ABSTRACT

Hyper contraction of Smooth Muscle is related to various diseases like hypertension, asthma, coronary resistance. Most of the contractile pathways are related to endothelial dysfunction and rise in extracellular calcium. To counter the hyper contractile response of smooth muscles flavonoids act as relaxant molecules or inhibitors of contraction. In this paper we have reviewed the mechanism of action of some known flavonoids such as quercetin, Galetin 3-6-dimethyle ether, kaempferol, fisetin, catechin, apaginin wogonin, naringeinin, formonontin, genistein, daidzen, ayanin, pulicarin. These compounds show endothelium dependent relaxation and play role in enhancing nitric oxide (NO) and endothelium independent relaxation by blocking calcium channels and potassium channels. We have not limited our literature survey to organ bath related research papers but we have included both invivo and invitro studies in this review so that this review may help researcher to make correlation between both studies.

KEYWORDS: Smooth muscle relaxation and contraction, Calcium Channels; Cyclic Nucleotide; Quercetin; Relaxant Effects; Rutin; Galetin 3,6-dimethyl ether; phosphodiesterase; ERK1/2, Fisetin, MYPT1, Phorbol ester, Rho-kinase; L-NAME (N□-Nitro-L-arginine methyl ester).

INTRODUCTION

Smooth muscle cells originate in the walls of various organs and tubes including intestines, stomach, blood vessels, uterus, bladder airways and the penile clitoral, cavernosal sinuses. Smooth muscle cells are also attached to the iris, lens of the eyes and hairs of skin. To focus light on the retina, smooth muscle cells contract and causes change in the shape of lens; erection of hair also is due to contraction of smooth muscle bundles. Under normal
physiological conditions smooth muscles cells are under neural control, that activates autonomic receptors in normal healthy person. Diseases like hypertension, Oesophageal motility disorders\cite{2}, pulmonary disorders like asthma, penile dysfunction are all due to hyper contractility of smooth muscles. There are various synthetic drugs recommended as smooth muscle relaxants. These synthetic drugs have shown promising effect on relaxation of smooth muscles but certain side effects were also reported due to their consistent use.\cite{3} There was need to study ameliorative effect of natural compounds like flavonoids present in fruits, vegetables and in plant parts on hyper contractile muscles. In this review we have discussed the mechanism of some important flavonoids in relaxation of hyper contractile smooth muscles.

THE CONTRACTILE MECHANISM
The process of smooth muscle contraction is principally regulated by pharmacomechanic and electromechanic activation of the contractile proteins namely- myosin and actin.\cite{4} In smooth muscles, depolarization of the membrane’s resting potential occurs by the activation of stretch-dependent ion channels and voltage-operated Ca$^{2+}$ channels, or by the firing of action potentials. all these mechanisms trigger the influx of Ca$^{2+}$ and ultimately results in a contraction.\cite{5,6} Myogenic tone or pressure induced constriction of arterioles and small resistance arteries are dependent on electro mechanic activation. During the process of contraction, myosin light chain (MLC) kinase are activated by Ca$^{2+}$- calmodulin complex that phosphorylate Ser 19 residue of the 20-kDa regulatory MLC, thus enabling the molecular interaction of myosin with actin.\cite{7,8} Energy released from ATP by myosin ATPase activity during phosphorylation of MLC results in the cycling of the myosin cross-bridges with the actin thus causing contraction.\cite{9} So, contractile activity in smooth muscle cells is primarily determined by the phosphorylated state of the MLC.

Rho, a small GTP binding protein is involved in the myosin light chain (MLC) phosphatase activity.\cite{10} Rho-GDP is the inactive form which gets converted to Rho-GTP through the activity of Rho-GEF (Guanine Exchange Factor). Rho kinase phosphorylates and inhibits the MLC phosphatase and hence maintain the contracted state of myosin.\cite{4,11} Increased Rho/Rho Kinase dependent Ca$^{2+}$ sensitization contributes to hypercontraction, and blockage of Rho or Rho kinase reduces blood pressure.\cite{12} There are two isoforms of Rho kinase (ROCK): ROCK1 and ROCK2.\cite{13,14} Various inhibitors of ROCK are known such as fasudil, statins, Y-27632. Contraction of smooth muscle is regulated by various voltage gated ion channels like
voltage operated calcium channels (VOCC), receptor operated calcium channels (ROCC) and store operated calcium channels (SOCC) pathways (Fig 1). These ion channels are the primary source of activator calcium. Voltage gated ion channels include L-type voltage operated calcium channels and T-type voltage gated calcium channels. L-type voltage gated calcium channels function when agonist induces calcium influx caused by depolarization of the membrane. At relatively positive potential L-type Ca\(^{2+}\) is activated: threshold at (-30 to 40 mV) and high single channel conductance. Increased concentration of calcium activates PKC which phosphorylates MLC and causes contraction.\(^{[15,16]}\) T-type channels are mostly activated at negative membrane potentials (-60 to -70 mV); these channels show rapid voltage dependent inactivation and have small signal channel conductance. Endothelin-1 receptors present on the membrane also act through this mechanism. Interestingly, in the case of depolarisation, convergence of Ca\(^{2+}\) and Ca\(^{2+}\) sensitisation pathways induces Rho A / Rho Kinase activation, When L-type Ca\(^{2+}\) channels seem to play a role in Ca\(^{2+}\) induced Ca\(^{2+}\) release and maintenance of tonic contraction.\(^{[17]}\) Receptor operated calcium channels (ROCC) are activated after agonist bind to the receptor which activates G-protein coupled receptor (GPCR), which further activates phospholipase C (PLC) and leads to the generation of secondary messenger Diacylglycerol (DAG) and inositol triphosphate (IP3). IP3 release calcium from sarcoplasmic reticulum (SR) which activate PKC that phosphorylates MLC and hence causes contraction.\(^{[18,19]}\)

Store operated calcium channels (SOCC) channels are activated when intracellular calcium store is depleted. Calcium depletion leads to the activation of sarcoplasmic / endoplasmic reticulum calcium ATPase (SERCA) which is located on the SR membrane. SERCA pumps the calcium ions back in to the intracellular space and causes increased concentration of calcium which further activates PKC sand leads to the opening of Store operated calcium release activation Ca\(^{2+}\) channels (CRAC) and causes contraction.\(^{[20,21]}\) Potassium channels, which are present on the cell membrane, are involved in muscle contraction and vascular diseases. Closure of potassium channels causes the depolarization of the membrane and tends to open the voltage activated calcium channels which will lead to increased concentration of calcium levels and hence causes the contraction.\(^{[22]}\)
Figure 1: Signalling path of contracted smooth muscle. Contraction of smooth muscle occurs by calcium uptake through various smooth muscle channels like SOCC, ROCC and VOCC.

SMOOTH MUSCLE RELAXATION

Smooth muscle relaxation happens either as a result of exclusion of the contractile stimulus or by the direct action of a substance that stimulates inhibition of the contractile mechanism (e.g., atrial natriuretic factor is a vasodilator) thereby, leading to the decrease in the intracellular concentration of Ca\(^{2+}\) and an increase in MLC phosphatase activity (Fig. 2).\(^{[23, 24]}\) The mechanisms that remove intracellular Ca\(^{2+}\) and/or increase MLC phosphatase activity may become altered, leading to the abnormal smooth muscle response.

Increased uptake of Ca\(^{2+}\) is responsible for smooth muscle contraction and hence decrease in the intracellular concentration of Ca\(^{2+}\) induces smooth muscle cell relaxation. There are quite a lot of mechanisms responsible for the removal of cytosolic Ca\(^{2+}\) that involves sarcoplasmic reticulum and plasma membrane. It is well established fact that the Ca\(^{2+}\) uptake into the sarcoplasmic reticulum is dependent on ATP hydrolysis. The presence of phosphorylated Ca,Mg-ATPase in the sarcoplasmic reticulum is responsible for translocation of Ca\(^{2+}\) ions into luminal space and release them out. The Ca,Mg-ATPase is inhibited by many different pharmacological agents such as vanadate, thapsigargin and cyclopiazonic acid. Sarcoplasmic reticular Ca\(^{2+}\) binding proteins in smooth muscle such as calsequestrin and calreticulin also contribute in decreasing intracellular Ca\(^{2+}\) levels.\(^{[25]}\)
Figure 2. Relaxation of smooth muscle. Smooth muscle relaxation happens either as a result of elimination of the contractile stimulus or by the direct action of a substance that excites inhibition of the contractile mechanism.

Ca,Mg-ATPase is also present in plasma membrane and is responsible for reducing the intracellular concentration of Ca$^{2+}$. This enzyme has an auto inhibitory domain extra to sarcoplasmic reticular protein. This auto-inhibitory domain binds to calmodulin, causing stimulation of the plasma membrane Ca$^{2+}$ pump.$^{[25]}$

Smooth muscle relaxation is also elicited by various endogenous and exogenous compounds. These agents reduce smooth muscle contraction by different ways, like inhibition of the synthesis of contractile agonist and blockage of receptor that facilitates smooth muscle contraction. These compounds can also generate intracellular secondary messengers, by binding on specific receptors located on cell membrane or its cytoplasm. Cyclic AMP mediated smooth muscle relaxation is mediated by β adrenergic drugs; while cGMP mediated relaxation is prompted by nitric oxide release and natriuretic peptides. Other ways of smooth muscle relaxation is activation of Ca$^{2+}$ activated K$^+$ channels. Smooth muscle cells express a family of proteins called Ca$^{2+}$ activated K$^+$ channels (KCa channels); these channels cause efflux of K. Once activated, these channels act as negative feedback regulators, hyperpolarizing the cell membrane and blocking the entrance of calcium through voltage gated calcium channels. It was earlier hypothesized that activation of KCa channels is reported to be through dephosphorylation process through activated protein phosphatase type 2A, PP2A, which is a serine threonine protein phosphatase, that is thereby phosphorylated and activated by PKG. But some of the researchers have dispute with that hypothesis.$^{[26]}$ The current
opinion is that vasoconstriction process is opposed by stimulation of calcium spark that leads to activation of big potassium channels (BK channels) in vascular smooth muscles. PKA or PKG1 are the two protein kinases which activate the BK channels by phosphorylation of Phospholamban, which upsurges SR calcium load by disinhibiting the SR calcium ATPase, resulting in an elevated calcium spark frequency and amplitude.[27-30] Apart from above mentioned mechanism other relaxant substances also play their role in relaxation of smooth muscle cells. These relaxant molecules include nitric oxide, prostacyclin and endothelium derived hyperpolarising factors.

**Nitric oxide as Vasodilator**
The endothelium of blood vessels must interact with ACh to induce arterial smooth muscle relaxation. A substance named "endothelium-derived relaxing factor" (EDRF) originates from a blood vessel with an intact endothelium, causes relaxation in an arterial ring with a denuded endothelium. Later, the EDRF was confirmed to be nitric oxide (NO).[30,31] NO is a gaseous free radical which is synthesized from the amino acid, L-arginine catalysed by the family of enzymes known as nitric oxide synthase (NOSs). NO relaxes vascular smooth muscle cells (VSMCs) by increasing the production of cyclic guanosine 3',5'-monophosphate (cGMP), thereby increases the flow of oxygen-bearing blood to the heart muscle. Normally, endothelium constantly releases small amounts of NO and extra amount of NO is released in response to physiological stimuli such as increased shear, stress, reduced oxygen tension, chemical substances such as ACh, bradykinin, histamine, thrombin, ADP, ATP and the substance P. So far, NO, the most potent vasodilator, is reported to inhibit platelet aggregation, neutrophil adhesion to the endothelium, VSMC proliferation and expression of adhesion molecule. NO synthesis is impaired in many diseases e.g. in hypertension, diabetes, hypercholesterolemia and atherosclerosis Pharmacologically, NO synthesis can be blocked by L-arginine analogs such as N -Nitro-L-arginine methyl ester (L-NAME).[32]
FIGURE 3. Relaxation of vascular smooth muscle cells by diffusible vasodilator substances from endothelial cells. Prostacyclin (PGI₂) activates adenylate cyclase, leading to increased production of cyclic AMP. Nitric oxide (NO) activates soluble guanylate cyclase, yielding increased levels of cyclic GMP.\[33]\n
Prostacyclin

Prostacyclin (also called prostaglandin or PGI₂) is formed from arachidonic acid (AA) by the action of enzyme cyclo-oxygenase. The endothelial cells are the highest producers of PGI₂, but VSMCs and fibroblast are also able to synthesize PGI₂. It is produced in response to shear stress and to substances that stimulate NO formation. PGI₂ has less potency for vasodilation as compared to NO. However, PGI₂ inhibits platelet aggregation and promotes fibrinolysis. Estrogen stimulate PGI₂ synthesis in cultured human endothelial cells and in the rat endothelium whereas testosterone reduces it.\[34,35]\n
Endothelium-derived Hyperpolarizing Factors

Endothelium-dependent relaxations and hyperpolarizations are partially or totally resistant to the inhibitors of cyclo-oxygenase and NO synthetase, suggesting the existence of an additional endothelial relaxing mechanism. NO-and PGI₂-independent relaxations occur without an increase in the intracellular levels of cyclic nucleotides in smooth muscle cells, and the relaxations are antagonized by apamin and ChTX, which are inhibitors of Ca²⁺ sensitive K⁺ channels (KCa).\[23]\n
Therefore, it has been suggested that the hyperpolarization of smooth muscle cells caused by the opening of K⁺ channels is responsible for these relaxations and the relaxing agent is called an endothelium-derived hyperpolarizing factor (EDHF). EDHF is an 11,12-epoxyeicosatrienoic acid formed by cytochrome P450 2C from arachidonic acid, at least in the porcine coronary artery.\[36]
Role of flavonoids in smooth muscle relaxation

Flavonoid

Flavonoids belong to a large group of naturally occurring low molecular weight polyphenolic compounds found in fruit, vegetables, grains, bark, roots, stems, flowers, tea and wine. These compounds have basic $C_6$-$C_3$-$C_6$ ring skeleton. Flavonoids are divided into various classes depending upon their orientation of the substituents (hydroxyl and/or methyl etc.), the degree of unsaturation, the type of sugar moiety attached and the position of the benzenoid substitution.

Flavonoids are known to exhibit various biological effects such as inhibiting platelet aggregation, scavenging free radicals, preventing cell proliferation and reducing low-density lipoproteins levels in plasma. In addition, these compounds are reported to modulate vascular tone. Several epidemiological studies have revealed the inverse association between flavonoid intake and reduction in occurrence of cardiovascular diseases, such as myocardial infarction. The relaxant effects of flavonoids are reported to have the following order of potency: flavonols > flavones > flavanols. However, Chan et al have reported equal potency of flavones and flavonols in causing vasorelaxation.

Quercetin

Quercetin is the most abundant flavonols in the human diet, accounting about 60% of the total intake. It is a major flavonoid present mostly in the form of glycosides found in several plant products such as red wine, vegetables, fruits and grape juice. There are almost 180 different glycosides of quercetin described in nature, with rutin (quercetin-3-O-rutinoside) being one of the most common. Based on its polyphenol structure (Figure 4), quercetin exerts many beneficial health effects, such as improvement of cardiovascular health, reduces risk for cancer, protection against osteoporosis. Quercetin has also prevented morphological and functional changes in kidney, heart, vessels and causes decrease in blood pressure.
On the other hand in vitro animal studies were performed to understand the effect of quercetin and its metabolites in isolated blood vessel preparations. Acute treatment to isolated rat aorta with quercetin produced a vasodilator effect in healthy coronary arteries,[51] resistance vessels[52], rat aortic rings[53,54] mesenteric, pulmonary arteries[55] and portal veins.[56] Similar vasodilatory effects have also been reported in isolated blood vessels from spontaneously hypertensive rats[57] and subchronic diabetic rats.[58] Quercetin shows both endothelium independent[43,56,58] as well as endothelium –dependent vasodilatory effect.[53,54,58] Endothelium dependent mechanism generally involves increase in nitric oxide production and enhanced eNOS activity and cyclic eNOS production. Khoo et al and benito et al[53,59] showed that vaso-relaxation caused by quercetin in rat aortic ring segments is due to increased cGMP levels. Moreover Khoo et al have revealed during in-vitro experiments in bovine aortic endothelial cells quercetin treatment rapidly increased Ca\(^{2+}\) and stimulated eNOS phosphorylation at Ser1179 and causes increased production of NO.[53] Quercetin induces dose dependent phosphorylation of eNOS at Ser 1179 and increases production of NO by an AKT independent and cAMP/PKA dependent manner in isolated rat portal vein.[56] Various studies on hypertensive rat models have reported that quercetin significantly reduced endothelium-dependent vasodilatory capacity with accompanying increased NO metabolites[60-63] KCl-induced contractions occurs as a result of an increased Ca\(^{2+}\)influx through voltage-stimulated L-type Ca\(^{2+}\) channels[64] and are specifically inhibited by Ca\(^{2+}\) antagonists.[65] Quercetin inhibits the contractions induced by an increase in extracellular Ca\(^{2+}\) in KCl-depolarized aorta and suppress the spontaneous myogenic contractions recorded in portal veins.[43]
Quercetin has been reported to interact with the Ca\(^{2+}\)-calmodulin complex\(^{66}\) in rat aorta. Calmodulin antagonists inhibit PMA (phorbol 12-myristate 13-acetate)-induced contractions when added before the agonist but, in contrast to flavonoids, they produce a weak relaxation when applied during the plateau of PMA-induced contractions and had almost no relaxant effect on high K- and NA-induced contractions.\(^{67}\) Quercetin not only inhibits calmodulin activity and has also been reported to be involved in blockage of Ca\(^{2+}\) towards sarcoplasmic reticulum by blocking ATP dependent calcium transport.\(^{68}\) However, this possibility was ruled out since quercetin inhibited PMA-induced contraction in Ca-free PSS containing EDTA after depletion of noradrenaline-sensitive intracellular Ca\(^{2+}\) stores with the same potency as it did in the presence of Ca\(^{2+}\).\(^{43}\)

Relaxation of isolated human bronchus is clearly observed in the presence of quercetin in a concentration-dependent manner, against the contractions induced by acetylcholine and histamine. In addition, concentration-dependent relaxation is observed with increasing cumulative concentrations of rutin, the quercetin-3-O-rutinosidein, in human bronchial preparations pre-contracted with histamine and acetylcholine. In Contrast with quercetin, the relaxation effect of rutin is feebly potentiated on acetylcholine and histamine contracted human airways.\(^{69}\) This may be due to the hydrophilicity or the presence of bulky oligosaccharide “rutinose”, that makes rutin unable to infiltrate the cell membrane and might account for its ineffectiveness.\(^{70,71}\) Quercetin are known to inhibit KCl and Ca\(^{2+}\) dependent contractions of human bronchial rings in an effective and concentration dependent ways. K\(^+\)-induced contraction is result of an increase in Ca\(^{2+}\) influx through L-type voltage-dependent calcium channels (VDCC).\(^{72,73}\) Other related compounds like apigenin and 3-O- methyl quercetin are calcium channel blockers in vascular smooth muscle. In contrast Duarte et al\(^{74}\) have reported that vascular effects of quercetin causes inhibition of Ca\(^{2+}\)-sensitising mechanism smooth muscle contraction such as protein kinase C, as no change was noticed in cytosolic Ca\(^{2+}\) level. Where as rutin has no inhibitory effect on the K\(^+\)-induced contraction in human bronchial rings.\(^{73,75}\)

Quercetin scavenges NO too\(^{76}\) and inhibits the expression and/or the activity of the inducible NO synthase.\(^{77}\) The relaxation effect of isoprenaline on acetylcholine-contracted human bronchi is significantly potentiated by quercetin (3×10\(^{-5}\)M) in terms of potency and efficacy. Furthermore, on acetylcholine-contracted human bronchi, quercetin (10\(^{-5}\) M) significantly increased the potency as well as the efficacy of the relaxation elicited by sodium...
nitroprusside (SNP). This potentiating effect of quercetin on relaxation might be mediated via inhibition of cAMP-phosphodiesterase (PDE) and inhibition of cAMP-phosphodiesterase.\cite{78} Indeed, the 3-O-methylquercetin, a metabolite of quercetin, potentiates the relaxing effect of forskolin and SNP on the trachea of guinea pig and significantly inhibits the activity of cAMP- and cGMP-phosphodiesterase.\cite{79} The potentiating effect of quercetin on SNP-elicited relaxation is due to an increased rate of cAMP formation.\cite{43} This subsequently activates the cAMP-dependent protein kinase and inhibits the myosin light chain kinase by phosphorylation and reducing the contraction.\cite{80} However, rutin (10^{-4} M) has no potentiating effect on relaxation evoked by isoprenaline and sodium nitroprusside. Serotonin or 5-hydroxytryptamine (5HT) is regarded as potential candidate in development of cardiac hypertrophy.\cite{81} Quercetin inhibits the decrease in Kv currents and down regulates over expression of 5HT2A receptors that might contribute to reduced arterial remodelling.\cite{82} Quercetin is pan inhibitor of MAPKs and TGF-β pathway\cite{83} and interferes with activity of both PI3K and mTOR.\cite{84} Endothelial dysfunction is associated with elevated levels of ET-1. Quercetin also prevents ET-1 induced contraction and down regulation of P47Phox therefore preventing endothelial dysfunction by inhibiting PKC. In human umbilical vein endothelial cells, quercetin (0.1-1µM) decreased oxidative stress induced by ET-1 mRNA expression and ET-1 secretion.\cite{85} Quercetin is a competitive inhibitor of PI3K; the design of another inhibitor of PI3K, LY294004 (2-(4-morpholinyl)-8-phenyl-1(4H)- benzopyran-4-one) was based on the structure of quercetin: the IC 50 for PI3K and LY294004 are similar: 1.4 and 3.8 mol/L, respectively.\cite{86} Chirumbolo et al have suggested more complex role for quercetin as a vaso-relaxant agent. Tropomysin receptor kinase A, TrkA/AKT, signaling pathway may play an important role in regulating pulmonary artery smooth muscle cells (PASMC) proliferation and migration under hypoxic conditions.\cite{87} The effects of quercetin on hypoxia-mediated PASMC proliferation and migration are most likely due to its direct action on the TrkA/AKT pathway.\cite{88} Quercetin can also attenuate TNFα stimulated adhesion molecule expression in human aortic endothelial cells.\cite{89} An abdominal aortic aneurysm (AAA) is a localized, permanent dilatation of the aorta that affects ~8% of males >65 years-old. The role of inflammation in the pathogenesis of AAA is well established. Infiltrating inflammatory cells enter the aorta, release cytokines and proteases, inducing apoptosis of vascular smooth muscle cells and ultimately, lead to destruction of the vascular wall. Wang et al has reported quercetin prevented CaCl₂ induced abdominal aortic aneurysm (AAA) by blocking aneurysm formation which may occur via the mediation of JNK/AP-1 pathway and MMP.\cite{90} Choe et al have studied direct vascular action of quercetin in aorta from renal hypertensive rats.
Experimental model system 2 kidney, 1 clip (2k1C renal hypertension), control rats were treated with sham. They have reported quercetin treatment has augmented endothelium dependent relaxation in response to acetylcholine in 2K1C rats. But quercetin did not affected relaxant response to acetylcholine in sham rats. They have suggested that quercetin promotes endothelial derived NO mediated response in 2K1C hypertensive aorta. Quercetin did not affect phenylephrine induced contraction in presence of L-NAME in either 2K1C or Sham rats.[91]

Ozlem et al used quercetin along with SOD; they found that there was increase in relaxation in presence of SOD. This effect was seen at lower doses of quercetin (1 µM, 5 µM and 10 µM). Further they have studied the effect NOS inhibitor L-NNA on lower concentration of quercetin (1 µM and 5 µM) and observed inhibition of relaxation; while at higher concentration of quercetin (10 µM)-induced relaxant response was not affected.[92] The effect of quercetin 3′-sulphate and quercetin-3-glucuronide was studied on porcine isolated coronary arteries. Quercetin-3-sulphate did not affect KCl induced contraction at lower concentration but at increased concentration inhibited both U46619 and endothelin-1-induced contractions crucially. This effect of quercetin was shared by its major metabolite quercetin 3′-sulphate, as quercetin also selectively enhanced the cyclic-GMP-dependent vasodilator glyceryl tri-nitrate by two different mechanisms, one of which is mimicked by quercetin 3′-sulphate.[93]

**Galetin 3,6-dimethyl ether (FGAL)**

![Chemical structure of FGAL](Fig 5.)

Galetin 3,6-dimethyl ether (FGAL) is a flavonoid found in plant *Piptadenia stipulacea* (Benth.) Ducke, exhibits some pharmacological activities, such as antiviral,[94] antinociceptive and anti-inflammatory activities in mice,[95] as well as non-selective spasmolytic activity in smooth muscles (e.g., rat uterus and aorta and guinea-pig ileum and trachea). Moreover, it shows the highest relaxant potency in rat aorta and this effect is independent of endothelium-derived relaxant factors (EDRF).[96] In blood vessels, the endothelium plays an
important role in regulating vascular smooth muscle tone by releasing endothelium-derived relaxing factors (EDRF)\textsuperscript{[97]}, including endothelium-derived hyperpolarizing factor (EDHF), nitric oxide (NO), prostacyclins and epoxygenesatrienoic acids.\textsuperscript{[98]} In spite of this, it is demonstrated that the vaso-relaxation induced by FGAL is independent of EDRF, since it relaxes rat aorta both in the absence and presence of endothelium in an equipotent manner.\textsuperscript{[99]} On the other hand, several mechanisms are involved in endothelium-independent vaso-relaxation, such as Ca\textsuperscript{2+} channel blockade, K\textsuperscript{+} channel opening, protein kinase C (PKC) inhibition, attenuation of Ca\textsuperscript{2+} release from the sarcoplasmic reticulum (SR) and phosphodiesterase (PDE) pathway inhibition.\textsuperscript{[100]} It has also been demonstrated that flavonoids can produce vasorelaxation by different mechanisms, such as NO release from endothelium.\textsuperscript{[101]} PKC and PDE inhibition\textsuperscript{[85,102]} blockade of Ca\textsuperscript{2+} influx through voltage-sensitive Ca\textsuperscript{2+} channels (CaV)\textsuperscript{[103]} and K\textsuperscript{+} channel activation (IKCa and BKCa).\textsuperscript{[53,104]}

PKC is a key protein in vascular smooth muscle contraction.\textsuperscript{[25]} This protein kinase can activate both voltage-sensitive Ca\textsuperscript{2+} channels and inhibit K\textsuperscript{+} channels, leading to Ca\textsuperscript{2+} influx and thus contributing to the contractile process. Phorbol esters, such as phorbol 12-myristate 13-acetate (PMA), which is described as PKC stimulator, is used as an exogenous activators of this protein kinase. Also, they have been known to induce sustained contraction in several arterial tissues.\textsuperscript{[105]} FGAL can also relax rat aorta pre-contracted with phenylephrine (PE); it is 16 folded more potent in relaxing Phenylephrine induced contraction than with PMA. FGAL at concentrations of 10\textsuperscript{-8} to 10\textsuperscript{-3} M can activate K\textsuperscript{+} channels and show about 10-fold greater relaxant potency in rat aorta pre-contracted with 30 mM than with 80 mM KCl. FGAL shows a profile of non-competitive antagonism acting on \(\alpha_1\)-adrenergic receptors and includes the non-selective opening of K\textsuperscript{+} channels, the inhibition of Ca\textsuperscript{2+} influx through voltage-gated Ca\textsuperscript{2+} channel and the inhibition of Ca\textsuperscript{2+} influx, which may be by direct or indirect mechanisms. Additionally, it involves inhibition of cyclic nucleotide pathways particularly through phosphodiesterase type 5 (PDE V) inhibition. FGAL is a promising flavonoid used in the treatment of conditions associated with vascular smooth muscle disorders, such as hypertension or ischemia.\textsuperscript{[99]} FGAL (0.1 nM to 0.1 mM,) showed equipotency in relaxing the ileum pre-contracted by 40 mM KCl (EC\textsubscript{50} 2.6± 0.5 x 10\textsuperscript{-5} M) or 10 mM carbachol (CCh) (EC\textsuperscript{50}=1.8±0.4x 10\textsuperscript{-6} M). FGAL (3 mM, 10 mM, 30 mM and 0.1 mM) inhibited the histamine-induced cumulative contractions in a concentration dependent manner. T these concentrations, FGAL inhibited CaCl\textsubscript{2}-induced cumulative contractions in a depolarizing medium (70 mM KCl) nominally Ca\textsuperscript{2+}-free. FGAL (0.1 nM to 0.1 mM) relaxed
ileum pre-contracted with 0.3 mM S-(−)-Bay K8644, selective Cav1 agonist (EC_{50}=9.5±1.9 x10^{-6} M) in a concentration-dependent manner. FGAL shows high potency when histamine was employed as the contractile agent but is equipotent in relaxing the ileum pre-contracted with KCl or CCh.\textsuperscript{106}

**Kaempferol**

![Chemical structure of Kaempferol](image)

Fig 6.

Kaempferol is a naturally occurring polyphenolic compound present in a great amount in tea, broccoli, propolis and grape fruit. It is a very well-known to possess antioxidant\textsuperscript{107} anti-inflammatory\textsuperscript{108}, anticancer,\textsuperscript{109} neuroprotective\textsuperscript{110} and anti-diabetic properties.\textsuperscript{111} Influence of kaempferol, a flavonoid compound, on membrane-bound ATPases in streptozotocin-induced diabetic rats.\textsuperscript{75} Kaempferol causes endothelium independent relaxation in different animals. However, kaempferol and isorhamnetin have also been reported to inhibit Ca^{2+}-sensitizing mechanisms for smooth muscle contraction such as protein kinase C.\textsuperscript{73} Kaempferol (3 to 60 µM) induces concentration-dependent relaxation on KCl-induced tonic contraction. It produces transcriptional events that is involved in the relaxation of rat uterine smooth muscle through cAMP.\textsuperscript{112} Kaempferol can cause both NO-dependent and NO-independent relaxations and which is mediated by activation of KCa1.1 channels in the coronary vascular smooth muscle cells. Endothelium-dependent relaxation enhanced by kaempferol is mediated by NO or endothelium-dependent hyperpolarization (EDH). More ever kaempferol enhances relaxation of isolated porcine coronary arteries to bradykinin, irrespective of their mediation by either endothelium-derived NO or EDH signalling. Xu et al has studied beneficial effect of kaempferol in opening of KCa1.1 channels.\textsuperscript{113} Previous studies demonstrate that inhibition of PKA inhibits kaempferol-induced increases in the KCa1.1 current in HUVECs.\textsuperscript{114}

**Fisetin**

Fisetin (3,3′,4′,7-tetrahydroxyflavone)is abundant in strawberries and other edible fruits or vegetables.\textsuperscript{115} It has a wide variety of pharmacological activities such as anti-allergic and
neuroprotective activities$^{[71,116,117]}$ and also possesses the anti-cancer effect through its anti-proliferative, antioxidant and ROS generating activities$^{[118,119]}$ and recently through increased generation of NO and elevated Ca$^{2+}$ entry activating the caspase dependent apoptotic pathways.$^{[120]}$

![Chemical structure of Fiestin](image)

**Fig 7.**

Endothelium-Independent effect of fisetin on the agonist-induced regulation of vascular contractility has been studied. The mechanism by which phorbol ester activates MEK/ERK and causes vascular contraction has been established.$^{[121]}$ On the other hand, previous studies that inspected the mechanisms underlying arterial contractions induced by fluoride or thromboxane A2 have reported variable findings with regard to the contraction triggered by Rho-kinase activation.$^{[122,123]}$ These findings are consistent with the notion that fisetin can decrease phorbol ester or fluoride-induced contraction by inhibiting MEK or Rho kinase activity. The phosphorylation of caldesmon by MEK/ERK seems to regulate smooth muscle contraction.$^{[96]}$ In this process MEK/ERK is activated by PKC which in turn is stimulated by phorbol esters or GPCR receptor agonists. Fisetin reforms/rectifies the maximal or submaximal contraction induced by vasoconstrictor fluoride or phorbol ester endothelium-independently and ameliorative mechanism involves the MEK/ERK and RhoA/Rho-kinase pathway. A study by Je et al has demonstrated that fisetin at a low concentration (0.03mM, 0.01mM, 0.1mM) significantly inhibits phorbol ester- or fluoride-induced contraction regardless of endothelial function. Furthermore, fisetin decrease phosphorylation of extracellular signal-regulated kinase (ERK)1/2 induced by phorbol ester and significantly decrease the phosphorylation of myosin phosphatase targeting subunit 1 (MYPT1) at Thr855 induced by fluoride causing relaxation, thereby inhibiting Rho-kinase or MEK activity.$^{[124]}$
Catechin

Catechin is well known flavonoid found in many food plants and often utilised by naturopaths for symptomatic treatment. Catechin causes dose dependent relaxation and relaxes high KCl induced contraction in rabbit jejunum. In intact smooth muscles catechin causes inhibition of high K⁺ induced contraction from rat stomach, fundus, guinea pig ileum, guinea pig trachea. When compared with verapamil, a potent calcium channel blocker, catechin is potent in relaxing K⁺ induced contraction. Further catechin can mimic the effect of verapamil in a dose dependent manner. Catechin causes inhibition of K⁺ induced contractions and PE induced contractions at similar dose ranges.\[^{125}\] Catechin, at lower concentration of 10 µM, lacks NO scavenging effect. Catechin is also reported to prevent the effect of quercetin via an antioxidant effect when given synergistically.\[^{125}\]

Apaginin

Apaginin is polyphenolic bi-flavone (4,5,7-trihydroxyflavone), isolated from Cynara cardunculus L, is regarded as non-mutagenic chemopreventive agent present in a variety of green leafy vegetables and Chinese herbs, especially in parsley, thyme, peppermint, olives. Besides showing chemo preventive action by inhibitory action on phosphorylation of MAPKs, such as ERK\[^{126}\] \[^{127}\], apigenin is reported to improve endothelial function by increasing endothelial nitric oxide synthase NOS activity.\[^{83}\] The increase in nitric oxide bio-availability, together with its anti-inflammatory properties, makes this flavanone an interesting target for the prevention of cardiovascular diseases. Apigenin is reported to modulate vascular tone\[^{40,128}\] and decreases contraction of smooth muscle\[^{129,130}\] and vas deferens.\[^{131}\] Researchers have demonstrated endothelial dependent vaso-relaxing effect of
Apigenin on isolated aortic rings\textsuperscript{[132-134]}, apigenin induced vasodilation is blocked by L-NAME\textsuperscript{[83]} Rossoni et al has shown increased endothelial function on invivo rat models fed with apigenin rich diet\textsuperscript{[133]} Apigenin is reported to activate BK\textsubscript{Ca} and SK\textsubscript{Ca} leading to cell hyperpolarisation followed by an influx of extracellular Calcium and calcium is from ER; that activates NOS; and the activated NOS is responsible for the inhibition of Akt phosphorylation\textsuperscript{[135]} It has also been reported that apigenin at very low concentration of 7µM is responsible for restoring normal vascular reactivity in isolated aorta from either insulin deficient or insulin resistance animals and that inhibition of PKC is the mechanism behind this restoration\textsuperscript{[136]}

**Wogonin**

![Chemical structure of Wogonin](Fig 10.)

Wogonin is a flavone derivative present in traditional Chinese drug used as an anti-inflammatory and for management of dysmenorrhea and is isolated from *Scutellaria baicalensis* Georgi (Labiatae) root. Wogonin shows antioxidant\textsuperscript{[137]} antiviral\textsuperscript{[138]} antithrombotic\textsuperscript{[139]} and anti-inflammatory\textsuperscript{[140]} activities. In the case of smooth muscle Huey-Chuan Shih et al has reported that wogonin has multiple effects on the uterine smooth muscles. The possible mechanism can be that it can inhibit extracellular calcium inflowing into cells via cell membrane and intracellular release of calcium ions. In addition, wogonin also induced relaxation responses in uterus smooth muscle can be correlate with activation of Kv and BK\textsubscript{Ca} potassium channels. Wogonin exhibits a dose-dependent relaxant effect on the rat isolated uterus precontracted with PGF\textsubscript{2α} (0.1 mM), ACh (1 mM) and OXT (0.01U/ml). The uterine contractions induced by K\textsuperscript{+} depolarization were gradually inhibited with increasing concentrations of wogonin reaching the significant level at 10, 25, 50 and 100 µM.\textsuperscript{[141]}
Naringenin

Naringenin is one of the most commonly consumed flavonoid compound.\cite{142} Hammad and Abdullah have reported that naringenin reduces gastric tone i.e. it causes inhibitory action on tonic and phasic contraction of isolated ilium.\cite{143} Another study has reported reduction in peristaltic movement by naringenin at concentration of 0.1–20 mol/l. Flavonoid naringenin inhibited colonic spontaneous contraction both in vitro and in vivo.\cite{144} BK\textsubscript{Ca} channels activation ability and relaxant activity of naringenin provide insight into the treatment of GI motility disorders. Intracellular IP3R-mediated Ca\textsuperscript{2+} release is not affected by naringenin. Yang et al have conducted both invitro studies and invivo studies; in case of invitro studies naringenin activates BK\textsubscript{Ca} channel and in invivo studies naringenin at different doses like 25 mg/kg and 50 mg/kg inhibits neostigmine enhanced intestinal transit. Naringenin-induced membrane potential hyperpolarization could be reduced by tetraethylammonium (TEA) and specific BK\textsubscript{Ca} channel inhibitor iberiotoxin.\cite{145}

Formononetin

Formononetin, an O-methylated isoflavone, is contained in the roots of \textit{Astragalusmembranaceus}\cite{146,147,148} and liquorice root.\cite{149} It causes dose dependent (0.1-100 μM) vasorelaxant activity in isolated rat aorta rings in endothelium intact and endothelium denuded aortic rings, Formononetin in 100 μM causes increase in NO production much higher than that by acetylcholine and also by up-regulating enos mRNA expression. The endothelium independent component of formononetin induces relaxation is coupled with the
activation of both $K_{ATP}$ and $BK_{Ca}$ channels of the vascular tissues.\textsuperscript{[150]} Formononetin is reported to cause endothelium-independent pathway, probably due to inhibition of voltage-dependent Ca\textsuperscript{2+} channels and intracellular Ca\textsuperscript{2+} release.\textsuperscript{[151]} Formononetin is very potent antioxidant flavonone and is reported to increase the activity of superoxide dismutase SOD, catalase, GSHPX glutathione peroxidise; but it shows weaker effect on activity of malonaldehyde (MDA).\textsuperscript{[152]}

Genistein and Daidzein

![Structure of Daidzein and Genistein](image)

**Fig 13. Structure of Daidzein and Genestein.**

Genistein and daidzein are plant derived compounds and are naturally occurring isoflavones, present in red clover. Soy beans and soy products are known to be the richest sources of these two estrogenic active isoflavones.\textsuperscript{[153]} It is reported that genistein and daidzein are significantly present in Red clover.\textsuperscript{[152]} Genistein and daidzein are isoflavones which have been reported to show many physiological properties including anti-menopausal (female) osteoporosis, anti-aging and antitumour. They also improve learning and memory skills in menopausal women and are used to treat various diseases like heart disease, diabetes and Kawasaki disease (KD).\textsuperscript{[154]} In vascular system genistein and daidzein induce endothelium independent relaxation in noradrenaline and KCl contracted rat mesenteric artery. In rat aortic rings, genistein strongly inhibits tyrosine kinase and also potentiates the relaxation of other substances like adrenergic $\beta$1 and $\beta$2 receptors agonists by inhibiting of cyclic AMP Phosphodiesterase.\textsuperscript{[155]} Genistein causes inhibition of contractile activity of isolated gastrointestinal smooth muscle. The effects are due to activation of alpha adrenergic receptor via NO, cAMP pathways ATP-sensitive $K^+$ channels ($K_{ATP}$) channels and inhibition of L-type channels.\textsuperscript{[156]} Takayuki Matsunoto has modified genistein and named as GII and GVI. These molecules show more relaxant effect than genistein on aorta. The rank of order of relaxation is GVI>GII>genistein. But GVI and GII induced-relaxation in presence of ODQ (a heme-site inhibitor of soluble guanylyl cyclase) are similar to the genistein induced relaxation; the two compounds are also inhibitor of tyrosine kinase and act as NO donor.\textsuperscript{[157]} Previous studies have shown that daidzein has a relaxant effect in rat isolated aorta or mesenteric artery.
Daidzein is also reported to dilate rat isolated cerebral basilar artery ring via endothelium independent mechanism that involves activation of BK$_{Ca}$.\textsuperscript{[155]} Along with hesperetin, daidzein synergistically shows relaxant effect of histamine-induced tonic contraction in non-sensitized guinea-pig trachea.\textsuperscript{[158]} At a concentration of 100 μM both genistein and daidzein almost completely suppress U46619- or KCl-induced GTP-Rho. These isoflavonones attenuate vascular contraction by inhibiting RhoA/Rho-kinase signaling.\textsuperscript{[159]}

**Ayanin**

![Chemical structure of Ayanin](Fig14)

Ayanin (quercetin 3,4′,7-trimethyl ether) is isolated from *Croton schiedeams* Schlech(*Euphorbiaceae*), species. It induces endothelium-dependent relaxation in Wistar rat aorta that is mainly related to NO cGMP pathway. In case of aortic rings pre contracted with phenylephrine, ayanin induces a greater concentration-dependent relaxation.\textsuperscript{[160]} M. F. Guerrero et al has reported vasorelaxant effect of other structurally related compounds that were also isolated from *Croton schiedeams* Schlech(*Euphorbiaceae like Quercetin 3′,7′ dimethyle ether, while quercetin 3,3′,4′,7, tetremethyle ether are modified compounds. Their results showed the potency order of relaxation as Quercetin 3′,7′ dimethyle > Quercetin > ayanin > quercetin 3,3′,4′,7, tetremethyle ether.\textsuperscript{[160]} Other studies have also reported that ayanin causes endothelium dependent relaxation related to NO/cGMP pathways and endothelial vasorelaxant factors related COX dependent pathway, but they have not seen any role of ayanin in blockage of Voltage dependent calcium channels and in activation of K$^{+}_{ATP}$channel. They have also observed that Ayanin does not have enhanced relaxant response elicited by acetylcholine (ACh), while ayanin weakly decreases the relaxation induced by sodium nitroprusside.\textsuperscript{[161]}
**Pulicarin**

![Chemical structure of Pulicarin](image)

Fig 15.

Pulicarin has been isolated from the epigeal part of *Pulicaria salviifolia* Bunge and its scientific name is 3′,6-dihydroxy-3,4′,5,7-tetramethoxyflavone. Its structure has been established on the basis of physicochemical and spectral parameters (UV, IR and mass spectra and PMR).[162] Pulicarin relaxes rat aorta preparations, pre-cut hyperpotassium solution (50 mmol/L KCl), i.e. have a pronounced effect on relaxation and these effects are dose-dependent. The mechanisms by which pulicarin releases KCl-induced contraction is mainly due to its interaction with the potential dependent Ca$^{2+}$-channels of plasma membranes of SMC. As a result of this interaction, blocking of these channels occur, that leads to suppression of Ca$^{2+}$ entry and reducing their concentration in the cytoplasm of smooth muscle cell. The relaxant effect of pulicarin is endothelium-dependent and may be mediated by its interaction with the NO-synthase and its activation.[163]

**CONCLUSION**

Natural molecules act as potent smooth muscle relaxants as they can target more than one pathway. Molecules like quercetin are able to reduce generation of reactive oxygen species, blockage of calcium channels and release of nitric oxide. Besides quercetin other flavonoids are also able to reduce hyper contractility of smooth muscles. Quercetin is reported to cause potent effect on aortic and bronchial smooth muscles while a few papers have reported role of quercetin in relaxation of ilium. FGAL is potent smooth muscle relaxant and is reported to cause its effect by releasing Nitric oxide also by acting as inhibitor of PKC and PDE. On the other hand FGAL causes K$^{+}$ activation as well as blockage of various calcium channels. Fisetin has a different mechanism in smooth muscle relaxation; it is reported to cause inhibition of MEK/ERK and Rho kinase pathways. Apigenin activates BK$_{Ca}$ and SK$_{Ca}$ channels leading to cell hyperpolarisation followed by an influx of extracellular calcium and intercellular calcium; that activates NOS; and the activated NOS is responsible for the inhibition of Akt phosphorylation. Wogonin is reported to cause its relaxant effect on uterine contraction by inhibiting K$^{+}$ depolarization and extracellular calcium inflow. Naringenin is
showing multiple effects on isolated ilium by activating BK\textsubscript{Ca} channels and it is also reported that IP3R-mediated Ca\textsuperscript{2+} release is not affected by naringenin. Ayanin is one of the flavonoid that is reported to cause release of nitric oxide through cGMP pathway and by COX dependent pathway. So these flavonoids can be used to treat diseases related to hyper contractility as chemically synthesised drugs are unable to target multiple pathways. Further research is needed to study the prophylactic and ameliorative nature of these flavonoids on diseases related to hyper contraction on higher experimental animal models on various smooth muscles. Thus these molecules can be further processed to synthesise drugs with high potential and least side effects.

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