the grid coordinates (X, Y, and Z). Ligand tethering of the protein was performed by regulating the genetic algorithm (GA) parameters, using 10 runs of the GA criteria. The docking analyses were performed by both Autodock Vina, Pymol version 1.7.4.5 Edu and Biovia Discovery Studio 4.5.

On the basis of docking study total 09 (as given above) compound has been prioritized the docking energy and binding sites are given bellow-

Table 1. Protein target structures and active site amino acids and the native ligand structure

			Comp 1	Comp 2	Comp 3	Comp 4	Comp 5	Comp 6	Comp 7	Comp 8	Comp 9
	SARS-CoV-2										
1	3C-Like Protease (3CL-pro)	5RHC	-6.97	-7.02	-7.39	-3.37	-3.88	-6.18	-6.33	-6.33	-6.55
2	Spike Glycoprotein 6LYEST	6VYB	-6.82	-6.06	-6.72	-3.09	-4.52	-4.90	-5.43	-5.25	-9.42
3	Envelope Glycoprotein	6X6P	-6.41	-6.51	-6.74	-3.87	-4.15	-4.70	-4.91	-5.44	-9.02
4	Nucleocapsid Protein	6M3M	-5.90	-5.91	-5.67	-3.01	-3.64	-5.14	-5.11	-5.52	-7.20
5	Papain-Like Protease (PL-pro)	6W9C	-5.92	-5.64	-6.30	-2.83	-4.06	-5.80	-4.81	-5.32	-7.05
6	Rna-DependantRna Polymerase (RdRp)	7BV2	-6.47	-5.93	-6.17	-3.96	-4.23	-5.24	-5.40	-6.37	-7.37
7	Helicase Inhibitors	6WX4	-5.92	-5.91	-6.38	-4.04	-4.06	-5.13	-5.10	-5.40	-7.37
	Human Proteins										
8	Furin	6HZA	-7.29	-5.86	-6.72	-3.66	-4.47	-5.47	-5.63	-6.03	-6.36
9	DHODH	1D3G	-6.70	-7.20	-7.42	-3.21	-4.25	-6.69	-4.73	-8.22	-6.38
10	Angiotensin Converting Enzyme 2 (ACE2)	3SCI	-5.56	-6.64	-6.60	-4.48	-4.46	-5.89	-5.82	-5.87	-5.36
11	Serine protease Hepsin	5CE1	-6.16	-5.60	-5.71	-3.48	-4.00	-5.26	-4.94	-6.50	-6.78
12	AP2-associated protein kinase 1	5T30	-6.05	-5.66	-5.92	-2.97	-3.53	-4.91	-4.15	-5.10	-6.30
13	Structure of human ABL2	3GVU	-7.00	-6.37	-6.72	-3.43	-5.16	-5.77	-5.01	-6.74	-5.80
14	Angiotensin Converting Enzyme	1O86	-7.14	-6.38	-6.67	-3.71	-5.55	-7.26	-6.41	-6.35	-5.63
15	SARS-CoV-2 Spike D614G mutation	6XS6	-8.40	-6.88	-7.01	-4.58	-3.74	-5.59	-5.24	-5.49	-8.86
16	PALS1	4WSI	-6.54	-7.17	-6.96	-3.97	-5.50	-6.30	-6.56	-6.47	-6.10
17	GP41 mutation (HIV)	1SZT	-6.32	-6.17	-6.35	-3.62	-3.66	-5.04	-4.21	-5.14	-5.77
18	IL-6 (IL-6 - Interleukin 6)	4CNI	-6.85	-7.04	-6.95	-3.39	-4.44	-5.06	-4.88	-4.92	-6.88
19	IgG	2IG2	-5.89	-6.10	-6.07	-3.62	-4.03	-4.72	-5.65	-5.27	-6.81
20	IgM/IgA	1DN0	-5.68	-5.80	-6.51	-3.68	-4.04	-5.01	-5.09	-6.38	-6.12
21	C-reactive protein	1GNH	-6.25	-5.44	-6.14	-3.14	-4.16	-4.92	-4.93	-6.25	-5.26
22	Galectin-3	4LBJ	-5.84	-6.93	-6.13	-4.38	-3.97	-4.84	-5.01	-5.37	-6.26
23	Angiotensin II	6JOD	-7.40	-5.85	-6.02	-2.59	-4.72	-5.34	-5.83	-6.86	-9.13
24	Histamine (Histamine H1 receptor)	3RZE	-6.97	-5.64	-8.05	-3.94	-4.21	-4.72	-4.66	-5.26	-9.03
25	Serum amyloid A is name like C-reactive protein	4IP8	-6.90	-6.35	-6.97	-3.87	-4.28	-6.44	-4.57	-6.19	-5.96
26	Neuropilin 1 (NRP1)	2QQI	-6.03	-5.53	-5.62	-3.26	-3.43	-5.06	-4.37	-5.30	-7.50
27	Neuropilin-1	4RN5	-6.28	-6.31	-5.97	-4.07	-3.94	-5.61	-4.81	-5.28	-6.76
28	Emmprin (CD147) Thrombosis factor	3B5H	-7.73	-5.64	-5.53	-2.90	-4.15	-4.79	-4.76	-5.25	-6.04
29	GRP78	5E84	-7.07	-6.09	-6.46	-4.08	-4.63	-5.45	-5.58	-6.05	-7.29
30	Antithrombotic	4EL1	-7.48	-6.23	-6.56	-3.42	-4.24	-5.03	-5.55	-5.55	-6.82
31	Heparan sulfate proteoglycan (HSPG)	3SH5	-5.97	-6.40	-6.17	-2.92	-3.85	-5.23	-4.83	-5.30	-5.62
32	Dipeptidyl Peptidase-4 (DPP-4) Inhibitor	6B1E	-7.03	-6.75	-6.90	-3.77	-4.75	-6.18	-5.77	-5.91	-7.10
	SARS-CoV-2 Mutation										
33	Spike Glycoprotein (7KDJ-Mutant)	D614G	-7.08	-7.06	-6.41	-2.87	-4.34	-4.83	-5.40	-5.14	-8.57
34	Spike Glycoprotein	G476S	-7.43	-7.40	-7.01	-4.18	-5.06	-6.48	-6.80	-6.60	-7.76
35	Spike Glycoprotein	V483G	-7.70	-7.29	-7.54	-4.35	-5.59	-6.97	-6.36	-7.72	-8.00
36	Spike Glycoprotein	E484K	-7.18	-6.56	-6.83	-3.13	-4.41	-4.72	-5.62	-6.92	-6.79
37	Spike Glycoprotein	P681H	-6.80	-6.17	-6.92	-4.76	-4.43	-6.07	-6.24	-6.03	-6.94
38	Spike Glycoprotein	K417N	-7.26	-7.35	-7.54	3.81	-6.65	-6.89	-6.89	-7.38	-7.28
39	Spike Glycoprotein	N501Y	-7.39	-7.35	-7.06	-4.75	-5.72	-6.43	-7.25	-6.86	-7.88
40	Spike Glycoprotein	N440K	-7.55	-7.96	-7.21	-4.91	-6.31	-6.98	-7.07	-7.40	-7.69

In Summary:

In the above sections we discuss nine different herbal compounds, each with a long history of human safety, each of which were shown to have antiviral, immuno-modulatory and anti-coagulation properties. All the selected compounds are with optimum nutrition, non-toxic, natural herbal compounds, easy to digest, have health protective and will rejuvenate functions to ameliorate the symptoms and/or syndromes of COVID-19 and will promote general health and well-being of COVID-19 patients. In nut shell, our multiple herbal compounds have anti SARS-CoV-2 activities by blocking Viral enzymes, structural and non-structural proteins, by blocking the host and virus docking receptors and at the same time selected compounds enhance the immune response of the host at great extent. Following is the summary of activities of each herbal compound -

1) compound 1 will act on COVID-19 patients by targeting 15different pathways
2) compound 2 will act on COVID-19 patients by targeting 20different pathways
3) compound 3 will act on COVID-19 patients by targeting 14different pathways
4) compound 4 will act on COVID-19 patients by targeting 18 different pathways
5) compound 5 will act on COVID-19 patients by targeting 16 different pathways
6) compound 6 will act on COVID-19 patients by targeting 17 different pathways
7) compound 7 will act on COVID-19 patients by targeting 18 different pathways
8) compound 8 will act on COVID-19 patients by targeting 14 different pathways
9) compound 9 will act on COVID-19 patients by targeting 16different pathways

We have selected the herbal compounds with highest level of confidence and broad spectrum activity hence we are of opinion that suitable formulation prepared using above combination of herbal compounds will have devastating effect on SARS-CoV-2 while it will provide the optimum nutrition, health protective and immune system rejuvenating activity on the host.

Along with broad band Anti-Viraland immunomodulatory effects, we are confident that our identified molecules / compounds combined with nutrients and vitamins we recommend achieve followingat least

1 Drastically Reduce the replication rate of SARS_Cov_2 (**viral load):**

The proposed nutraceutical composition contains more than one Phyto-compounds that will attack the multiple target sites present in SARS-CoV-2 which includes Main Protease (MPro)/ 3-Chymotrypsin-Like Protease (3CLpro), Papain like Protease (PLpro), Angiotensin Converting Enzyme 2 (ACE 2), Helicase, RNA-dependant RNA polymerase (RdRp), Furin, Trypsin and various structural proteins like Spike Glycoprotein (S), Envelope Protein (E), Nucleocapsid (N) Protein.

2 Moderate and regulate the immune and inflammatory response (Cytokine storm) and avoid immune system destroying vital organs: Controlling the inflammatory response is extremelyimportant along side targeting the virus. Therapies inhibiting viral infection and regulation of dysfunctional immune responses may synergize to block pathologies at multiple steps. Our proposed herbal composition contains the nutraceutical compounds to provide the

supportive treatment to take care of the symptoms and/or syndromes appeared due to SARS-CoV-19 infection. Cytokine storm has ripple effects across the body. Elevated levels of cytokines such as TNF can cause septic shock and multi-organ failure. These may result in myocardial damage and circulatory failure observed in some patients. Older people (those aged over 60 years) and people with co-morbidities are more likely to develop such a dysfunctional immune response that causes pathology and fails to successfully eradicate the pathogen. Hence, we carefully chose the active constituents with well-established and effective anti-inflammatory and immuno-modulating agents.

3 Avoid Thrombosis (Blood clotting):

Biopsy studies across the world revealed that the one of death cause in COVID-19 is abnormal blood clotting. In addition to pneumonia affecting the small air sacs within the lungs, scientists also found the hundreds of small blood clots throughout the lungs. This scenario is not seen with other types of lung infection, and explains why blood oxygen levels fall dramatically in severe COVID-19 infection. Blood clots were observed in other parts of body as well. This is like a storm of blood clots. It is very necessary to stop blood clotting in order to avoid the death of the COVID-19 patients as mainly deaths are caused due to blood clotting. Interestingly our formulation also contains the compounds which help in reducing blood clotting thereby ultimately helpful for the treatment of COVID-19.

4 Protects Cardiovascular System:

Recent study published in The Lancet reports SARS-CoV-2 is a vasculotropic virus, meaning that it affects the blood vessels. In most of the severe cases inflammation of heart is also reported.40% of deaths from Covid-19 are related to cardiovascular complications, and hence the disease look like a vascular infection instead of a purely respiratory one because as explained above storm of blood clots is also major cause of deaths. The virus enters the lung, it destroys the lung tissue which leads to severe coughing. Severe coughing causes the destruction of the lung tissue breaks destroying and opening of some blood vessels. Then the Virus starts to infect endothelial cell after endothelial cell, creates a local immune response, and inflames the endothelium of blood vessels. The endothelial cell layer is in part responsible for clotting regulation, it inhibits clot formation through a variety of ways. If it gets disrupted, blood clotting is potentially promoted. Endothelial damage might account for the high rates of cardiovascular damage and seemingly spontaneous heart attacks in people with Covid-19, too. Damage to endothelial cells causes inflammation in the blood vessels, and that can cause any plaque that is accumulated to rupture, causing a heart attack and ultimately death. To protect the cardiovascular system inflammation of endothelium and blood clotting prevention is of prime importance. All of our components inhibit the inflammation and blood clotting and hence our formulation will have profound cardio-protective effects which will increase the survival rate of COVID-19 patients to great deal.

5 Synergy of action due to multiple targets sites:

As explained above, we have nine phyto-constituents working on ten different target sites of SARS-CoV-2 hence majority of compounds naturally work synergistically for example one of the well-established phyto-constituentis well known to enhance the biological activity of other three phyto-constituents. We have also ensured that all

possible targets involved in the entry and replication in to the human cell shall be targeted. Our scientific council has come up with nine phyto-constituents attacking ten target enzymes and proteins. Since all nine molecules can simultaneously inhibit multiple sites. Hence our composition will have synergistic activity by following four ways –

I) Natural synergy as explained above with example well established phyto-constituent.

II) One phyto-constituent will exert synergy by inhibiting multiple targets of the SARS-CoV-2 simultaneously.

III) Multiple phyto-constituents will exert synergy by inhibiting multiple targets of the SARS-CoV-2 simultaneously.

IV) Phyto-constituents will provide another synergy mechanism since they not only exert the anti-SARS-CoV-2 activity, but they also have anti-inflammatory, immunity enhancing and anti-coagulant activity which is of utmost important in the management of COVID-19.

As our composition has broad-spectrum anti-bacterial, anti-viral and anti-fungal activity, it will prevent bacterial co-infection in COVID-19 patients which already have weakened immune system as SARS-CoV-2 attacks T lymphocytes.

6. Dose determination

Each serving 50ml contains (approx.):		**%RDA as per ICMR**
Curcuma longa L.	1052.00 mg	#
Camellia sinensis	888.89 mg	#
Rutin	736.84 mg	#
Citrus reticulata Blanc.	666.67 mg	#
Glycyrrhiza glabra extract (as Glycyrrhizin 20%)	500.00 mg	#
Scutellaria baicalensis (as Baicalin 50%)	352.94 mg	#
Arachis hypogaea (As Luteolin 98%)	200.00 mg	#
Quercetin Powder	100.00 mg	#
Piper nigrum (As Piperine)	15.00 mg	#

Nutritional information per 50ml (approx.)		**% RDA as per ICMR**
Energy	76.20 kcal	4.01
Protein	1.09g	1.98
Carbohydrate	17.96g	#
(Total Sugars	19.23g)	
(Added Sugars	1.320g)	
Fat	0.00g	0.00
Sodium	66.58mg	3.5

% RDA expressed as per Sedentary Work Women
# % RDA Not established in ICMR

Ingredients: Purified water, Sweetener [INS 420(ii)], Honey, *Curcuma longa* Extract, *Camellia sinensis* Extract, Rutin, *Citrus reticulata* Blanc., *Glycyrrhiza glabra* Extract, Lubricant (INS 1503), *Scutellaria baicalensis*, Acidity Regulators [INS 331(i), INS 330], *Arachis hypogaea*, Thickening Agent (INS 466), Preservative (INS 211), Quercetin Powder, Thickening Agent (INS 415), Preservatives (INS 219 & INS 386), *Piper nigrum* Extract, Preservative (INS 217).

6. Toxicity study

6.1Acute toxicity study

Acute Toxicity Study of Vedicinal 9 by Oral Route in Sprague Dawley Rats.

Objectives: The objective of the study was to determine the possible health hazards likely to arise in Sprague Dawley healthy Rats following single oral exposure of VEDICINAL9 followed by a 14-day observation period. The study was estimated to provide LD50 cut-off value and LD50 range as per GHS. VEDICINAL9 was supplied by IGES efficiency solutions India Private Limited, Pune, India. The study was performed in accordance with OECD guideline for testing of chemicals, No. 423, entitled 'Acute Oral Toxicity- Acute toxic class method'

Based on the results of this study, i.e., 'Acute Toxicity Study of VEDICINAL9 by Oral Route in Sprague Dawley Rats', the Median Lethal Dose (LD50) of VEDICINAL9 upon a single oral administration to female Sprague Dawley rats, in accordance with Globally Harmonized Classification System is Category 5 (>5000 mg/kg of body weight).

The LD50 cut off value is 5000 mg/kg of body weight.Globally Harmonized Classification and Labelling of Chemicals: Category 5.

7 Days Repeated Dose Toxicity Study of VEDICINAL9 by Oral Route in Sprague Dawley Rat.

Objectives: The objective of this pilot study was to find the dose ranges of VEDICINAL9 for the subsequent 28-day toxicity study in Sprague Dawley rats following 7 days repeated oral dose administration. The study will provide information on the toxic effects and information for selection of doses for 28-day toxicity study of VEDICINAL9. The test item was supplied by IGES Efficiency solution Pvt. Ltd., Pune, India. The study was performed in accordance with New Drugs and Clinical Trials Rules (Gazette of India, 2019).
Gross pathological examination showed minimal focal congested area in glandular stomach of one 1000 mg/kg dose group animal. Microscopic observations revealed minimal focal congested blood vessels.

Based on the results obtained in the present study conditions, it can be concluded that VEDICINAL9 when administered repeatedly for 7 days via oral route to Sprague Dawley rats resulted in normal body weight gain, non-significant increase in values of WBCs and RBCs, increase in spleen and thymus weights. These changes do not reveal any abnormal effects which could be correlated to the treatment of VEDICINAL9. In the present study conditions, it can be concluded that doses up to 1200 mg/kg are safe when administered for 7 days consecutively to SD rats.

28 Days Repeated Dose Toxicity Study of VEDICINAL9 by Oral Route in Sprague Dawley Rat with 14 Days Recovery Period.

Objective: The objective of this study was to evaluate toxicity, if any of VEDICINAL9 after repeated daily oral administration to Sprague Dawley rats for a period of 28 consecutive days. The recovery group animals observed for a treatment free period of 14 more days to evaluate reversibility, persistence or delayed occurrence of toxic effects. The study also provided information on major toxic effects, target organs and No-Observed-Adverse-Effect-Level (NOAEL) of VEDICINAL9 in rats for establishing safety criteria in humans. VEDICINAL9 was supplied by IGES efficiency solutions India Private Limited., Pune, India. The study was performed in accordance with New Drugs and Clinical Trials Rules (Gazette of India, 2019).

No test item related adverse effects were observed in body weights and Feed consumption. There was significant decrease in PT in G4 females and PT and APTT in G4R males. PT values significantly increased in G4R females. Decreased clotting time observed might be related to the pharmacological effect of test item and not a toxic effect. Significant increase in GPT values in G3 males and GOT values in G4 females, were not considered to be related to treatment due to absence of dose dependency. Some changes observed in absolute weights of brain, testes and ovaries were not considered to be related to treatment due to lack of dose response and correlating histopathology. No treatment related histopathological changes were observed. Incidental observations of minimal severity were observed in both Control (G1) and High dose (G4) group animals. Based on the above findings, the NOAEL for VEDICINAL9 is considered to be 1000 mg/kg in both sexes after 28 days repeated oral administration in Sprague Dawley rats.

6.2 Subacute toxicity study

7 Days Repeated Dose Toxicity Study of Vedicinal 9 by Oral Route in Sprague Dawley Rat.

Objectives: The objective of this pilot study was to find the dose ranges of VEDICINAL9 for the subsequent 28-day toxicity study in Sprague Dawley rats following 7 days repeated oral dose administration. The study will provide information on the toxic effects and information for selection of doses for 28-day toxicity study of VEDICINAL9. The test item was supplied by IGES Efficiency solution Pvt. Ltd., Pune, India. The study was performed in accordance with New Drugs and Clinical Trials Rules (Gazette of India, 2019).

Gross pathological examination showed minimal focal congested area in glandular stomach of one 1000 mg/kg dose group animal. Microscopic observations revealed minimal focal congested blood vessels.

Based on the results obtained in the present study conditions, it can be concluded that VEDICINAL9 when administered repeatedly for 7 days via oral route to Sprague Dawley rats resulted in normal body weight gain, non-significant increase in values of WBCs and RBCs, increase in spleen and thymus weights. These changes do not reveal any abnormal effects which could be correlated to the treatment of VEDICINAL9. In the present study conditions, it can be concluded that doses up to 1200 mg/kg are safe when administered for 7 days consecutively to SD rats.

28 Days Repeated Dose Toxicity Study of VEDICINAL9 by Oral Route in Sprague Dawley Rat with 14 Days Recovery Period.

Objective: The objective of this study was to evaluate toxicity, if any of VEDICINAL9 after repeated daily oral administration to Sprague Dawley rats for a period of 28 consecutive days. The recovery group animals observed for a treatment free period of 14 more days to evaluate reversibility, persistence or delayed occurrence of toxic effects. The study also provided information on major toxic effects, target organs and No-Observed-Adverse-Effect-Level (NOAEL) of VEDICINAL9 in rats for establishing safety criteria in humans. VEDICINAL9 was supplied by IGES efficiency solutions India Private Limited., Pune, India. The study was performed in accordance with New Drugs and Clinical Trials Rules (Gazette of India, 2019).

No test item related adverse effects were observed in body weights and Feed consumption. There was significant decrease in PT in G4 females and PT and APTT in G4R males. PT values significantly increased in G4R females. Decreased clotting time observed might be related to the pharmacological effect of test item and not a toxic effect. Significant increase in GPT values in G3 males and GOT values in G4 females, were not considered to be related to treatment due to absence of dose dependency. Some changes observed in absolute weights of brain, testes and ovaries were not considered to be related to treatment due to lack of dose response and correlating histopathology. No treatment related histopathological changes were observed. Incidental observations of minimal severity were observed in both Control (G1) and High dose (G4) group animals. Based on the above findings, the NOAEL for VEDICINAL9 is considered to be 1000 mg/kg in both sexes after 28 days repeated oral administration in Sprague Dawley rats.