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Review Article

Potential Of Dietary Flavonoids In The Prevention And Therapy Of Covid-19 : Focusing In Mast Cell - Calcium Ion Channnel Axis

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Abstract: Syndrome Acute Respiratory Syndrome Corona Virus-2 (SARS COV2) is the virus that causes the COVID-19 disease and has caused more than 4 million deaths worldwide. This virus infects the host cell through the interaction between the virus's glycoprotein S molecule with the ACE2 which is the virus receptor, binding, undergoes membrane fusion and enters the cell and replicates in it. Currently, several strategies used in developing anti-viral compounds are targeting compounds that play a role in the process of entering the virus into host cells such as ACE2, S glycoprotein, and TMPRSS2, while some target main proteases such as RNA dependent Polymerase and N proteins. On the other hand, one of the causes for the worsening of COVID-19 cases is hyperinflammation. This condition can also be caused by an increase in calcium consumption activity which is responsible for the process of viral endocytosis, mast cell recruitment, and also the recruitment of surrounding cells to form syncytia. Under these conditions, virions that are trapped and accumulated in the syncytia can initiate the release of virions and pro-inflammatory molecules, leading to hyperinflammation and second week crash. This review will explain the importance of the role of calcium ions and mast cells in mediating inflammation as well as the prospect of inhibiting hyperinflammation in COVID-19 using flavonoid compounds contained in daily food ingredients.

Keywords: SARS-COV2; COVID-19; Mast Cells; Ion Calcium Channel.

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1. Introduction

Severe Acute Respiratory Syndrome Corona Virus-2 (SARS-CoV-2) that caused Corona Virus Disease-19 (COVID-19) has been infected more than 4 million cases and reached over 300,000 cases of death worldwide. Since 2020, COVID-19 has been declared as pandemic by the World Health Organization (WHO) [1]. Recently, several strategies have been implemented to prevent virus transmision, such as reduced mobility and physical contact. In addition, vaccine strategies is also implemented to achieve herd immunity. At least in April 2021, more than 700 doses have been administered worldwide in 169 countries [2]. However, limited information related to COVID-19 immunity, it still unclear how many people should be vaccinated to achieve herd immunity.

Beside vaccinations, exploration of anti viral agent are also done extinsively. Several agents that have been recommended by the WHO is remdesivir [3], while several agents are evaluated such as azitromisin, anti malarial drugs hydroxychloroquine and chloroquine [4], anti helminthic ivermectin [5], and HIV protease inhibitor such as Lopinavir [6]. However, some agent shown undesirable outcome. For instance, chloroquine was only efficient in vitro [7] and not in vivo [8], this may due to the virus was fused with the cell through TMPRSS2 and not endocytosis. This was strengthened by recent evidence that administrations of camostate mesilate, which TMPRSS2 inhibtor was able to reduced viral load [9]. However, it has been proposed that anti viral agent should

be supported by the administration of anti inflammatory agent. Since it has been well known, that COVID-19 severity were triggered by hyperinflammation response, therefore several anti inflammatory agents has been adminstered in patients such as non steroidal anti inflammatory drugs (NSAID) and anti-IL6 actemra.

During infections, glycans molecul can deceive the immune system as well as play role to enter the cells, similar to that HIV and influenza [10]. A computational study showed that glycan N343 of SARS-CoV-2 RBD act as a wrench that pulled up the RBD position which facilitate access to the host's ACE2 receptor [11]. Such analogy is similar to the tip of bike pump that pulled and locked the tire valve and pushed air entering the valve. Similar to that, glycan caused spike proteins more flexible by forming switch like structure on the bottom part of spike protein. This allowed the virus easier to scan which part of host cell surface that can be interacted with [12] one of herbal such as quercetin has been shown to have interactions with the RBD.

The virus also infected the cell by activating the calcium channel. Activated calcium channel caused cell host releasing its lipid layers of the cells to bind with surrounding cells that not yet infected and formed a unified structured consisted with 20 nuclei called syncytia [12], [13]. Braga et al (2021) found that overexpression of TMEM16F, which known as anoktamin 6), an ion channel that activated by calcium and scrambelase was significantly provoke syncytia formation that induced by the S protein of SARS-CoV-2. in this study, it was revealed that anti helminthic drug niclosamide was able to suppressed TMEM16F activity that responsible to phosphatidil exposure on the cell surfaces [14].

Alteration of ion concentration inside and outside the cell such as Ca^{2+} is required to activate several immune cells such as cell mast. Arlt et al (2020) is characterized as functional ion channel TPC1 that selectively regulated free calcium concentration in mast cell, regulated mast cell activity, and histamine secretion [15], mast cell appears to play a role during SARS-COV2 infections. Wu et al (2021) reported that administration intranasal antigen of SARS-CoV-2 to inbride mice ACE2 induced cell mast degranulation in peri-bronchus [16]. This review will discuss calcium channel axis with mast cell during infections of SARS-CoV-2 that implied to hyperinflammations. We also discussed the potential of dietary flavonoid in prevention and therapy of COVID-19, in particular mass cell stabilizer and calcium channel inhibitor. This is based on Greene et al (2021) that previously founded negative association between mediterranean diet with the COVID-19 number of cases and death [17].

2. Role of calcium channel and mast cell during SARS-CoV-2 infection.

Bussani et al (2021) revealed that dysmorphic cells in the lungs that abundant and easier to detect in all patients that analyzed. These cells are abnormal pneumocytes that generally swallowing cytoplasm, inclusion bodies and frequently marked by bi- or tri nucleation in most of the patients, polynucleic syncytia was observed. Therefore, they were concluded that syncytia was also one of the characteristics of COVID-19 [13]. Furthermore, Zhang et al (2021) identified that syncytia was mostly consisted of CD45+ cells,which is a lymphocyte marker [18] Lin et al (2021) stated that syncytia formation in one side and facilitate viral transmission, while at the other hand, promotes pneumocytes multinuclear ability to swallow CD45+ cells and caused lymphopenia [19]. While Buchrieser et al (2020) showed that syncytia formations in cells that infected with SARS-CoV-2 and accelerated byTMPRSS2 protease and may inhibit by protein transmembrane that induced by interferon (Interferon Induced Transmembrane / IFITM) [20].

Braga et al (20121) studied effectivity of 3049 drugs that has been approved by the FDA/EMA to identify that able to block syncytia formation of vero cells fusion due to induction of S protein SARS-CoV-2. Interstingly, the study revealed that drugs with the ability to suppress the cell fusion also able to regulate intracelullar levels of Ca^{2+} ion [14]. Ca^{2+} ions so far is required by the virus to enter the cell hosts, viral gene expressions, viral protein processing, maturation and viral release [21]. To fulfill calcium requirement, many of pathogen viral induced enhancement of these ions to goes accross the cell membrane [22]. SARS-CoV-2 related infection causes hypocalcemia that has been

approved positively associated with severity of COVID-19 [23]. Hypocalcemia was also frequently observed in COVID-19 patients with mild to severe symptomps [23], [24]. This has become implied evidence that hipocalcemia was intrincly in COVID-19 and inhibited calcium ion channel as potential target therapy. All of these explanations may suggested role of calcium channel activation during SARS-CoV-2 infection.

Post mortem studies in COVID-19 (n=6) patients showed that number of mast cell increase at the wall of septum alveolar and lung parenchym of COVID-19 patients compared to pneumonia patients that induced by H1N1 (n = 10) or control speciment (n= 10) [25]. Other evidence showed by mast activations during SARS-COV2 infections was rapid viral interaction with mast cell (LAD2) cell a (5 minutes) triggered mast cell degranulation [16]. The Virus interaction with LAD2 cell via RBD interactions of spike proteins with ACE2 was hypothized to activation of ion calcium channel. This may due to extracellular Ca^{2+} is essential for release of cell mast mediators [26]. In mast cell, calcium signalling is initiated by production of 1,4,5-trisphosphate mediated phospholipase C that produced release of Ca^{2+} from reticulum endoplasma (ER) and Golgi, which later activating extracellular Ca^{2+} entering the cells. Importing of extracellular Ca^{2+} load help to reserve and maintain cytosolic Ca2+ concentrations is a mandatory signal for mast cell activation [27].

Role of calcium channel in triggering virus to enter the cell host and activating mast cell are most likely due to connection of activated TMEM16. Inhibitor TMEM16 are able to block syncytia formations in SARS COV2 induced S protein models [14]. This is similar to the phenomenon that observed in Human respiratory syncytial virus (HRSV) infection, which the inhibitor suppressed gene expression and viral release of HRSV occurred, showed prophylaxis activity after exposure [28]. Virus gene expression in cell membrane is required for syncytia formations as explained by Lin et al (2021) [19]. Furthermore, activated mast cell which release histamine and IL-4 has been known to induce expression and/or activity of TMEM16A [29], [30]. Viral Entry virus and syncytia formation by virus that mediated by calcium channel and TMEM 16 (scramblase) showed in Figure 1

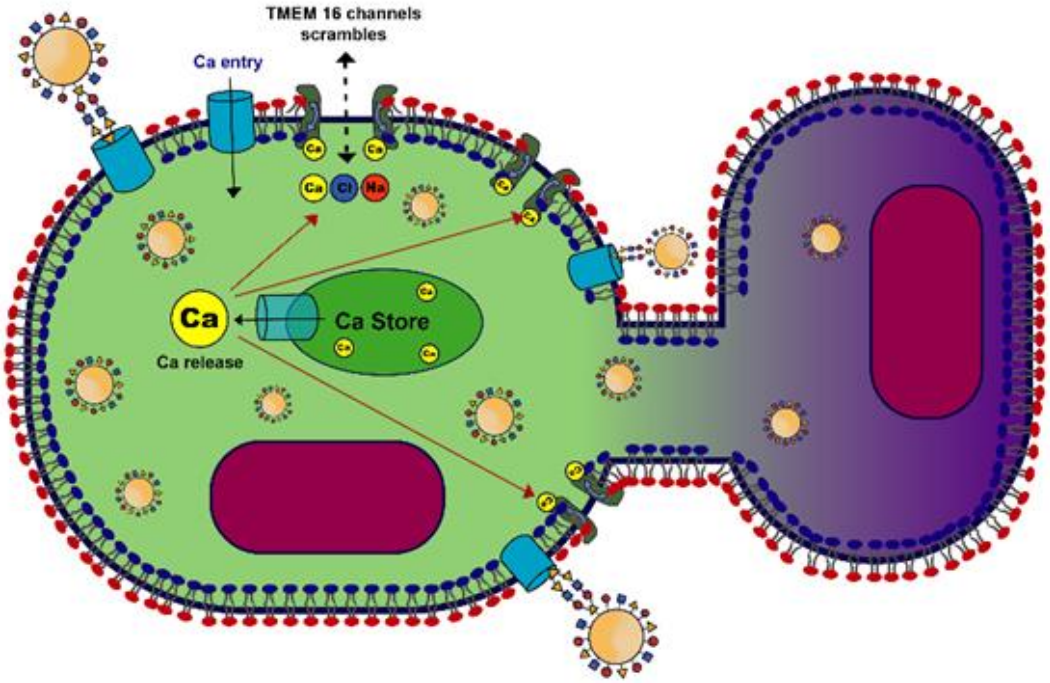

Figure 1. Viral Entry virus and Syncytia formation by virus is mediated by calcium channel and TMEM 16 (scramblase).

Virus activity in cells required thus depletes ca stock in Reticulum Endoplasmic (RE). This caused calcium channel active and cause calcium influx. Calcium Influx activated TMEM (scramblase) thus expossed lipoprotein/phosphatidil serine and interacted with healthy cell membrane surrounding the infected cell and formed syncytia. Syncytia is also occured when viral antigen expressed in cell membrane and interacted with ACE2 receptor in healthy cells. This phenomenon trigerred calcium influx kalsium due to formations extracelullar domain of TMPRSS2 required calcium ions.

3. Syncytia, Long COVID and second-week crash

Syncytia formations allow infected cells developed in long periods and produced more virion [15] this may cause due to syncytia swallowed lymphocytes and included lymphopenia, therefore affect immune response to the virus [18], [19]. Lymphopenia is a common symptomp of COVID-19. A National cohort study in South Korea on 5628 COVID-19 patients showed lymphopenia and severity can used as predictive factors of clinical outcome of COVID-19 including death, requirement of intensive treatment, and oxygen demand. The study showed that lymphopenia at early phase of COVID-19 is related to the disease poor prognosis [31].

Virus ability to form syncytia that swallowed lymphocytes caused the virus become persistent. Moreover, gene availability or virus antigen that expressed in mast cell which also occurted in HRSV infections - other respiratory virus that caused syncytia formation [32], assumed induced chronic and long term response. In case, the phenomenon was also occuring in mast cell after infections of SARS-CoV-2, this may explain Long COVID symptomps. This may cause due to mast cell become consistently activated, caused the release of inflammatory mediator, including histamine and implied to long COVID symptom. Some research team from the UK, recently revealed theraphetic benefit of histamine receptor antagonist to reduce long COVID [33]. In those studies, symptom burden was decreased in 60 % of Long COVID patients that treated with histamine antagonist receptor, including fatigue, fever, neurology and neuropsychology symptom , malaise after activity, chest pain, gastrointestinal symptom, and dermatology symptom, that mostly symptomps related to histamine signal

Syncytia formation that harbors many virion inside may lysed and cause a sudden arise of viral load in alveolar. Moreover, in case syncytia that contained virus was formed in mast cell. These may induce rapid and massive degranulation of mast cell in alveolar and released inflammation mediator. This may also explain '*second-week crash*' phenomenon in COVID-19 patients. *'Second-week crash'* is condition which patients were suddenly gotten worse in the second week after infections, that previously has mild or asymptomatic [34]. The relation between calcium influx and mast cell, related to symptoms and clinical features of SARS-CoV-2 infections shown in Figure 2.

Recent studies have shown that higher levels of plasma 2′–5′ oligoadenylate synthetase 1 (OAS1) in the non infectious state were associated with reduced COVID-19 susceptibility and severity and that the Neanderthal isoform of OAS1 in individuals of European descent conferred this protection [35]. Mast cells, OAS, apart from being known as viral recognition receptors and causing viral RNA degradation, are also known to be responsible for viral activation of human mast cells [36]. In LAD2 cells infected with vesicular stomatitis virus (VSV), LAD2 cells express several OAS proteins. and its expression level is regulated by viral infection [36]. This further strengthens the notion that the hyperinflammatory response that occurs in COVID-19 patients is influenced by mast cell activity, so that mast cell stabilizing agents such as flavonoids can be useful in inhibiting mast cell degranulation.

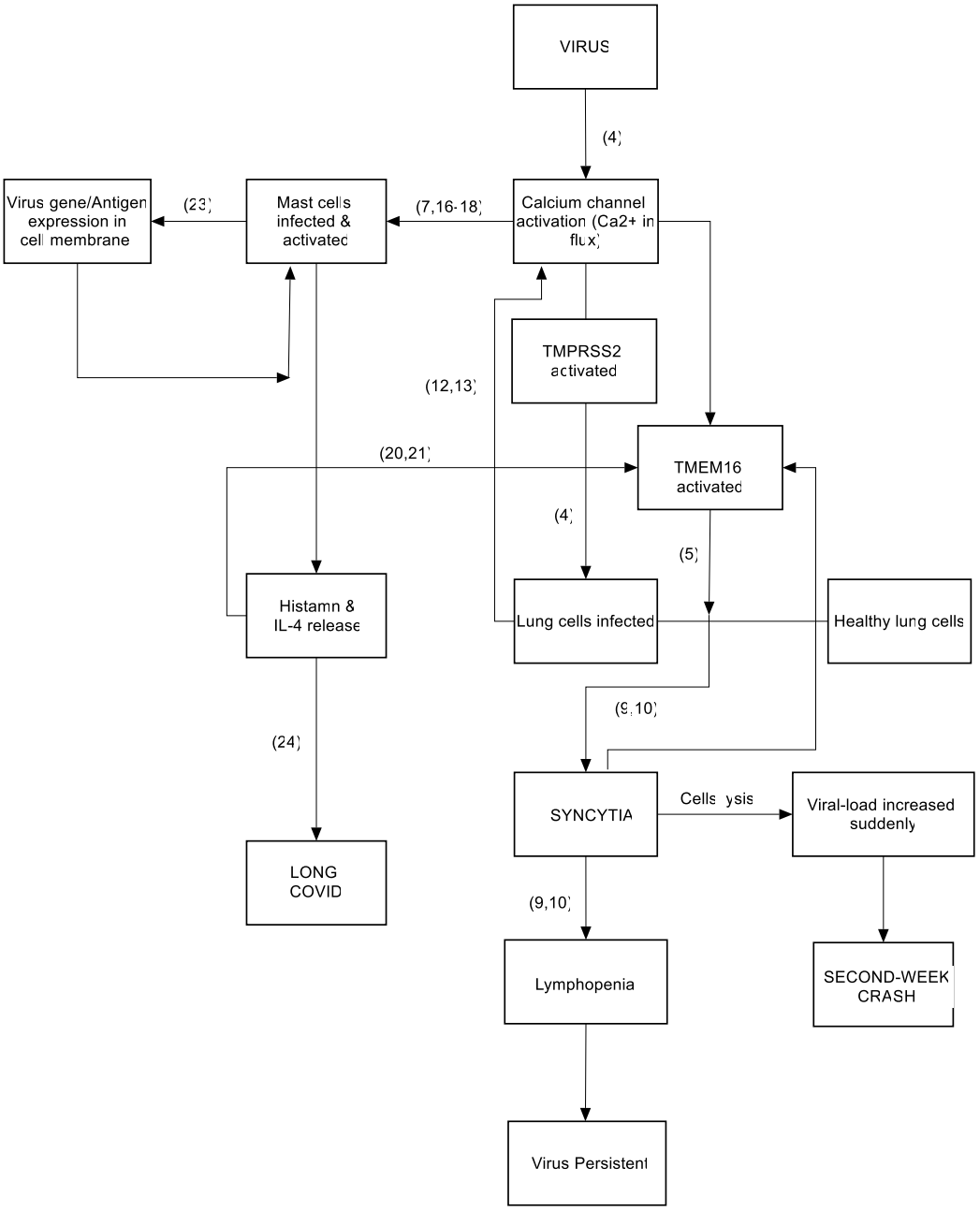

Figure 2. Relationship between calcium channels and mast cells, related symptoms and clinical features of SARS-CoV-2 infection.

The presence of the virus will activate calcium channels that cause virus entry into cells (by activating TMPRSS2) and trigger the formation of syncytia through activation of TMEM16. Substrate recognition of TMPRSS2 requires a cysteine-rich scavenger receptor (SRCR) and LDL receptor A (LDLRA) domain that requires calcium for activation. Viral gene expression, viral protein processing, viral maturation, and release then deplete calcium stores in the Endoplasmic Reticulum (ER) and Golgi, and trigger calcium influx. Scramblase activity (transposition of phospholipids between 2 cell membranes to form cell fusion/syncytia) by TMEM16 is also activated by calcium. Viruses can also infect mast cells. The increased amount of calcium in the cytoplasm of mast cells then degranulates the mast cells which triggers the release of histamine and IL-4 which activates TMEM16. Circular grooves that pivot on calcium channels and mast cells are thought to contribute to viral persistence, eliciting long covid symptoms, and hyperinflammation triggered by lysis of the syncytia.

4. Potency of Flavonoid diet to Control Hyperinflammatory State in COVID-19

Table 1. Mechanism and Literature's doses of Flavonoid in Calcium Ion channel – mast cell axis pathways.

Mechanism	Flavonoids	Doses	References
TMEM16 inhibitor	Liquiritigenin, luteolin, galangin, quercetin and fisetin.	4.5 to 15 μM (IC50)	(44)
	liquiritigenin	12.89 μM (IC50)	(45)
	Gallotanin	6 μM (IC50)	(46)
	Silibinin	30.90 μM (IC50)	(47)
	EGCG	100 μM (IC50)	(48)
Iinflux calcium channel inhibitor	Quercetin, kaempferol, myricetin, morin	10 μM (IC50) -Quercetin, kaempferol, myricetin 100 μM (IC50)- morin	(49)
	Naringenin	100 μM (IC50)	(49)
Mast cell degranulation inhibitor.	Quercetin, kaempferol, myricetin, dan morin	10 μM -decreased release of IL-8 (quercetin: 63%, and kaempferol: 49%) 100 μM- decrease release of IL-8 (quercetin: 87%, kaempferol: 82%, myricetin: 36%, morin: 55%) 10 μM- decreased release of TNFα (quercetin: 83%, kaempferol: 60%, myricetin: 41%, morin: 48%) 100 μM- decreased release of TNFα (quercetin: 90%, kaempferol: 90%, myricetin: 51%, morin: 70%) 10 μM- decreased release of triptase (quercetin: 50%, myricetin: 50%) 100 μM- decreased release of triptase (quercetin: 79%, kaempferol: 90%, myricetin: 95%, morin: 36%) 10 μM- decreased release of histamin (quercetin: 29%, myricetin: 37%) 100 μM- decreased release of histamin (quercetin: 52%, kaempferol: 67%, myricetin: 95%, morin: 28%)	(49)
	Luteolin, apigenin	< 1 μM (IC50)- decreased release of TNFα (luteolin) 1-2 μM (IC50) - *decreased release of* IL-4 & IL-13 (luteolin dan apigenin)	(50)

Greene et al (2021) showed a negative association between mediterranean diet with a number of case and death of COVID-19 [17]. Flavonoid is a component of mediterranean diet, in particular from fruits, seeds, and vegetables [37]. Several flavonoid has been found can stabilize the mast cell, inhibit calcium channels and inhibitors of TMEM16. Recent studies have shown that higher level of serum 2′–5′ oligoadenylate synthetase 1 (OAS1) in non-communicable conditions and linked to decrease of susceptibility and severity of COVID-19 and isoform of Neanderthal of OAS1 in European decendant shown protections of these [35]. The OAS1 known as a viral recognition receptor in mast cell that leading to degradation of viral RNA, it has been responsible to human mast cell activation by a virus [36]. One studies that suggesting this was LAD2

cells that infected with Stomatitis Vesicular Virus (VSV), the LAD2 expressed several OAS proteins and demonstrated that its expression level was regulated by viral infections [36]. These was strengthen our hypothesize that hyperinflammator response in COVID-19 patients was regulated by the activity of mast cells, thus the stabilisator egents such as flavonoid is potential to inhibit mast cell degranulation.

Based on the table 1, determinations of plants with those compounds can be performed. We provide of which plants has compounds using a database of "IJAH analytics" (http://ijah.apps.cs.ipb.ac.id) [43]. Compounds in food including herbs, seeds, fruits and vegetables was provided using "foodb" database (http://foodb.ca by considering daily diets). Keywords of compounds shown in Table 1 while the results shown in Table 2.

Table 2. Types of flavonoids and plant sources*

Flavonoids	Mechanism of Action	Plant Sources
Apigenin	Mast cell degranulation Inhibitor	Parsley (h/s), Malabar spinach (v), Celery leaves (h/s), Kumquat (f), Wild celery (h/s), Wild carrot (v), Common oregano (h/s), Juniperus communis (h/s), Peppermint (h/s), Pak choy (v)
EGCG	TMEM16 inhibitor	Black tea, Green tea, Red tea, Herbal tea
Fisetin	TMEM16 inhibitor	Soy bean (g)
Galangin	TMEM16 inhibitor	Mexican oregano (h/s)
Gallotanin	TMEM16 inhibitor	Nance (f), Red tea, Herbal tea, Green tea, Black tea, Black walnut, Pomegranate (f), Evening primrose (h/s), Bilberry (f), Guava (f)
Kaempferol	calcium channel inhibitor, mast cell degranulation inhibitor	Saffron (h/s), Capers (h/s), Cumin (h/s), Chinese mustard (v), Welsh onion (h/s), Cloves (h/s), Cabbage (v), Dill (h/s), Rocket salad (v), Caraway (h/s)
Liquiritigenin	Inhibitor TMEM16	Alfalfa (h/s), Chickpea (p), Common pea (p), Liquorice, Abiyuch (f), Acerola (f), Acorn (n), Adzuki bean (p/b), Apricot (f)
Luteolin	TMEM16 inhibitor, mast cell degranilation Inhibitor	Common oregano (h/s), Wild celery (h/s), Anise (h/s), Juniperus communis (v), Mexican oregano (h/s), Common thyme (h/s), Wild carrot (v), Angelica (h/s), Chicory (v), Celery leaves (h/s)
Morin	Calcium channel inhibitor, cell mast degranulation inhibitor	Strawberry (f)
Myricetin	Calcium channel inhibitor, cell mast degranulation inhibitor	Common walnut (n), Carob (v), Fennel (h/s), Bog bilberry, (f) Red wine/ dark-colored (black) grape (f) , Blackcurrant (f), European cranberry, Parsley (h/s), Dock (h/s), Blackberry (f)
Naringenin	calcium channel inhibitor	Sweet orange (f), Common oregano (h/s), Mexican oregano (h/s), Kumquat (f), Grapefruit (f), Pummelo (f), Rosemary (h/s), Globe artichoke (v), Mandarin orange (f), Tarragon (h/s)
Quercetin	TMEM16 inhibitor, calcium channel inhibitor, cell mast degranulation	Evening primrose (h/s), Garden onion (v), Lovage (h/s), Capers (h/s), Dock (h/s), Juniperus communis (v), Mexican oregano (h/s), Black elderberry (f), Angelica (h/s), Black chokeberry (f)
Silibinin	TMEM16 inhibitor	milk thistle (Silybum marianum)

*Taken from top 10 fruits (f), vegetables (v), herbs/spices (h/s), nuts (n), peas/beans (p/b) and grains (g) with the highest average content (http:/ /foodb.ca)

In addition, the mechanism of calcium influx as in myocytes occurs via calcium channels with voltage gates induced by oxidants (H_2O_2 and OH radicals) [38]. The formation of OH and H_2O_2 radicals is initiated by xanthine oxidase (XO) activity in lung epithelial cells, as shown in Figure 2. occurs in RSV infection [39], [40]. It is known that RSV infection can also form alveolar syncytia [41]. Nagao et al (1999) mentioned that planar flavones and flavonols with 7-hydroxyl groups such as chrysin, luteolin, kaempferol, quercetin, myricetin, and isorhamnetin inhibited XO activity at low concentrations (IC50 values from 0.40 to 5.02 m) in mixed type mode, whereas nonplanar flavonoids, isoflavones and anthocyanidins were less inhibitory [42]. Thus XO has the potential to be a therapeutic target for SARS-CoV- infection. 2. In addition, like the flavonoid diet, a diet rich in antioxidants such as those contained in fruits, vegetables, and whole grains, is beneficial during the COVID-19 pandemic.In table 1, we described types of flavonoids and its mechanism related to our previous explaination.

Conclusion and Future Perspective

In severe cases of COVID-19, hypocalcemia was observed. This may caused due to calcium influx into cells, which later to facilitate the process of viral endocytosis, activate mast cells and induce the formation of syncytia. In such conditions, hyperinflammatory conditions can be caused through several pathways as previously mentioned. Potential Flavonoid compounds are known to inhibit several pathways that activate calcium influx such as TMEM16. Thus, it is important to consider the use and research of calcium influx inhibition into cells as an effort to prevent or worsen COVID-19. In particular, potential of dietary flavonoids

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