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Review Article

AN OVERVIEW OF ANTI-INFLAMMATORY, ANTIOXIDANT, ANTI-CANCER, ANTI-HYPERLIPIDEMIC, NEUROPROTECTIVE AND MUSCLE RELAXANT EFFECTS OF NATURAL FLAVONOID, APIGENIN; A REVIEW

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Abstract: *Apigenin (4', 5, 7,-trihydroxyflavone), a natural glycoside belonging to a class of cyclone, is found in many different forms in plants and vegetables such as grapefruits, parsley, onions, oranges, tea, chamomile, wheat, sprouts, oregano, artichokes and in some seasonings. It effectively treats various anti-inflammatory disorders because it is less toxic, non-mutagenic, and potent against skeletal muscle sarcoplasmic reticulum. Furthermore in combination with allopathic drugs, its activity was enhanced as an anti-inflammatory, antioxidant, cardioprotective, neuroprotective, and anticancer agent. Little data was found that emphasized its role as an anti- hypoglycemic and sedative agent; however, it was found to promote hair growth by inhibiting the TGF-B1 pathway. Different characterization techniques were used to determine physicochemical properties, and various formulations of apigenin with other polymers have been done to enhance its biological role. The literature search used various scholarly search engines (Google Scholar, PubMed, Springer Link, etc.). A total of 355 research articles were reviewed, and it was observed that 34% of articles were about anti-inflammatory, 15% of articles were about neuroprotective, and 10% of studies were about anti-cancer use of apigenin. Thus, the current review focuses on its activity, characterization and limitations of apigenin role.*

Keywords: Apigenin, Anti-Inflammatory, Neuroprotective, Anticancer, Anti-Oxidant, Anti-Hyperlipidemia, Muscle Relaxant

Introduction

Apigenin, chemically known as four ′, 5, 7,-trihydroxyflavone, occurs as a yellow crystalline powder belonging to the flavone class that is alcyone of several naturally occurring glycosides. It is insoluble in water but organic solvents (Salehi, Venditti et al. 2019). It is yellow with needle shape crystals, with molecular formula of $C_{15}H_{10}O_5$ with molecular weight of 270.24 g/mol. (Cannataro, Fazio et al. 2021). Apigenin is available in many derivative forms, such as apigenin 7-O-B-D-glucopyranoside, apigenin 7-O (6-O-acetyle-b-d-glucopyranoside), apigeni-6-C-glucoside, apigenin 8-C-glucoside and apigenin-7-B-D glucuronide and many more (Nozhat, Heydarzadeh et al. 2021). Dihydroxy functionality increases the radical stability due to H-bonds formation which favors hydrogen atom abstraction. Bond dissociation energy and ionization potential were determined to investigate its anti-oxidant profile via an h-atom or an electron transfer mechanism (Leopoldini, Pitarch, et al. 2004). The presence of solvents can alter the preferred thermodynamic mechanism of anti-oxidative progress for apigenin. For anti-oxidative progress, hydrogen atom transfer is the thermodynamically dominant mechanism in a vacuum, carbon tetrachloride (CCl_4), and chloroform phases, while sequential proton loss electron transfer is more favored in pyridine, ethanol, acetonitrile, DMSO, and water phases (Zheng, Zhou, et al. 2017). Figure 1 shows the biosynthesis of apigenin from phenylalanine.

Apigenin has shown promising effects in chronic inflammatory diseases as it is less toxic and non-mutagenic. Apigenin showed its anti-inflammatory effect by decreasing reactive oxygen species and inflammatory mediators. Moreover, it also hinders various signaling pathways, including NF-kB, nuclear factor kappa, and MAPK-mitogen-activated protein kinase (Ginwala, Bhavsar, et al. 2019). Another known mechanism of anti-inflammatory action of apigenin includes inhibitory action by TNF- α induction NF-KB transcription activation. These suppressions may inhibit the transcriptional activation of GAL-4-NFkB p65 apigenin and the TNF-α induced expression of CCL2/MCP-1 and CXCL1/KC inhibited due to the apigenin mechanism of action. The conclusion suggested all these mechanisms support the anti-inflammatory effect of apigenin (Funakoshi-Tago, Nakamura et al. 2011). Apigenin scavenging effects against skin inflammation were noticed when inserted with glucose oxidase xanthine oxidase/hypoxanthine and cumene hydroperoxide intradermally, which contributed to stopping the liposomal application of apigenin 7-glucoside in a dose-dependent manner contributing to from antioxidant to the anti-inflammatory effect (Fuchs & Milbradt, 1993).
When used in combination with resveratrol and apigenin, anti-inflammatory activity was enhanced on 264.7 raw cells, resulting in hindrance of nitric oxide production, prostaglandin E2, interleukin-1B, interleukin six and TNF-a while the effects were not shown by the metabolites of apigenin further co- metabolites of apigenin and resveratrol

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also inhibit the inducible nitric oxide synthase expression and cyclooxygenase-2 (Lee, Ha et al. 2015).
The lipopolysaccharide induces inflammation in acute lung injury due to the primary inhibition of Cox-2, and NF-KB was treated with apigenin. (Wang, Liu et al. 2014). Also, it showed anti-inflammatory activity by inhibiting COX-2 adhesion of monocytes to humans' umbilical vein endothelial inhibition of the collagenase (suppressed LPS induced NO production) and expression of cellular adhesion molecule in treating rheumatoid arthritis and the in a dose-dependent manner (Lee, Zhou, et al. 2007). Furthermore, it also inhibited phosphoinositide 3-kinase MAPKS, P38, and JNK and protein kinase-C inhibitors, which blocks the anti-inflammatory effect of apigenin in nicotine and LPS treated cells, indicating apigenin use in preventing and treating periodontal disease, which is related to smoking and dental plaque (Jeong, Lee, et al. 2009). This shows that apigenin can be used for a variety of anti-inflammatory disorders.

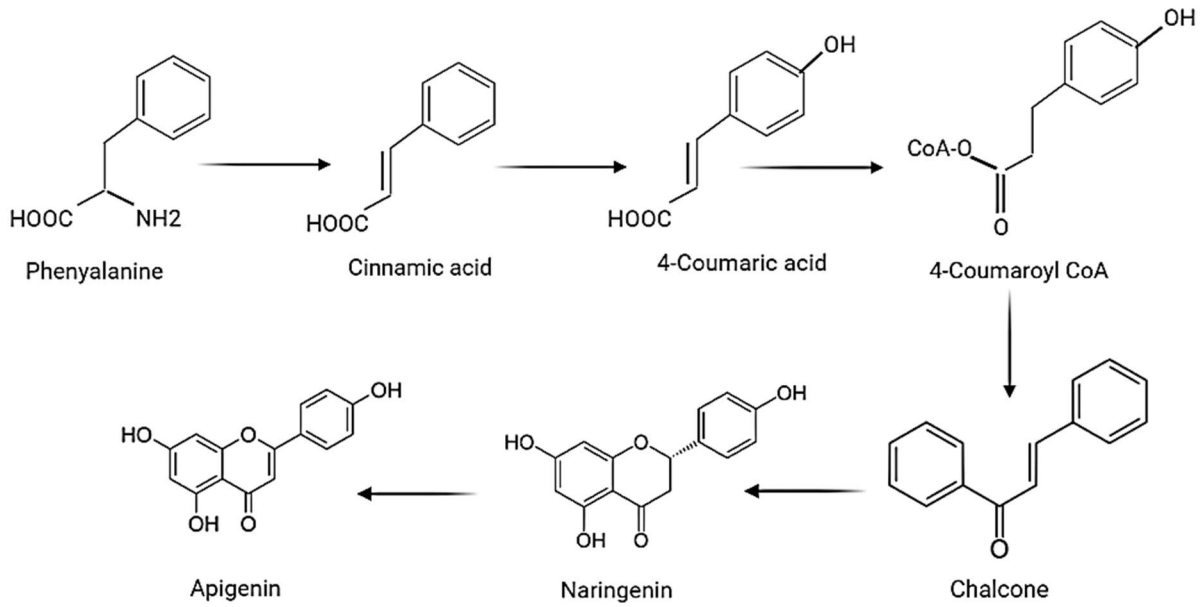

Figure 1 Apigenin biosynthesis and its chemical structure.

Flavones exert their neuroprotective effect by direct interaction with the apoptotic caspase pathway without disturbing anti-oxidant action (Kang, Lee, et al. 2004). Apigenin has shown a neuroprotective effect against amyloid-β 25-35 by decreasing the expression of GSK-3β with the consequence of lowering the hyper-phosphorylation of tau protein and suppressing BACE1 Expression (Alsadat, Nikbakht et al. 2021). Also, it has shown activity against OGD/R injury, and the protective effect is associated with its ability to improve sodium pump action (Ding, Lin et al. 2020).
Apigenin has the potential to restore the neurotrophic ERK/CREB/BDNF pathway in the cerebral cortex by ameliorating AD-associated learning and memory impairment through relieving Aβ burden suppressing amyloidogenic process inhibiting oxidative stress and restoring ERK/CREB/BDNF pathway (Zhao, Wang, et al. 2013). Anti-epileptic drugs have shown some side effects associated with learning and memory behavior, but apigenin is a safe flavone with anti-oxidant properties. However, there is little information about the beneficial effects of apigenin on cognitive therapy in epilepsy (Hashemi, Babaei, et al., 2019).
Apigenin has also shown anti-depressive effects when used in 20mg/kg quantity in streptozocin interceded depression. (Bijani, Dizaji et al. 2022). A combination of apigenin and rivastigmine developed with multi-target ligands has shown effective treatment against Alzheimer's disease. The combination affected aluminum chloride-induced zebrafish Alzheimer's disease and defended zebrafish vascular injury caused by Aβ1-40. Moreover, it also ameliorated memory impairment interceded with scopolamine. (Sang, Wang et al.2020). It also has shown metastasis suppression in a dose-dependent manner at 0-40 um implanted in A2780 cells, indicating increased bioavailability by nanoparticle delivery (Ashrafizadeh, Bakhoda et al. 2020). Combined with etoposide and cyclophosphamide, Apigenin produces a synergetic effect, induces apoptosis, and decreases drug resistance in treating apoptosis-related gene and protein leukemia cells (Mahbub et al., et al. 2022).
Among flavonoids, apigenin is the most potent inhibitor of the skeletal muscle sarcoplasmic reticulum (Sosa, Chaves et al. 2004). Its muscle relaxing property in chamomile causes a slow relaxation of isolated blood vessels by affecting the calcium influx. Umbelliferon produces a rapid and transient relaxation dependent upon the release of nitric oxide from the endothelium (Roberts, Allen et al. 2013).
Asdaq, S.M.B reported that its combination effect with skeletal muscle relaxant properties along with sedative and anti-depressive effects at doses of 50mg per kg caused a decrease in muscle strength and muscle tone (Asdaq, Mannasaheb et al. 2021). It also produces enhanced smooth

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muscle relaxant activity when combined with acetylcholine. (Sadraei, Ghanadian, et al., 2019).

Apigenin is more potent in producing gastric myorelaxant effects due to calcium influx that is negative modulation through the voltage dependent Ca+2 Channel. Flavonoids cause murine gastric relaxation (Rotondo, Serio et al. 2009). The apigenin relaxation effect is notantagonized by indomethacin or methylene blue in the presence of nifedipine, not affecting the cAMP, nor were cGMP levels. However, apigenin did not affect the formation of inositol monophosphate caused by NE and the phasic contraction induced by caffeine in the Ca2+-free solution. 45Ca2+ influx caused by either NE or K+ was inhibited by apigenin concentration-dependently. Apigenin relaxes the thoracic aorta mainly by suppressing the Ca2+ influx through both the voltage and receptor-operated calcium Channels (Ko, Huang et al. 1991).

The antioxidant activity of flavonoids depends on several hydroxyl and phenolic groups. Also, the compounds with the enol groups are better than non-enolic groups in anti-inflammatory and antioxidant activity. Therefore, quercetin is a better anti-oxidant and anti-inflammatory flavanol than apigenin, luteolin, and kaempferol (Kim, Jung, et al. 2019). Apigenin's effective antioxidant activity is observed by delaying the peripheral neurodegeneration process against oxidative stress by scavenging free radicals in neurological disorders, including cerebral ischemia, including four principal phenotypes (axonal degradation, myelin fragmentation, trans-dedifferentiation, and proliferation of Schwann cells via Krox20- and extracellular signal-regulated kinase-independent processes) (Tian, Liu, et al. 2021).

Apigenin extracted from the flower of Gentiana veitchiorum can decrease total cholesterol and triglyceride levels as well as enhance the activity of superoxide dismutase by decreasing the lipid deposition and lipid vacuoles, further reducing the high fat-induced oxidative damage through low-density lipoprotein receptor and lecithin-cholesterol acyltransferase pathway (Dou, Zhou et al. 2020). It also has low intrinsic toxicity. Therefore, it treats muscle diseases and hypokinesia due to damaged and degraded myocytes by enhancing proliferation, anti- inflammatory, anti-oxidant metabolism, and signaling pathways, reducing atrophy (Huang, Yu et al. 2022).

1.1 Sources of Apigenin

The sources of apigenin include plants such as chamomile, spinach, oregano, marjoram, rosemary, parsley, etc., and also in some other food sources including pistachio, rutabaga, artichoke juniper berries, peppermint, onion, broccoli, blueberry and many more (Cannataro, Fazio, et al. 2021). As discussed earlier, apigenin is found in many fruits and vegetables in the form of a single active drug in a chamomile plant and isolated from the dried flower of the Martica chamomilla, which is the herb native to Europe and West Asia modified in Australia, United States and Britain (Singh, Khanam, et al. 2011). Table 1 shows the amount of apigenin found in food sources (Cannataro, Fazio, et al. 2021):

Table 1 Amount (mg/ml) of apigenin found in fruits and vegetables.

Food source	Phenol-Explorer mg/100g-mg/100ml	USDA mg/100g-mg/100ml
Broccoli	7.2	7.84
Spinach	55.0	6.38
Onion	4.5	4.1
Blueberry	3.50	1.66
Marjoram	4.40	3.5
Sage	2.40	1.20
Pistachio	0.03	0.00
Oregano	3.50	0.00
Chamomile	3-5	Not present
Celery seeds	78.65	78.65

1.2 Apigenin Physicochemical Properties Determination

The chemical and physical properties of apigenin are determined by different methods (ultraviolet (UV) -visible spectrometry, infrared spectrometry (IR), scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and X-ray diffractometry). However, a synthetic process cannot break the apigenin-conjugated structure because the complex is held together by Hydrogen bonding and van der Waals force and processes new physical and chemical characteristics. Table 2 shows the study of the physicochemical properties of apigenin by various methods (Brad & Zhang, 2018)

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Table 2 Methods used to study physicochemical properties of apigenin.

Sr.	Techniques	Results
1.	UV spectrometry	No distinct difference was examined between the complex and physical mixture.
2.	Fourier transform-IR	The absorption peaks of lecithin subdued characteristic absorption peaks of apigenin.
3.	SEM	The irregular form of the complex.
4.	DSC	Thermogram of complex the characteristics endothermic peak belonging to apigenin disappears.
5.	X-ray	The apparent amorphous properties are shown in the X-ray X-diffractograms of the complex.

1.3 Search Strategy

An extensive data search was conducted between September and November 2022 on various scholarly search engines (PubMed, Science Direct, Springer, Books, Wiley online library, and Google Scholar) for newly published articles to be part of the review. Search terms included Apigenin, apigenin extraction, anti-inflammatory, neuroprotective, anticancer, anti-oxidant, anti-hyperlipidemic, and muscle relaxant. A total of 355 articles were assessed, and after detailed screening, 63 articles were chosen for the review, and the remaining 292 research articles were excluded. No limitations on sample size, study design, and outcome measurements were part of the review.

2.0 Properties of Apigenin

2.1 Anti-inflammatory

Murat Karamese et al. reported the anti-inflammatory and antioxidant effects of apigenin in sepsis-induced rat model to elaborate the immunological, biochemical, and histopathological study aiming to find out the protective activity of apigenin in polymicrobial sepsis through cecal ligation and puncture. Sixty-four female albino rats were selected and were divided into eight groups. The anti-inflammatory and pro-inflammatory cytokines were calculated by using an assay. CD3, CD69, and NF-KB positivity rates were also measured by using immuno-histochemical methods, and oxidative stress was seen by tissue biochemistry. The major activity was shown as sepsis may cause an increase in TNF-α, IL-1B, IL-6, and TGF-B. At the same time, IL-10 decreases, which may enhance the CD3, CD68, and NF-KB positivity rates, and oxidative stress levels further? Apigenin reduces the inflammatory action. It was concluded that apigenin prevents sepsis and is an anti-inflammatory agent to protect against organ failure. (Karamese, Erol et al. 2016).

Shi-Qing Cai et al. reported the anti-inflammatory effect of apigenin and genistein in rat intestinal epithelial cells (IEC-6), which TNF-α stimulated in response to heat treatment. Pretreatment of cells before TNF-α provokes maintenance of cell morphology and represses the PGE2 and two pro-inflammatory cytokines, IL-1β and IL-6. Meanwhile, provoking the production of two anti-inflammatory cytokines, TGF-β (transforming growth factor-β) and IL-10. Furthermore, blocking the movement of NF-κβ (nuclear factor kappa B) p65 and decreasing phosphorylated IRB-α and p65 stimulated by TNF-α. So, the study concluded that both apigenin and genistein showed anti-inflammatory effects to TNF-α stimulated IEC-6 cells by deactivating the NF-κβ pathway, and heat treatment produced a negative effect on these anti-inflammatory effects, revealing heated apigenin and some flavonoids are less effective than unheated. (Cai, Tang et al. 2022).

Salida Mirzoeva et al. demonstrated that UVB exposure causes transient skin inflammation that encourages angiogenesis and vasodilation, two favorable factors for the development of cancer. Research shows TKO mice are less susceptible to apigenin's chemopreventive effects. Importantly, the enhanced cutaneous inflammation, characterized by heightened infiltration of neutrophils and macrophages and higher levels of local and systemic inflammatory cytokines, notably IL-6 and IL-12, was the primary cause of the reduced chemopreventive activity of apigenin. Results were in line with earlier research that demonstrated the profound influence of TSP1 receptors CD36 and CD47 on inflammatory responses, albeit these results may not be directly applicable to the skin's reactions to UVB light. UVB radiation has been shown by Salida Mirzoeva et al. to generate acute skin irritation, which encourages vasodilation (Mirzoeva, Tong, et al. 2018).

Che-Hwon Park et al. studied apigenin's anti-allergic, anti-inflammatory, and skin protective effects. The study's objective was to evaluate the effect of apigenin in improving skin diseases and its use in cosmetics as the main ingredient. The study was performed on RAW264.7 murine macrophage, rat basophilic leukemia-2H3, and human immortalized keratinocyte cells. The results indicated that

100μM apigenin ceased nitric oxide, IL-1β, IL6, COX-2, and inducible nitric oxide synthase. Moreover, RAW264.7 cells also ceased the phosphorylation of mitogen-activated protein kinase signal molecules, including extracellular signal-regulated kinase and c-Jun N-terminal protein kinase. However,30μM apigenin stopped the phosphorylation of signaling molecules Lyn, Syk, phospholipase Cγ1, ERK, and JNK, and the expression of high-affinity IgE receptor FcεRIα and cytokines TNF-α, IL-4, IL-5, IL-6, IL-13, and COX-2 that cause inflammation and allergic reaction in rat basophilic leukemia-2H3 cells. Moreover,20μM apigenin prompted the expression of filaggrin, loricrin, aquaporin-3, hyaluronic acid, hyaluronic acid synthase HAS-1, HAS-2, and HAS-3 in HaCaT further, it induced the expression of human β-defensin HBD-1, HBD-2, HBD-3, and cathelicidin LL-37 in HaCaT cells. All these peptides are important chemical barriers to skin. Hence, in RAW264.7 and RBL cells, apigenin reduced the inflammatory and allergic reactions. It also enhances the physical and chemical barriers of the skin. So, effective in skin diseases like psoriasis, acne, and atopic dermatitis (Park, Min et al. 2020).
Alexandros Charalabopoulos et al. studied the anti-inflammatory activity of apigenin in acute pancreatitis. Wistar rats were used and divided into three groups. One group is the experimental group that undertook laparotomy with no treatment; the other is the control group that undertook laparotomy along with the joining of the biliopancreatic duct to induce pancreatitis with no apigenin treatment, and the third group is treated with apigenin. The animals were bled at 6, 12, 24, 48, and 72 hours postoperatively. The results indicated that in the control group, there was increased expression of TNF alpha concerning postoperative time, while in the apigenin group, there was decreased expression of TNF alpha concerning postoperative time. At 72 hours, apigenin caused a reduction in the expression of TNF alpha along with averted pancreatic necrosis. Thus, apigenin effectively treats acute pancreatitis as an adjuvant (Charalabopoulos, Davakis, et al. 2019).

2.2 Neuroprotective

Paria Hashemi et al. studied the role of apigenin in temporal lobe epilepsy as a neuroprotective, anticonvulsant, and cognition-enhancing agent. The main objective was to evaluate the effect of apigenin in epilepsy as an alternative because of the side effects of anti-epileptic drugs on memory and cognitive behavior. The Morris water maze and Y-maze task evaluated the results. The study was performed on male Wistar rats, divided into four groups: a control-vehicle group, an apigenin-treated sham group, a kainic acid group, and an apigenin-treated kainic acid group. The results indicated that apigenin rehabilitated the kainic acid, prompted memory deficit, and enhanced living neurons in hilus. Hence, plays a role in the inhibition of the intrinsic apoptotic pathway. So, it is concluded that apigenin protects kainite-induced memory deficit via the anticonvulsant and anti-apoptotic properties (Hashemi, Babaei, et al. 2019).
Balez Rachelle et al. explained the neuroprotective action of apigenin they have used on human induced pluripotent stem cell model of familial and sporadic AD to assess the neuroprotective activity of apigenin. The IPSC (Induced Pluripotent Stem Cells) derived AD neurons demonstrated a hyper-excitable calcium signaling phenotype with elevated levels of nitrate, increased cytotoxicity and apoptosis, reduced neuritis length, and increased susceptibility to inflammatory stress challenge from activated murine microglia to control neurons. Studies showed it has potent anti-inflammatory properties and can protect neuritis and cell viability by promoting a global down-regulation of cytokine and nitric oxide release in inflammatory cells. In addition, apigenin's ability to protect iPSC-derived AD neurons via multiple means by reducing the frequency of spontaneous Ca2+ signals and caspase-3/7 mediated apoptosis was observed. These data demonstrate the broad neuroprotective action of Apigenin against AD pathogenesis in a human disease model (Balez, Steiner et al. 2016).
Dourado Naiara Silva et al. explained the neuroprotective and neuro-immunomodulatory action of apigenin in their study, which evaluated the neuroprotective and neuro-immunomodulatory potential of apigenin using in vitro models of neuro-inflammation associated with Alzheimer's disease. Co-cultures of neurons and glial cells were obtained from the cortex of newborn and embryonic rats. After 26 days in vitro, cultures were exposed to lipopolysaccharide or IL-1β for 24 hours to Aβ oligomers for 4 hr and then treated with apigenin for 24 hr. It was observed that the treatment with apigenin preserved neurons and astrocytes integrity determined by Rosenfeld's staining and immunocytochemistry for β-tubulin III and GFAP, respectively (Dourado, Souza et al. 2020).
Jingwen Wang et al. reported that rats' primary cultured cortical neurons were challenged by oxygen and glucose deprivation and then treated with apigenin. Cell viability phosphorylation of GSK-β at Ser9 or nuclear expression of Nrf2 was measured. Male Sprague Dawley rat challenged by 2-h middle Cerebral artery occlusion was treated with 50 mg/kg apigenin, and the neurological score infarct volume, phosphorylation of GSK-3β, or nuclear expression of Nrf2 was analyzed. The neuroprotective effect of apigenin and the expression levels of antioxidant enzymes and oxidative products were also examined in the presence and absence of Nrf2-siRNA and PI3K inhibitors. Apigenin reduced the apoptotic proportion, attenuated the LDH release, or increased cell viability in vivo. Apigenin improved neurological scores or reduced infarct volume. Apigenin increased GSK-3β phosphorylation or Nrf2 nuclear translocation, while this effect was prevented by PI3K inhibitors or Nrf2-siRNA treatment in both OGD/R cell cultures and ischemic rats. These findings proved that GSK-3β phosphorylation-mediated Nrf2 activation is involved in the neuroprotective effects of apigenin (Wang, Wang et al. 2020).
Chengli Ling et al. studied the neuroprotective effect of apigenin against cerebral ischemia in vitro and in-vivo models. A multifunctional microplate reader, high content cytometer analysis, TTC staining, and neurological deficit scores were used. The PCl2 cells were treated with cobalt

chloride to cause oxidative stress. The results showed that in-vitro, ten mcg/ml of apigenin caused an increase in the neurological deficit scores and lowered infarct areas in rats. Hence, apigenin is effective in cerebral ischemia as a neuroprotective agent both in vitro and in vivo (Ling, Lei et al. 2020).

Sen Zhang et al. studied the neuroprotective effects of a new derivative of apigenin, 6-O-succinyl apigenin, which is highly water-soluble. In male rats, the infraction size and neurological deficits were determined by magnetic resonance imaging and assessed neurological scores after 2 hours of occlusion and 24 hours of reperfusion. The antioxidative mechanism of this novel compound was determined by investigating the levels of nuclear factor E2-related factor 2, Kelch-like ECH-associated protein1, heme oxygenase-1, and extracellular-signal-regulated kinase. In-vivo results indicated that 6''-O-succinylapigenin caused a reduction in infarct volume and neurological scores. Moreover, it enhanced heme oxygenase-1 and nuclear factor E2-related factor 2. Hence, it has anti ischemic effect via extracellular-signal-regulated kinase, nuclear factor E2-related factor 2, and heme oxygenase-1 pathway activation (Zhang, Xu et al. 2019).

Pinar Kuru Bektasoglu et al. investigated apigenin's anti-inflammatory, antioxidant, and neuroprotective effects in mild traumatic brain injury. Male Wistar albino rats were selected and divided into groups as control, traumatic brain injury, traumatic brain injury+ vehicle, and traumatic brain injury + apigenin (20 and 40 mg/kg, immediately after trauma). Traumatic brain injury was induced by falling weight of 300g from 1m height onto the skull of anesthetized rats. The results indicated that apigenin 20mg and 40mg have neuroprotective activity, reducing luminol and lucigenin levels and enhancing interleukin-10. Moreover, 20mg apigenin improved the cortical tissue damage caused by trauma (Kuru et al. et al., 2022).

2.3 Anti-cancer

Xiaohui Yan et al. explained the anti-cancer properties of apigenin and the mechanics through which it arrests cancer cells. In-vivo studies were performed on rats as a test animal as apigenin belongs to class II according to BCS; it has low solubility in water, reducing its bioavailability. Therefore, a solid dispersion system (novel carbon nano-powder) was used as a carrier in Wister rats (60mg /kg). The mechanism included anticancer activity by induction of apoptosis, cell cycle modulation, autophagy, PI3K/AKT, mTOR pathway, NF-KB, MAPK/ERK signaling, and Jak-Stat. The radio-labeled apigenin showed that 51% radioactivity recovered in urine and 12% in feces in 10 days, indicating slow distribution, slow elimination, and the accumulation of Apigenin in the body. They noted when doses (25, 50, 100, and 200 mg /kg) were used, doses (100 mg and 200 mg) caused liver damage. Hence, doses and routes must be adjusted (Yan, Qi et al. 2017).

Susu Thae Hnit et al. focused on apigenin anti-cancer activity in prostate cancer cells by inhibiting the proliferation of cancer cells by G2/M arrest induction. The experiment was performed by lymph node metastasis-derived prostate cancer cell line LNCAp (CRL-1740) and bone metastasis prostate cancer line PC-3 grown in RPMI within 10 percent v/v fetal bovine serum. Apigenin (20 mg) was taken, which was pure, more than 98% dissolved in DMSO than SYBR Green assay, flow cytometric analysis, and reversed transcription quantitative PCR (RT qPCR) were done. Results indicated that changes in DNA content in LNCAP and PC-3 cells occurred with the SYBR Green assay.

Apigenin decreased the net gain of DNA content in Prostate cancer cells as dose-dependent. The events of essential regulation for G2 to M transition were assessed, which showed that apigenin decreased Cyclin B1 PLK1 in both LCAP and PC-3 cells. As a result, apigenin suppressed the transcription of essential regulators that govern the G2- M phase (Hnit, Yao, et al. 2022).

Hwan Here Lee and Hyosun Cho explained the anti-cancer effect of apigenin on human breast carcinoma MDA-MB - 231 through the cell cycle arrest and apoptosis, and for that purpose, apigenin about 95% pure was taken and dissolved in DMSO, diluted with a medium that was made for human breast carcinoma MDA MB 231 and this was obtained from ATCC HTB26. Later, MTT assay, cell cycle analysis, and cell apoptosis were performed to test the anti-cancer effect, and dilutions (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 um) of apigenin were prepared. Human breast carcinoma cells were treated with apigenin for the duration (24hr and 72hr), and results showed that apigenin works in a dose-dependent and time-dependent manner. As the concentration of apigenin increases, its proliferation inhibitory effect increases (Lee & Cho, 2019).

Sung B et al. explained the role of apigenin in cancer prevention via the induction of apoptosis and autophagy apigenin (4, 5, 7-trihydroxyflavone). According to the studies, it is thought that the anticancer activity of apigenin was due to cellular response (oxidative stress, damage of DNA, and angiogenesis), and other mechanics stated that apigenin promotes cell cycle arrest and apoptosis via the P53 pathway. It is thought to be cancer preventive by inducing autophagy, a self-eating induction process. Mice were used to test apigenin anticancer activity doses (20 and 50ug) per mouse for 20 weeks. Results after 20 weeks showed a decrease in tumor volume, then hamsters were also used in the experiment. They were given a dose of 2.5mg per kg, and after 15 15-week periods, tumor volume was reduced in them. In conclusion, evidence from both in-vivo and in-vitro studies supports that apigenin can induce apoptosis (Sung, Chung et al. 2016).

According to Thu Hua Pham et al., apigenin, a partial antagonist of the Estrogen receptor (ER), inhibits ER-positive breast cancer cells by proliferation through AKT/FOXM1 signaling. To determine the effect of apigenin on cell proliferation, the MCF-7 and MCF-7/AKT cells were cultured with apigenin alone and with the E2. Results showed that apigenin alone slowly proliferates MCF-7 cells, and when with E2, it proliferates with four folds, indicating an enhancement in its anticancer when used in combination (Pham, Page, et al. 2021). A summary properties of rutin is shown in Table 3.

Table 3 Summary of uses of apigenin as an anti-inflammatory, neuroprotective, anticancer, antioxidant, antihyperlipidemia, and muscle relaxant.

Uses of apigenin	Therapeutic agent	Activity performed	Outcomes	Reference
Anti-inflammatory activity	Apigenin alone	Sepsis-induced rats	Apigenin reduced Inflammation	(Karamese, Erol *et al*. 2016)
	Apigenin and genistein	Intestinal epithelial cells of a rat	Heated apigenin and other flavonoids areless effective than non-heated	(Yu, Jiang *et al*. 2022)
	Apigenin and 4-O-diisocyanate	Acrolein-induced inflammatory cellsof human vein endothelium	Effective against acrolein-inducedinflammation	(Mirzoeva, Tong *et al*. 2018)
	Apigenin alone	RAW 264.7 murine macrophage, rat basophilic leukemia -2H3, and humanimmortalized keratinocyte cells	Showed effect on skin diseases	(Park, Min *et al*. 2020)
	Apigenin alone	Pancreatic cells	Effective as an adjuvant in acutepancreatitis	(Charalabopoulos ,Davakis, *et al*., 2019)

Table 3 (Continued)

Uses of apigenin	Therapeutic agent	Activity performed	Outcomes	Reference
Neuroprotective activity	Apigenin alone	Male Wistar rats	Protective effect in kainite-induced memory deficit by anti-convulsant and anti-apoptotic activity	(Hashemi, Babaei *et al*. 2019)
	Apigenin alone	Pluripotent cells	Showed neuroprotective action	(Balez, Steiner *et al*.
	Apigenin alone	In vitro model	Showed neuroprotective action	(Dourado, Souza *et al*.
	Apigenin alone	Cortical neurons of rat	Showed neuroprotective action by GSK-3β phosphorylation-mediated Nrf2 activation	(Wang, Wang*et al*. 2020)
	Apigenin alone	Cerebral ischemia in vivo and in vitro	Has neuroprotective effect in-vivo and in vitro	(Ling, Lei *et al*. 2020)
	Apigenin 6-O-succinyl 7-apigenin	Male rats	Has anti-ischemic effect	(Zhang, Xu *et al*., 2019)
	Apigenin alone	Male wistar Albino rats	Showed neuroprotective effect	(Kuru Bektaşoğlu, Demir *et al*.
Anti-cancer activity	Apigenin alone	Wister rats	Showed anti-cancer activity	(Yan, Qi *et al*. 2017)
	Apigenin alone	Prostate cancer cells	Showed anti-cancer activity	
	Apigenin alone	Human breast cancer cells	Showed apigenin work in a dose-dependent manner	(Hnit, Yao, *et al*. 2022)
	Apigenin alone	Mice	Anti-cancer activity by apoptosis	(Lee and Cho, 2019)
	Apigenin alone	ER-positive Breast cancer cells	Enhanced anti-cancer activity by E2	(Sung, Chung*et al*. 2016)
				(Pham, Page,*et al*. 2021)

Table 3 Continued)

Uses of apigenin	Therapeutic agent	Activity performed	Outcomes	Reference
Antioxidant activity	Apigenin-solid dispersion	In vitro and in-vivo mouse retina model	Antioxidant enzymes upregulation by Nrf2 pathway upregulation of autophagy Decrease in retinal damage and inhibiting it	(Ma, Ni *et al*. 2020)
	Apigenin oral supplement	2 and 6 months mice model	Decreased oxidative stress, decrease in age-related intrinsic aortic wall stiffness	(Clayton, Hutton *et al*. 2021)
	Potassium salt derivative apigenin	Human keratinocytes $CaCO_2$ cell monolayer	Increased topical activity against skin damage	(Sánchez-Marzo, Pérez-Sánchez *et al*. 2019)
	Apigenin alone	Male mice	Reduction in age-induced skeletal muscle atrophy, oxidative stress, hyperactive autophagy, and apoptosis inhibition	(Wang, Yang *et al*. 2020)
Anti-hyperlipidemic activity	Apigenin alone	Intracellular fat accumulation model cells and high-fat diet-fed model mice	Improves disorder of lipid metabolism	(Wu, Guo *et al*. 2021)
	Apigenin alone	HepG2 cells	Reduced the deposition of lipids in the liver	(Lu, Meng *et al*. 2019)
	Apigenin alone	RAW264.7 macrophages and mice	Reduced atherosclerotic lesion	(Ren, Jiang *et al*. 2018)

Table 3 (Continued)

Uses of Apigenin

	Therapeutic agent	Activity performed	Outcomes	Reference
uscle Relaxant				
	Perilla frutescens var acuta (PFA) leaves	Ciliary muscle cells isolated from Sprague Dawley (SD) rats' eye	Ciliary smooth muscle relaxation	(Kim, Kang *et al*. 2018)
	Apigenin alone	Sprague Dawley model intra-renal arteries	Non-specific and non-competitive effect by antagonizing vasoconstriction	(Jing, Dong *et al*. 2018)
	Roman Chamomile dried flower extract and essentialoil	Humans and animals (pigs and rats) have smooth muscles	Essential oil showed relaxation activity. The extract showed a smooth muscle relaxation effect.	(Sándor, Mottaghipisheh *et al*. 2018)

2.4 Antioxidant

Yuanzhang Zhang et al. confirmed apigenin (AP) activity against oxidative damage and regulation of the Nrf2 pathway and autophagy in the mouse retina. Apigenin is poorly soluble in water and fat; therefore, solid (dispersion of apigenin) AP-SD is prepared, significantly increasing solubility and dissolution of AP in vitro and enhancing absorption of AP in-vivo studies were performed on the mouse retina model with dry age-related macular degeneration (AMI). The experiment was assessed using fundus autofluorescence, optical coherence tomography, and electron microscopy. Results showed that AP-SD improved the bioavailability of AP. Further, an important decrease in retinal oxidative injury by increasing Nrf2 levels HO-1 and NQO-1 target genes levels, SOD, and GSH-PX levels. Antioxidant enzyme upregulation occurs through the Nrf2 pathway upregulating autophagy, resulting in declining retinal damage and inhibiting it (Ma, Ni et al. 2020).

Zachary S. Clayton et al. confirmed oral supplement Apigenin improves oxidative stress, reverses stiffed aorta, and reduces vascular inflammation with aging, leading to restoration of endothelium function; studies were performed on six months and two months mice model (arterial aging), which were divided into groups and allowed to consume water containing vehicle methyl cellulose and water containing apigenin. It was observed that apigenin in water didn't cause any changes in the intake of water and energy. The results indicated that with age, apigenin oral supplements reversed vascular endothelium dysfunction, large elastic artery stiffening, and decline in cell formation in the developed phase of atherosclerosis. Furthermore, apigenin performed this function by increasing NO bioavailability, normalizing age-related arterial ROS production increase, oxidation stress, oxidants, and antioxidants enzyme, and by reducing age-related aortic intrinsic wall stiffness, adverse extracellular matrix remodeling, and vascular inflammation (Clayton, Hutton, et al. 2021).

Noelia Sanchez-Marzo et al. reported apigenin antioxidant and photoprotective action, potassium salt derivative of apigenin in human keratinocytes, and absorption in CaCO2 cell monolayer. The studies observed UVA (Ultraviolet A) and UVB (Ultraviolet B) effects on human keratinocytes, and antioxidant activity was determined by TEAC (Trolox et al. Capacity), FRAP (Ferric Reducing Antioxidant Power) and ORAS (Oxygen et al. Capacity Assays). In vitro results in CaCO2 model. Both apigenins showed a protective effect in cell viability, approximately 50% at (5J/m2) UVA and 90% at (500J/m2) UVB radiation, also similar intestinal absorption apparent permeations (1.81×10-5cm/s and 1.78 ×10-5 cm/s). In conclusion, both apigenin is suitable for developing nutraceuticals and photoprotective topical active ingredients for protection against skin damage caused by UVA and UVB. However, apigenin keratinocytes' increase in water solubility makes it a better candidate than the CaCO2 model (Sánchez-Marzo, Pérez-Sánchez, et al. 2019). Dongtao Wang et al. confirmed the antioxidant activity of apigenin caused by the inhibition of oxidative stress, hyperactive milo graph, and apoptosis resulting in age- induced muscle atrophy. This research was performed on male mice (aged 16 months approx.) randomly divided into four groups (each group containing 12 mice). After administration, apigenin showed significant improvement in the antioxidant action of glutathione peroxidase and superoxide dismutase enzyme. A remarkable increase in ATP peroxisome proliferator-activated receptor y (co- activator 1α), mitochondrial respiratory factor A, nuclear respiratory factor I, and ATP5B while Bcl-29 (adenovirus E1B), 19KD (interacting protein-3), and DNA fragmentation inhibition were observed. On the whole, results showed a reduction in age-induced skeletal muscle atrophy, a decline in oxidative stress and hyperactive autophagy, and apoptosis inhibition by apigenin (Wang, Yang, et al. 2020).

2.5 Anti-hyperlipidemia

Lilling Wu et al. reported that apigenin improved lipid accumulation and insulin resistance. They established intracellular fat accumulation model cells and high-fat diet-fed model mice using palmitate and high-fat diet. They first observed apigenin downregulated sterol regulatory element binding protein-2 and sterol regulatory element binding protein-1c, stearyl-CoA desaturases-1, fatty acid synthase, and 3-hydroxy-3-methyl-glutaryl CoA reductase in mice and palmitate (PA) induce hyperlipidemic cells. They showed that apigenin markedly reduces endoplasmic reticulum stress, reducing blood lipid-inducing hyperlipidemic cells. So results concluded that apigenin significantly improves the disorder of lipid metabolism, which was related to a reduction in sterol regulatory element binding protein-1C and sterol regulatory element binding protein-2, inhibit endoplasmic reticulum stress, downstream genes, and decrease of lipids and insulin resistance (Wu, Guo et al. 2021).

Jing Lu et al. studied the effect of apigenin in reducing the increased buildup of lipids. The purpose of the study was to investigate the anti-adipogenic activity of apigenin. HepG2 cells were used. For 24 hours, HepG2 cells were treated with varying concentrations of apigenin. It reduces the total cholesterol and triglyceride, which were palmitic acid-induced and reduces intracellular aggregation of lipids. Moreover, apigenin enhanced the phosphorylated-AMP-activated protein kinase. It reduced the 3-hydroxy-3-methylglutaryl CoA reductase, sterol regulatory element binding protein-1, fatty acid synthase, and sterol regulatory element binding protein-2 in a concentration-dependent manner. Thus, apigenin reduces the deposition of lipids in the liver via the activation of phosphorylated-AMP-activated protein kinase and sterol regulatory element binding protein pathways (Lu, Meng, et al. 2019).

Kun Ren et al. studied the effect of apigenin on atherosclerosis. The study's objective was to evaluate the mechanism of action of apigenin on ABCA1-mediated cellular cholesterol efflux and LPS-stimulated inflammation. RT-PCR, Liquid scintillation counting, HPLC assay, and ELISA assay were used. Further, Oil Red O staining was used to measure the size of atherosclerotic

lesions, and immunochemistry was used to evaluate the contents of macrophages and smooth muscle cells in atherosclerotic lesions. The study was performed on RAW264.7 macrophages and mice. The results indicated that apigenin enhanced the expression of ABCA1 via miR-33suppression in dose and time dependent manner. It enhanced the ABCA1-mediated cholesterol efflux and decreased TC, FC, and CE levels in macrophage-derived foam cells. Moreover, levels of TLR-4, MyD88, and p-IκB-α, along with nuclear NF-κB p65, were reduced by epigenin in LPS-treated macrophages. It also reduced pro-inflammatory cytokines. In LPS-challenged mice, apigenin increased the expression of ABCA1, reduced macrophages and smooth muscle cells in atherosclerotic lesions, and decreased miR-33, TLR-4, and NF-κB p65 levels. Apigenin reduces atherosclerotic lesion size by enhancing the plasma lipid profile and comforting inflammation (Ren, Jiang, et al. 2018). Figure 2 depicts the role of apigenin as an anti-inflammatory, antioxidant, anti-hyperlipidemia, neuroprotective and muscle relaxant.

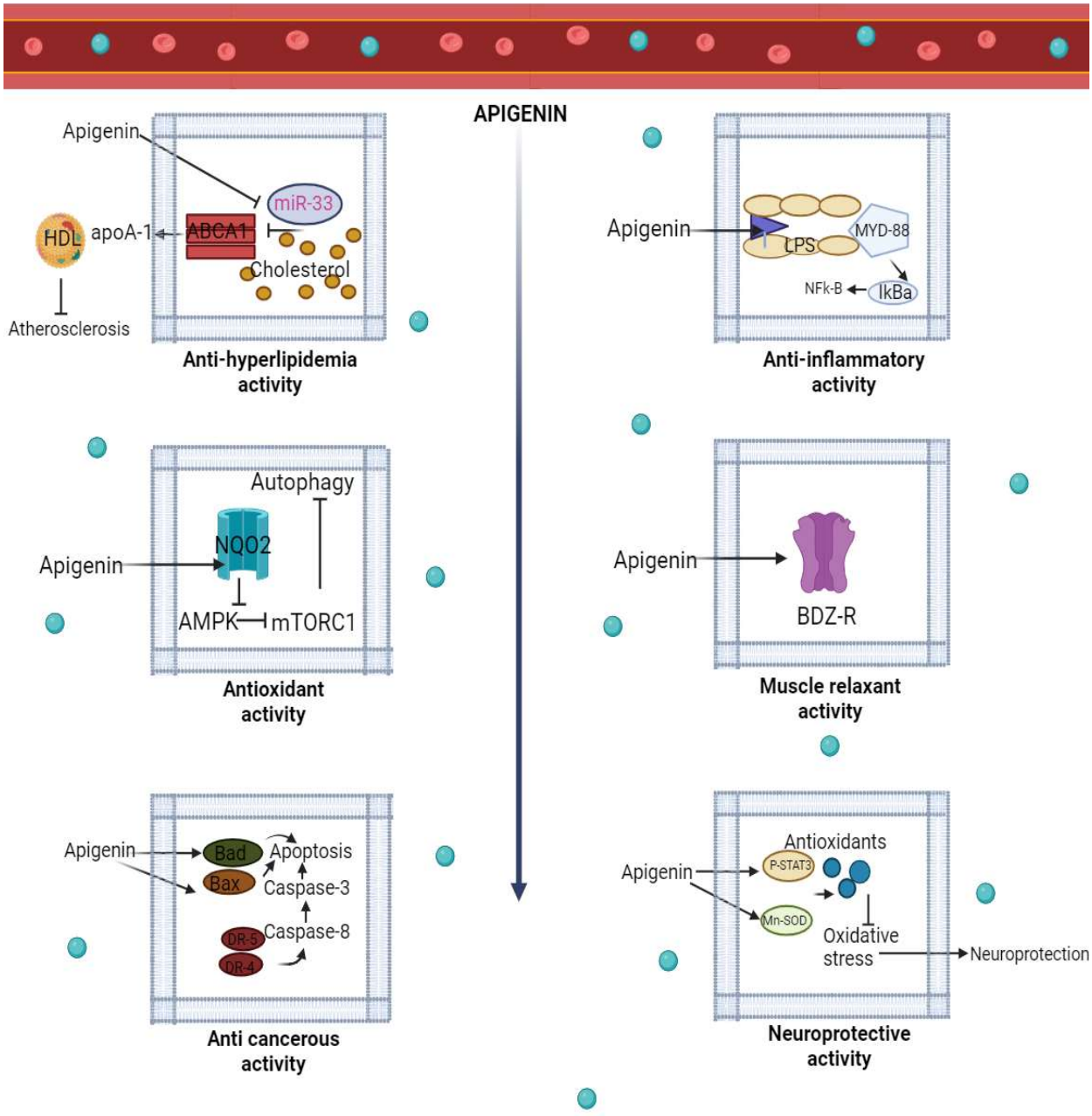

Figure 2 Cellular mechanism of apigenin as an anti-inflammatory, antioxidant, anti-hyperlipidemia, neuroprotective, and muscle relaxant

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2.6 Muscle Relaxant

Jaeyong Kim et al. reported that Perilla frutescens var acuta (PFA) leaves are an herbal remedy for ciliary smooth muscle relaxation in Korea, Japan, and China. This study analyzed active constituents (mainly apigenin-7-O-diglucuronide and luteolin-7-O-diglucuronide) by NMR and MS obtained from leaves aqueous extract in-vitro and in-vivo study by enhancing NO/cGMP contents. The freeze-dried and spray-dried compositions of PFA were almost the same. Ciliary muscle cells isolated from Sprague Dawley (SD) rats' eyes were treated with freeze-drying PFA compositions: 50 microgram/ml, 100 microgram/ml, and 20 microgram/ml. An increase in NO and cGMP content while a decrease in Ca+2in a dose-dependent condition was observed. However, a significant increase in cGMP was observed when SD rats were treated orally with 200mg/kg freeze-dried PFA. In conclusion, PFA extract (apigenin-7-odiglucuronide and luteolin-7-O-diglucuroniude) effectively treats eye fatigue (Kim, Kang, et al. 2018).

Yixin Jing et al. reported vasospasmolytic and electro-physiological activity of API involuntary Cl- and K+ channels in Sprague Dawley model intra-renal arteries (IRAS), whose vascular tone was recorded using a monograph. Also, a patch clamp study was performed on smooth muscles (fresh isolation from arteries) to record Cl-current across Ca+2 activated Cl- channels inward rectifier K+ channel and voltage-gated K+ current. KCL-induced contraction was lowered by 10-100um of apigenin when pre-incubated also phenyl ephedrine, vasopressin (VP), U46619, 11α- methanol epoxy prostaglandin, and 9,11-dideoxy-9α decline was observed. Furthermore, apigenin acute application provided immediate vasorelaxation in intrarenal arteries and noted values of ICSO (13.27-26.26um) and RC50 (5.80-24,33um). However, the apigenin vasorelaxation effect was reduced by Ca+2 activated Cl- channels, K+ voltage-gated channels blockers Cl- loss and NO synthase inhibitor, and inward rectifier K+ channel did not reduce API effect. In conclusion, apigenin shows non-specific and non-competitive effects by antagonizing vasoconstriction (Jing, Dong et al. 2018).

Zsolt Sandor et al. informed hydro-ethanol extract of Roman Chamomile dried flowers (Chamaemelum nobile (L.)) effectiveness as a smooth muscle relaxant in-vitro studies. The essential extract containing Apigenin, luteolin, hispidulin, and eupafolin were used, and HPLC-DAD, GC, and GC-MS were used to quantify and characterize them. The experiment was performed on human and animal (pigs and rats) smooth muscle preparations in an isolated organ bath. It was observed that gut walls cholinergic neurons pig ileum were activated resulting in prompt moderate and short-time contraction when extract was administered. However, essential oil showed relaxation and no contraction activity, but extract showed a sustained effect. Moreover, the extract showed smooth muscle relaxation in rat tissues (gastrointestinal) and human small intestine (strip preparations). As a result, traditional medicine Chamaemelum nobile extract and essential oil show sustained and direct smooth muscle relaxation effects (Sándor, Mottaghipisheh, et al. 2018).

Conclusion

This current review concluded that apigenin, a natural flavonoid, is effective as an anti-inflammatory, neuroprotective, anticancer, antioxidant, anti-hyperlipidemic, and muscle relaxant agent. Apigenin or its various derivatives are used alone or in combination therapy in pharmaceutical and nutritional fields to combat different diseases worldwide For example, 6-O-succinyl apigenin, a new derivative of apigenin, is highly water soluble and has a better neuroprotective effect. It is also used as an herbal remedy for muscle relaxant effects. Apigenin can be formulated in different formulations such as solid dispersion systems. The main problem associated with the use of using apigenin is that it is not heat resistant and loses its stability at high temperatures, requiring special storage conditions. So, in order to maintain its stability and overcome this drawback, different pharmaceutical approaches should be adopted during the manufacturing, formulating, packaging, and storing of drugs containing apigenin as the active ingredient.

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Declarations

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All data generated or analyzed during the study are included in the manuscript.

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Consent for publication

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