The role of flavonoids in maintaining epithelial barrier : A review

〔REVIEW〕

The role of flavonoids in maintaining epithelial barrier : A review

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Abstract

Flavonoids are natural compounds found in several fruits, flowers and vegetables. It has broad pharmacological properties such as anti－inflammatory, anti－oxidants, anti−carcinogenic, anti−diabetic, neuroprotective and anti－lipidemic. The epithelium maintains its barrier integrity through epithelial junctional molecules. However, with increasing inflammatory stimuli, the expression of epithelial junctional molecules alters thereby affecting the barrier function. The changes in epithelial junctional molecules are mostly due to high oxidative stress. The anti−inflammatory and anti−oxidative effect of flavonoids could be useful in improving the barrier function of epithelium. The aim of this study is to review the role of various flavonoids in maintaining epithelial barrier integrity.

Introduction

Flavonoids are a group of natural substances with different phenolic structures extracted from several fruits, flowers and vegetables (Panche et al., 2016). The interest on flavonoids is growing among researchers because of its diverse pharmacological benefit such as anti－inflammatory, anti－oxidants, anti－carcinogenic, anti－diabetic, neuroprotective and anti−lipidemic effect (Brodowska KM, 2017). Their anti−oxidative and anti−inflammatory properties are well documented among all these. Flavonoids can effectively oxidize the free radicals generated beyond physiological limit and reduce the oxidative stress thereby limiting the inflammatory cascade (Tsuji et al., 2013).

The epithelium is a major defense system of any tissue acting as a barrier to various insults. The integrity of epithelium is maintained by epithelial junctional molecules (Balda & Matter, 2008). However, with increasing inflammatory stimuli or aging process, these epithelial junctional molecules alter their expression resulting in diminished function of the tissue. These changes in epithelial junctional molecules are mostly due to high oxidative stress during the processes (Poljsak et al., 2013). Anti−oxidative and anti−inflammatory properties of flavonoids could contribute in safeguarding epithelial barrier junctions (Brodowska KM, 2017). Although the effectiveness of flavonoid on epithelial junctional molecules in various tissues have been studied, a comprehensive and focused review is still lacking. In this review, we will summarize various types of flavonoids, its anti−oxidative and anti−inflammatory effect and focus on its effect on epithelial junctional molecules.

Flavonoids : Polyphenols, a group of bioactive molecules, have been studied for their health promoting effect and their results are promising (Panche et al., 2016). Polyphenols have two general classes ; flavonoids and phenolic acids. Flavonoids are abundantly found in foods and beverages of plant origin, such as fruits, vegetables, tea, cocoa and wine ;

hence termed as dietary flavonoids. More than 8000 flavonoid molecules have already been identified which are broadly grouped under six types ; flavones, flavonones, flavonols, flavan−3−ols, isoflavones and anthocyanides (Panche et al., 2016) (Fig.1). The flavonoids can directly interfere with signal transduction enzymes such as tyrosine, protein and phosphatase kinases. This interference leads to wide biological activities such as anti−oxidant, anti−inflammatory, anti−carcinogenic, anti−bacterial, anti−viral and anti−lipidemic properties (Brodowska KM, 2017). Epidemiological evidence suggest that a high intake of plant products is associated with a lower risk of chronic diseases such as cardiovascular diseases, metabolic diseases such as diabetes and hyperuricemia, inflammatory colitis etc., (Eddouks et al., 2014). These properties make flavonoid a candidate natural compound which could be used for diverse health benefit.

Epithelial barrier function : Epithelium is a dynamic cellular layer that serves as a barrier to entry of allergen, toxin and pathogens. The barrier function is mainly maintained by epithelial junctional molecules, a part of an interconnected network of transmembrane protein complexes that include tight junctions (TJ), adherens junctions, gap junctions and desmosomes (Giepmans & van IJzendoorn, 2009). These are connected to actin cytoskeleton through scaffold proteins such as zona occludens (ZO−1, −2, −3). TJ, which are composed of complex network of protein interactions, are the primary determinants of barrier function in paracellular space. Claudins are the major proteins of TJ and 27 types of claudins have been discovered till date. Claudins are further divided into pore sealing and pore forming proteins. Claudins−1, −3, −5, −10a, −11, −14, and −19 are sealing proteins and are responsible for watertight stability of the cell. Claudins −2, −10b, −15 and −17 are pore forming proteins and allow passage of cations (Balda & Matter, 2008). Adherens junction are calcium−based junctions typically involved in development and tissue homeostasis. E−cadherin is a core protein of adherence junctions that is required for maintaining stability and homeostasis. Gap junctions acts as a bridge between adjacent cells and are composed of connexin protein subunits that allow intercellular communications (González−Mariscal et al., 2003). These proteins are affected under several pathophysiological conditions including epithelial barrier related diseases such as inflammatory bowel disease, Crohn's disease, ulcerative colitis, and epithelial malignancies.

Flavonoids in maintaining epithelial barrier integrity

Flavonoids mainly act through modulation of oxidative stress and inflammatory cytokines mediated pathway to maintain the epithelial barrier integrity. The anti−oxidant activity of flavonoids depends upon the arrangement of functional groups about the nuclear structure. The configuration, substitution, and total number of hydroxyl groups substantially influence several mechanisms of antioxidant activity such as radical scavenging and metal ion chelation ability (Kumar & Pandey, 2013). The antioxidant capacity of flavonoids is basically through direct or indirect mechanism. Direct mechanism includes the scavenging of toxic compounds such as ROS or chelating metal ions to produce inert and non−toxic compounds. These actions are mediated by

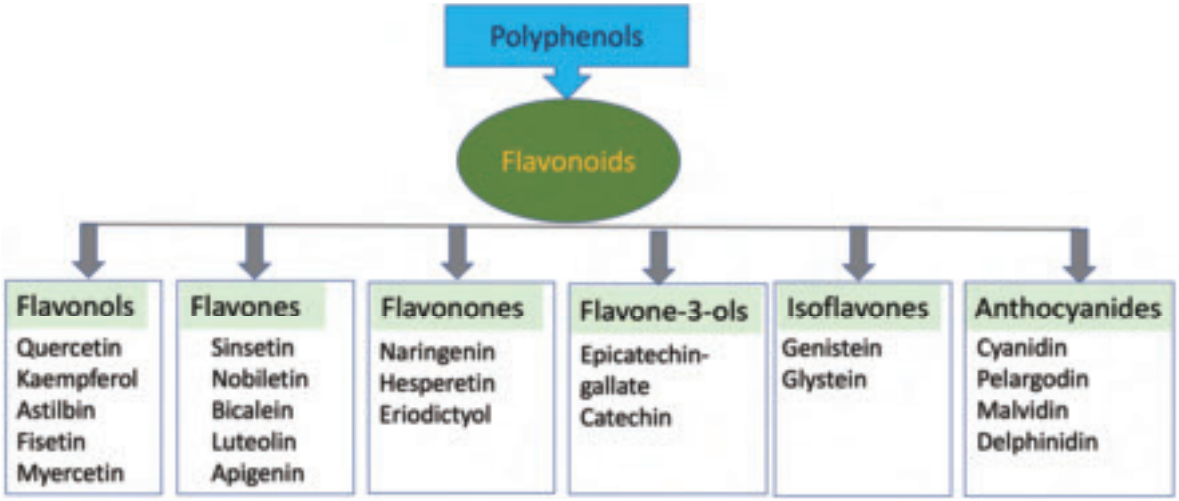

Fig. 1 : Six major groups of flavonoids. Few known molecules are listed for each flavonoid group.

enzymes such as superoxide dismutase (SOD), glutathione transferase (GTX) and Catalases (CAT). Indirect mechanism converts the remaining toxic compounds into water soluble compounds through series of enzymatic reactions (Tsuji et al., 2013). The indirect mechanism is mediated by phase II enzyme such as glutathione S−transferases (GST), uridine 5'−diphospho−glucoronyl transferases (UGTs) and sulfotransferases (fig 2).

The imbalance in oxidative cycle generates production of great amount of nitric oxide and leukotrienes. These mediator releases cytokines such as interleukin (IL)−1b, IL−6 and tumor necrosis factor alpha (TNF− α), chemokines or adhesion molecules responsible for the activation of inflammatory signal transduction pathway (Rao, 2008). These cytokines could phagocyte epithelial barrier molecules by internalization, as a micropinocytosis (Al−Sadi, 2009). Several inflammatory cytokines were responsible for activation of myosin light chain kinase (MLCK) pathway involved in degradation of barrier molecules (Al−Sadi, 2009). Flavonoids may contribute to maintain epithelial integrity via inhibition of inflammatory cascade and activation of phase II anti−oxidant detoxifying enzymes. Several flavonoids inhibited pro−inflammatory enzymes such as cyclooxygenase−2, lipoxygenase and inducible nitric oxide and activate mitogen activated protein kinase (MAPK), protein kinase C pathway and nuclear erythroid factor 2 pathways (Serafini et al, 2010).

Different flavonoid molecules have been studied for their role in maintaining epithelial barrier integrity (Table. 1). Their anti−oxidant and anti−inflammatory properties have been mainly discussed related to the maintenance of epithelial barrier.

Quercetin : Quercetin the most abundant flavonoid, in vegetables (such as onion and tomato), fruits (such as apple, berry and grape), beverages and medicinal plants (Xu et al., 2019). Quercetin greatly acts as a free radical scavenger (Xu et al., 2019). The preventive effects on certain diseases such as chronic inflammation, atherosclerosis and cancer have been shown (Min et al., 2007). The quercetin enhanced epithelial barrier functions in human colonic epithelial cells by increasing the expression of TJ molecule such as Claudin−1 and Claudin−4. (Suzuki & Hara, 2009 ; Amasheh et al., 2008). Enhancement of endometrial barrier function was accompanied by the increased expression of Claudin−2, −4, −5

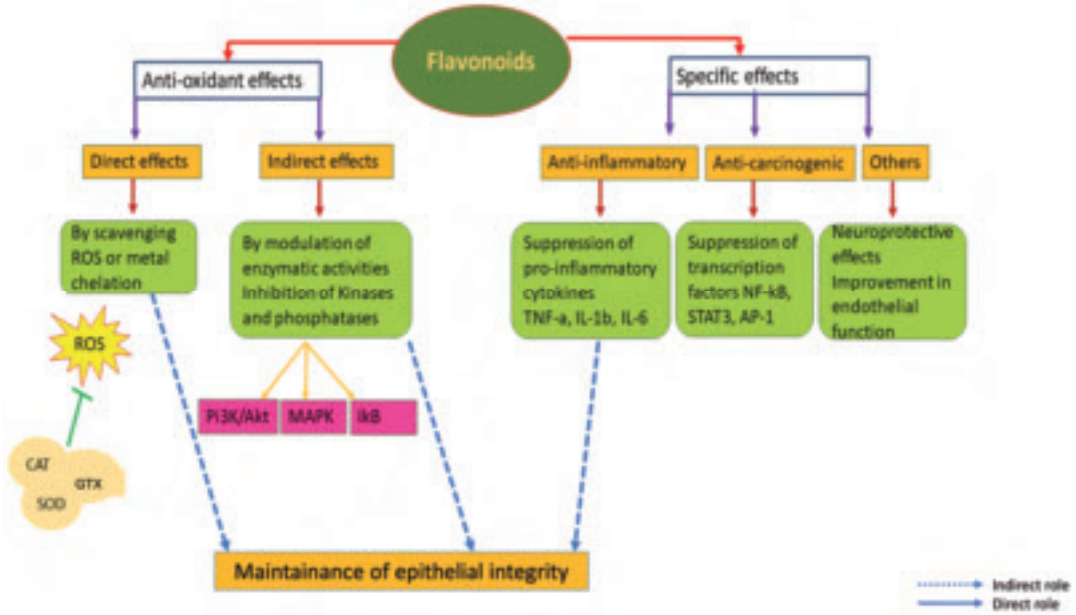

Fig. 2 : Various biological effects of flavonoid. The biological effects have been divided into anti−oxidant effects and specific effects. Anti−oxidant effect acts through direct and indirect effect. The direct effect scavenges the reactive oxygen species (ROS) produced during oxidative stress or chelates the metal ion to produce inert and non−toxic compounds. This effect is mediated by enzymes such as superoxide dismutase (SOD), glutathione transferase (GTX) and Catalases (CAT). The indirect effect converts the toxic compounds into water soluble compounds mediated by phase II enzyme such as glutathione S−transferases, uridine 5'−diphospho−glucoronyl transferases and sulfotransferases. Other specific effects such as anti−inflammatory, anti−carcinogenic and neuroprotective are mediated through various pathways as shown in the figure.

Table 1 : Flavonoid molecules, their source and studies in focus to epithelial barrier function

Flavonoid subclass	Plant source	Epithelial barrier functions		References
		In vitro study	*In vivo* study	
Quercetin	Onion, red wine, kale, olive oil, apples, cherries, berries	Treatment of quercetin in Caco−2 cells resulted in increased TER and decreased flux rate. TJ proteins Cld−1,2,3,4,5,7, occludin and ZO−2 were significantly upregulated in different studies.		(Amasheh et al., 2008)
Kaempferol	Apples, broccoli, tomatoes and tea	Kaempferol treatment in Caco−2 cells increase TJ protein Cld−1,3, occludin, ZO−1 ZO−2, with significant increase in TER		(Suzuki, Tanabe, & Hara, 2010)
Astibilin	Red wine, kale, olive oil, broccoli apples, cherries, berries, and grapefruit and tea	Astilbin treatment in Caco−2 cells increase the mRNA expression of Cld−1 and ZO−2 with significant increase in TER value		(Nakahara, Nishitani, Nishiumi, Yoshida, & Azuma, 2017)
Fisetin	Strawberries, apples, persimmons, onions and cucumbers			
Sinsetin	Orange peel	.	20−80mg/kg of Sinsetin were fed to colitis model rats for 14 days. Sinsetin restored epithelial TJ barrier integrity by inhibiting apoptosis and increasing cld−2 degradation.	(Xiong et al., 2019)
Nobiletin	Citrus fruits peel	Nobiletin restored the barrier function with significant rise in TER in Caco−2 cell treated with LPS. It significantly inhibited the MLCK pathway. However, its effects on TJ proteins were not analyzed.	20,40mg/kg of Nobiletin was administered in colitis model rats for 7 days. Significant rise in TER was seen	(Xiong et al., 2015)
Baicalin	Roots of *Scutellaria lateriflora* and *Scutellaria baicalensis*	Baicalin inhibited TNF−α−induced ZO−1 depletion by down−regulating miR−191a		(Wang, Zhang, Chen, Wu, & Kuang, 2017)
Naringenin	Grapefruit, sour oranges, tomatoes, tart cherries, beans	Treatment of Caco−2 cells with 100μM naringenin significantly raised the TER value. The raise in TER value was due to increase in protein level of cld−1, 4, occludin and ZO−2. Naringenin enhanced barrier function via activation of the transcriptional factor, Sp1		(Noda, Tanabe, & Suzuki, 2013)
Theaflavins	Black tea	TF increases occludin, Cld−1 and ZO−1 against Pg mediated periodontitis model in human gingival keratinocyte cell line and Caco−2 cell line. However, the molecular mechanism is unknown		(Park, Kunitake, Hirasaki, Tanaka, & Matsui, 2015)
Theobroma cacao	Cacao beans	Significant increase in TER after treatment of Caco−2 cells with theobromine cacao powder. However, increase in TER was independent to TJ protein expression.	There was significant increase in TER value in rat fed with cocoa powder	(Yamamoto et al., 2014)
Catechin	Green tea extracted from plant *Camellia sinensis*	Increases occludin, cld−1 and ZO−1 against Pg mediated periodontitis model in human gingival keratinocyte cell line and Caco−2 cell line. However, the molecular mechanism is unknown		(Lagha, Groeger, Meyle, &amp Grenier, 2018)
Genistein	Soybean	Genistein prevents the oxidative stress induced co−immunoprecipitation of occludin, ZO−1 and e−cadherin.		(Rao, Basuroy, Rao, Karnaky, & Gupta, 2002)
Calycosin	Active component in Astragali Radix	Calycosin suppressed LPS−induced activation of TLR4−mediated NF−κB signaling pathway thereby decreasing the expression of occludin and ZO−1	HacaT Cell	(Tao et al., 2017)

TER : Transepithelial resistance ; TJ : Tight junction ; Cld : Claudin ; ZO : Zona occludens ; LPS : Lipopolysachharide ; Pg : *Porphyromonas gingivalis*

and ZO−1 after quercetin treatment in porcine glandular endometrial epithelial cells (Poonyachoti S and Deachapunya C, 2017). The quercetin improved TJ barrier functions attributed via various signalling pathways such as PI−3 kinase, novel PKCδ, a protein kinase isoform, NF−kB, tyrosine kinase and MAPKs pathways (Min et al., 2007 ; Suzuki & Hara, 2009).

Kaempferol : Kaempferol is a natural flavonol mostly found in apples, grapes, broccolis, tomatoes and tea. Its anti−oxidants and anti−inflammatory activities allowed effectiveness in cancer chemotherapy and cardio− and neuro− protective effects (Hofer et al., 2020). The anti−oxidant property of kaempferol increased ZO−1 and occludin expressions, which restored the bacterial disruption of epithelial barrier integrity in human intestinal cell (Wallace et al., 2013). The kaempferol improved in barrier functions by increasing the expression of Claudin−3 and Occludin as well (Suzuki et al., 2010). Its anti−inflammatory properties inhibited the expression of inflammatory cytokines including IL−1b, TNF−a and IL−8 in macrophage cells and HEK 293 cells (Chen & Chen, 2013), and lipopolysaccharides (LPS)− derived NF−kB phosphorylation in intestinal microvasculature cells (Bian et al., 2018). In addition, the kaempferol downregulated MAPK, JNK, AKT and NF−kB pathways in microglial BV2 cells treated with LPS. Therefore, the kaempferol might enhance epithelial barrier by its anti−oxidant and anti−inflammatory properties.

Astilbin : Astilbin, a polyphenolic compound, isolated from the leaves of *Engelhardtia chrysolepis* Hance (Chinese name, huang−qui), found in various French wines, champagnes and turtle jelly. The anti−oxidant, anti−depressant, immunosuppressive and anti−inflammatory activity of astilbin have been demonstrated in various studies (Wang et al., 2018). However, only a few studies showed the significant effects of astilbin on epithelial barrier function. Astilbin improved the barrier functions by increasing the expression of Claudin−1 and ZO−2 in intestinal cells (Nakahara et al., 2017). Many studies showed its anti−oxidant and anti−inflammatory potencies (Zhao et al., 2020). Astilbin reduced the expression of NF−kB induced signalling events in rat glomerular mesangial cells (Chen et al., 2018). Decreased expressions of IL−6, IL−1β and PGE2 via inhibition of TLR 4/MyD88 and NF−kB signaling pathways were observed in macrophages cells by the stimulation with astilbin (Sharma et al., 2020). Therefore, anti−oxidant and anti−inflammatory properties of astilbin may enhance epithelial barrier function.

Fisetin : Fisetin is a bioactive flavonol molecule found in fruits and vegetables such as strawberry, kiwi, apple, persimmon, grape, onion, and cucumber (Sun et al., 2018). Fisetin is a potent anti−oxidant, anti−proliferative, anti−carcinogenic and anti−aging agent which have been studied both *in vitro* and *in vivo* (Kim et al., 2012 ; Giri et al., 2021 ; Prasath & Subramanian, 2014 ; Sun et al., 2018). No direct evidence for involvement of fisetin in epithelial barrier functions has been shown. Fisetin inhibited cytokine expressions via PI3K−Akt−mTOR and p38/JNK pathways, suggesting its anti−inflammatory activity (Chamcheu et al., 2019). As stated in the other flavonoid, anti−inflammatory property contributes to the maintenance of epithelial barrier. The anti−inflammatory actions by Fisetin may beneficially affect epithelial barrier. Fisetin acted as an anti−carcinogenesis by inhibiting p38 MAPK−dependent NF−kB signaling pathway in cervical cancer cells (Chou et al., 2013). Fisetin regulated signalling pathways involved in anti−carcinogenic actions often are involved in the enhancement of epithelial barrier too (Sun et al., 2018). Therefore, Fisetin may fulfill a function of the enhancement of epithelial barrier. Further studies are needed to prove these hypotheses.

Sinensetin and Nobiletin : Both sinensetin and nobiletin are polymethoxylated flavonoid isolated from orange peel. A previous study in rat colitis model showed that sinensetin administration for 14 days restored intestinal epithelial TJ barrier function by decreasing claudin−2 which is a pore forming protein (Xiong et al., 2019). The restoration of barrier integrity was due to autophagy by activating the 5' adenosine monophosphate activated protein kinase (AMPK) pathway (Xiong et al., 2019). The anti−inflammatory effect of sinensetin was also shown in LPS− stimulated RAW 264.7 cells via inhibition of NF−kB activation (SHIN et al., 2012). Nobiletin also shows potent anti−oxidant and anti−aging effect (Nohara et al., 2019 ; Yang et al., 2020). Nobiletin restored barrier functions in LPS− induced colitis in mouse model. The barrier function was improved with increase in electrical resistance between adjacent epithelial cells, accompanied by inhibition of NF−kB activation (Xiong et al., 2015). However, no studies till date showed the direct effect of Nobiletin on the expression of barrier molecules. Further studies are needed to prove its efficacy as

an epithelial barrier enhancer.

Apigenin : Apigenin is a natural product belonging to flavone class. It is mostly found in Grapefruits, plants derived beverages and vegetables such as celery, onions, oranges, maize, rice, tea etc., (Ali et al., 2016). It has shown its anti−oxidants and anti−inflammatory role in treating various inflammatory diseases. Various *in vitro* and *in vivo* studies have suggested its therapeutic benefit in rheumatoid arthritis, autoimmune disorders, Parkinson's disease, Alzheimer's disease, and various type of cancers (Ginwala et al., 2019) (Kim et al., 2019).

Baicalin : Baicalein is a flavone originally isolated from the roots of *Scutellaria lateriflora* and *Scutellaria baicalensis*. The *in vitro* studies have shown its anti−inflammatory, anti−oxidants and anti−carcinogenic potency. Baicalein significantly reversed TNF−α mediated ZO−1 depletion by down-regulating miR−191a in intestinal epithelial cells (Wang et al., 2017). Moreover, Baicalin promoted cancerous cell apoptosis by inhibiting PI3/AKT pathway and NF−kB activity in cervical cancer cell and human colon cancer cells (Fig. 3) (Koosha et al., 2016). Further studies are thus needed to explore it as an epithelial barrier enhancer as its anti−inflammatory and anti−carcinogenic pathways are involved in regulating barrier functions.

Naringenin : Naringenin is flavonone subclass of flavonoid predominantly found in citrus, grapefruit, sour orange, tomato, tart cherry as well as beans. Naringenin has shown its anti−oxidative, anti−inflammatory, anti−carcinogenic, anti−diabetic and anti−lipidemic effects in numerous in vitro studies (Azuma et al., 2013). A recent study showed that naringenin maintained epithelial barrier integrity in rat colitis model (Azuma et al., 2013). Naringenin restored the epithelial barrier of human intestinal cells by upregulating the expression of TJ proteins Claudin−1, 4 and Occludin partially through Sp1 pathway which a transcriptional activator containing binding sites for Claudin−1 and 4 (Noda et al., 2013). Further studies are needed to know its direct effect on enhancing epithelial barrier integrity.

Theaflavins, Theobroma and Catechin : Theaflavin, theobroma and catechin fall under flavonol subclass. Theaflavins (TFs) is one of the major pigments in black tea formed from the fermentation of *Camellia sinensis*. Theaflavins enhanced intestinal barrier function by increasing the

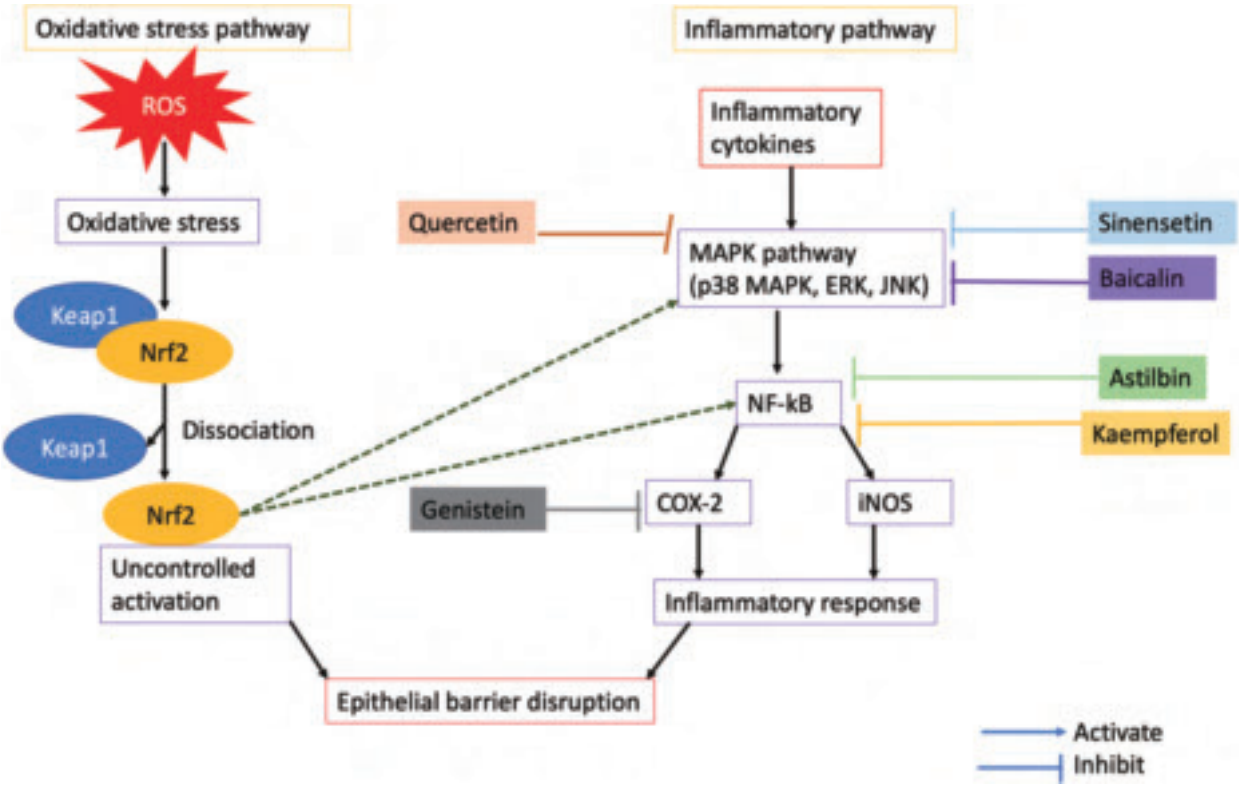

Fig. 3 : Various flavonoid components involvement in blocking inflammatory pathways. Flavonoid components such as quercetin, sinensetin, astilbin, kaempferol and genistein can act at different inflammatory pathways and inhibit those pathways.

expression of TJ proteins such as Occludin, Claudin and ZO−1 through the activation of AMPK (Park et al., 2015). Theaflavin functioned as an anti−inflammatory−in macrophage like cells by blocking the activation of the NF−kB (Lagha & Grenier, 2016). Theobroma containing a large quantity of caffeine, improved the epithelial barrier function by increasing the electrophysiological resistance between adjacent cells (Yamamoto et al., 2014). Epigallocatechin−3−gallate (EGCG) is the major catechin substance in green tea, grapes and red wine. Several epidemiological studies have shown that green tea may reduce risk of many chronic diseases such as neurodegenerative diseases, cancer, cardiovascular diseases and diabetes (Bernatoniene & Kopustinskiene, 2018). The anti−inflammatory, anti−oxidant, anti−aging and anti−carcinogenic capacity of catechin have already been demonstrated (Braicu et al., 2013). Catechins are capable of both scavenging and generating free radicals, and may function as their beneficial effects through a direct and indirect anti−oxidant defense mechanism (Youn et al., 2006). Catechin improved the gingival epithelial barrier integrity reduced by *Porphyromonas gingivalis (Pg)* administration attributed to increased expression of ZO−1 and Occluding, which was proved by increased electrophysiological resistance and decreased paracellular permeability. (Lagha et al., 2018). Catechin has also shown its ability to limit interferon −γ induced increase in epithelial permeability thereby restoring the association of occludin with ZO−1 (Watson et al., 2004). Owing to its greater use in daily life, dose and intensity for Theaflavins, Theobroma and Catechin intake with its impact on epithelial barrier integrity are yet needed to be determined.

Genistein : Genistein is an isoflavone and primarily found in soy−based foods and legumes. Genistein has been shown to contribute to maintain junctional barrier of intestinal epithelium. Genistein prevented TJ barriers from oxidative−stress (RAO et al., 2002) and LPS derived from pathogen (Kiatprasert, Deachapunya, Benjanirat, & Poonyachoti, 2015) in intestinal cells. During the processes, genistein provided increased redistribution of TJ−adherence junction complexes (Occludin−ZO−1 and E−cadherin−beta−catenin) (RAO et al., 2002), and decreased acetaldehyde−induced tyrosine phosphorylation of ZO−1 and Occludin (Atkinson & Rao, 2001). Also, genistein suppressed inflammatory cytokines such as IL−1β, IL−6, and TNF−α might and contribute to enhanced barrier function (Liu et al., 2019). It is of utmost important to identify its regulation mechanism on signalling pathway to direct its application for epithelial barrier enhancement.

Conclusion

Various flavonoid molecules can enhance the epithelial barrier system through their anti−oxidative and anti−inflammatory properties. Flavonoids could be a potential dietary supplement and help in prevention and treatment of epithelial barrier related diseases.

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