INTERNATIONAL JOURNAL FOR LEGAL RESEARCH AND ANALYSIS

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Peer Reviewed

www.ijlra.com

 ISSN:2582-6433

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 ISSN:2582-6433

EUPATILIN: A REVIEW OF ITS TRADITIONAL USES, PHARMACOLOGICAL ACTIVITIES, AND FUTURE PROSPECTS AS A THERAPEUTIC AGENT

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Abstract

Eupatilin (5,7-dihydroxy-3′,4′,6-trimethoxyflavone) is a pharmacologically active flavone which has been isolated from a variety of medicinal plants. Eupatilin is known to possess various pharmacological properties such as anti-cancer, anti-oxidant, and anti-inflammatory. It is speculated that eupatilin could be subjected to structural optimization for the synthesis of derivative analogs to reinforce its efficacy, to minimize toxicity, and to optimize absorption profiles, which will ultimately lead towards potent drug candidates. Although, reported data acclaim multiple pharmacological activities of eupatilin but further experimentations on its molecular mechanism of action are yet mandatory to elucidate full spectrum of its pharmacological activities. Eupatilin, a flavonoid compound derived from the Artemisia princeps plant, has been traditionally used in Asian medicine for its anti-inflammatory and anti-allergic properties. From a medicinal chemistry perspective, eupatilin's pharmacological activities can be attributed to its unique chemical structure, which consists of a flavone backbone with a hydroxyl group at position 5 and a methoxy group at position 4'. This review provides a comprehensive overview of eupatilin's traditional uses, pharmacological activities, and future prospects as a therapeutic agent.

Keywords eupatilin; anti-cancer; anti-inflammatory; anti-oxidant; medicinal plants; flavone

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Introduction

Natural products (NPs) have historically affirmed their worth not only as therapeutic agents but also as an important source of novel drug leads. The term "natural products" is usually prescribed as chemical entities that are mainly derived from the living species such as plants, microorganisms, and marine organisms. These naturally occurring compounds are known to possess complex chemical diversity with outstanding drug-like activities that contribute towards their multi-targeted action. Polyphenols are secondary metabolites that are found in numerous medicinal plants with potential anti-inflammatory, anti-cancer, anti-allergic, anti-microbial, and anti-oxidant activities.[1] Flavonoids represent an eminent group of plant-derived polyphenols, with greater than 8000 varying compounds (**Figure 01)**.

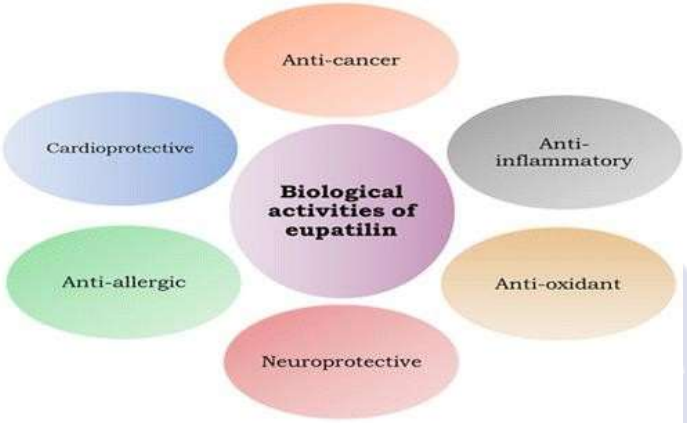

Figure 01: Biological activity of Eupatilin

Flavonoids are associated with multiple biological effects, including antitumor, anti-oxidation, anti-inflammation, antiviral and hepatoprotective activities, as well as in the prevention of cardiovascular diseases. Eupatilin (5,7-dihydroxy-3',4',6-trimethoxyflavone) is extracted from Artemisia asiatica (A. asiatica) Nakai, and this isolated flavonoid contains pharmacologically active ingredients. Eupatilin has been demonstrated to exert anticancer, anti-oxidative and anti-inflammatory effects. A previous report indicated that Stillen™ (DA-9601), produced from the ethanol extract of A. asiatica, contained the pharmacologically active flavonoid compound eupatilin. DA-9601 demonstrated cytoprotective effects against gastric mucosal damage and ulcerative proctitis. Eupatilin has exhibited positive effects in the treatment of oxidant-dependent gastric disorders.[2] Eupatilin, apigenin, wogonin and baicalein are all members of the same family of flavonoids. Although the flavones, apigenin, wogonin and baicalein, have previously been used in the treatment of OS, the molecular mechanisms underlying eupatilin-mediated apoptosis of the U-2 OS cell line have remained to be elucidated. Therefore, the present study aimed to aid the elucidation of the underlying mechanism involved in eupatilin-induced apoptosis of U-2 OS cells. This was achieved via cytotoxicity experiments, apoptosis

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studies and the analysis of changes in protein expression associated with apoptotic cell death.[3]

Eupatilin (5,7-dihydroxy-3′,4′,6-trimethoxyflavone), a pharmacologically active flavonoid mainly found in genus Artemisia, is known to possess auspicious anti-cancer, anti-inflammatory, anti-oxidant, neuroprotective, anti-allergic, and cardioprotective activities. Assorted studies on eupatilin demonstrated its potent bio-activities which have made further interest among the biologists and chemists to explore more about this bioactive natural flavone.[4]

To date, no comprehensive review has been available on the pharmacological and biological profile of eupatilin. The present review aims to compile the available information on eupatilin, along with signifying its current status.[5] This will hopefully help to ease the understanding of eupatilin pharmacological appearance in drug development, thus, proposing areas where further research work is mandatory. The literatures were screened through different e-sites; Elsevier Science Direct, Scopus, Springer Link, PubMed, and other medical journals. Key words that are being used for searching are “eupatilin”, “eupatilin and its biological activities”, “anti-cancer”, “anti-allergic”, “neuroprotective”, and “anti-inflammatory”. [6-9]

Biosynthesis of eupatilin

Eupatilin is a flavonoid compound that is biosynthesized in plants through the phenylpropanoid pathway. The biosynthesis of eupatilin begins with the conversion of phenylalanine into cinnamic acid by the enzyme phenylalanine ammonia-lyase (PAL). Cinnamic acid is then converted into p-coumaric acid by cinnamate 4-hydroxylase (C4H), which is subsequently converted into p-coumaroyl-CoA by 4-coumarate: CoA ligase (4CL). The enzyme chalcone synthase (CHS) then combines p-coumaroyl-CoA with malonyl-CoA to form naringenin chalcone, which is converted into naringenin by chalcone isomerase (CHI). Naringenin is then converted into eriodictyol by flavonoid 3'-hydroxylase (F3'H), and finally, eriodictyol is converted into eupatilin by flavonoid 3',5'-hydroxylase (F3'5'H).[10-12] The biosynthesis of eupatilin is regulated by various transcription factors and enzymes that control the expression of the genes involved in the phenylpropanoid pathway (**Figure 02)**.

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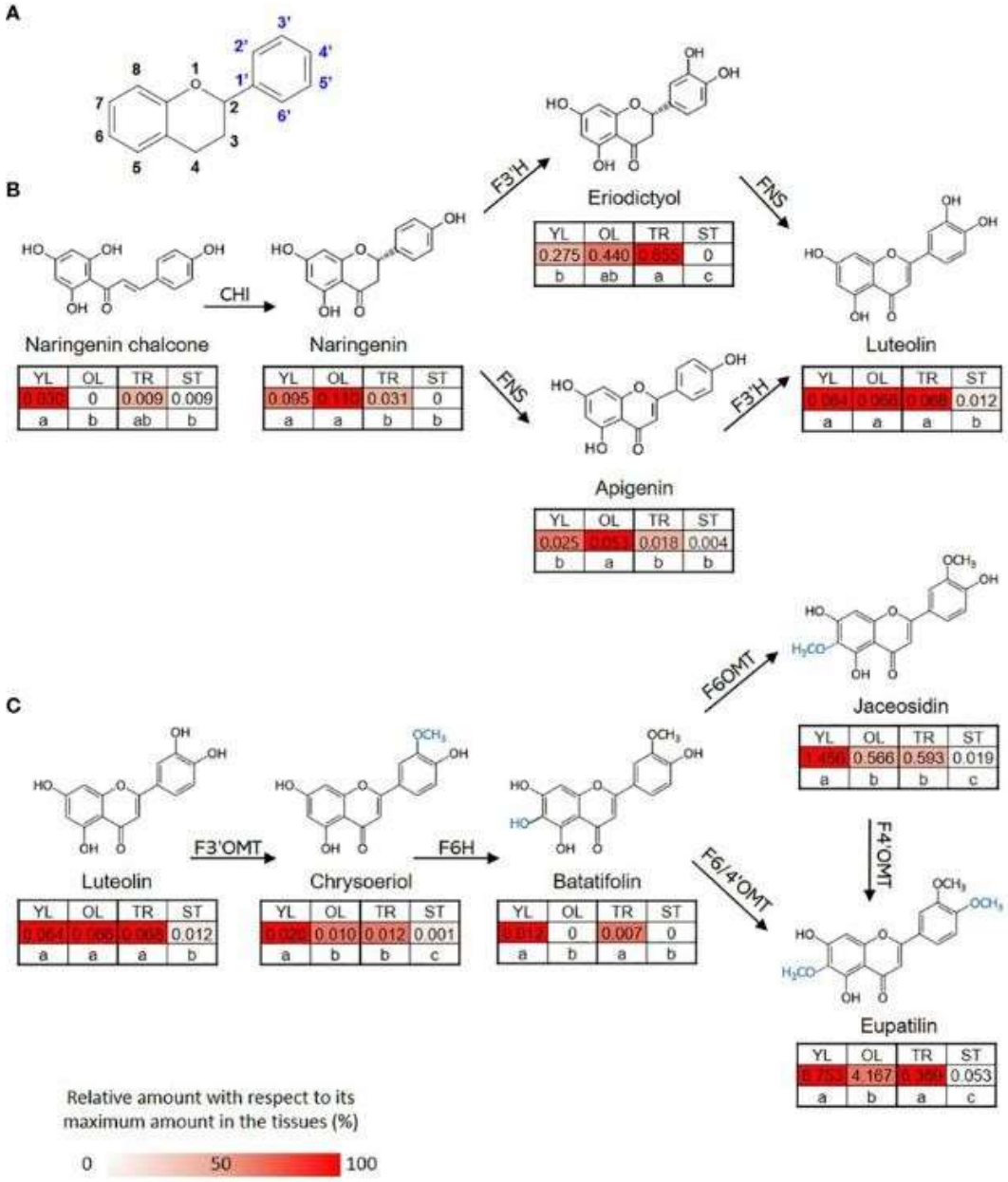

Figure 02: Biosynthesis pathway of eupatilin Method of Extraction of Eupatilin

Eupatilin is typically extracted from the leaves and flowers of the Artemisia princeps plant using various solvent extraction methods. The most common method involves soaking the plant material in a solvent such as ethanol, methanol, or acetone, followed by evaporation of the solvent to leave behind a crude extract containing eupatilin. Other methods include maceration, where the plant material is soaked in a solvent for an extended period, and ultrasound-assisted extraction, which uses high-frequency sound waves to enhance extraction.[12]

Microwave-assisted extraction, supercritical fluid extraction, and column chromatography are also used to extract and purify eupatilin. Finally, high-performance liquid chromatography (HPLC) is employed to separate and purify eupatilin, resulting in a highly concentrated and

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purified extract (**Figure 03)**.

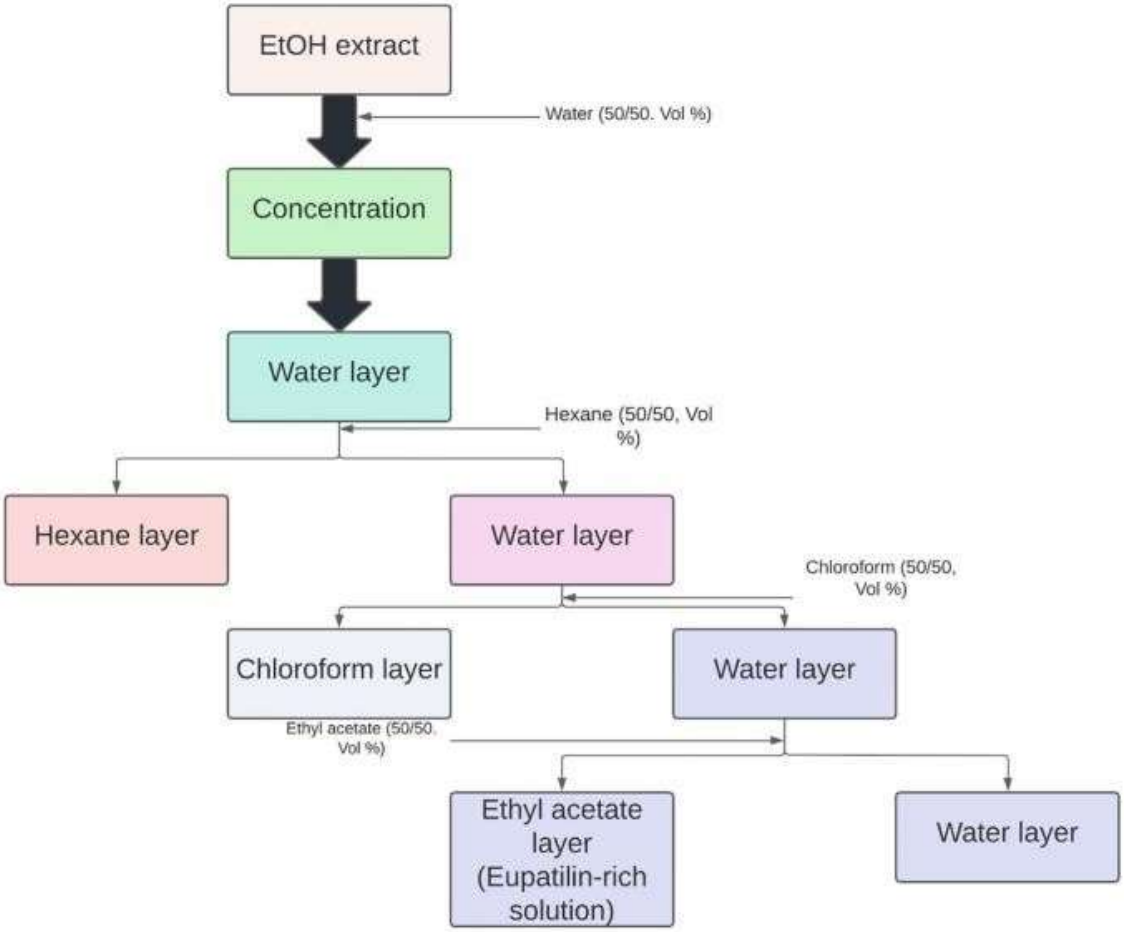

Figure 03: Method of Extraction of Eupatilin Eupatilin and its natural sources

Eupatilin, a bioactive flavone has been identified in many plants of family Asteraceae that comprises ~24,000 species. Of these, Artemisia is a diversified genus encompassing ~500 species in the temperate regions of Europe, Asia, and North America. It has been reported as an affluent source of flavonoids including eupatilin. Genus Centaurea incorporates more than 500 species, of which 45 grow instinctively in Algeria, with 7 species localized in the Sahara. Genus Tanacetum consists of ~160 species commonly known as tansies which are widely utilized in folk medicines for various treatment purposes. The aerial parts of T. vulgare are effective against neuralgia, rheumatism, and migraine. It also serves as an anthelmintic and anti-inflammatory agent. Besides them, eupatilin has been isolated from the plants of family Labiatae (Lamiaceae) generally known as “mint family”. Salvia is the most prominent genus of mint family with more than 900 species extensively distributed all over the world. S. plebeia is advantageous to cure asthma and skin inflammatory ailments in the Asian countries. Eupatilin was also extracted from the leaves of C. morifolium (Asteraceae) and L. dulcis (Verbenaceae) as modest anti-oxidative agent. The leaves of L. dulcis are also useful as

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traditional medicine for treating bronchitis and cough.[13-15] Eupatilin has notable pharmacological and biological characteristics of interest for the cure of different diseases including cancer, gastritis and many others (**Figure 04)**.

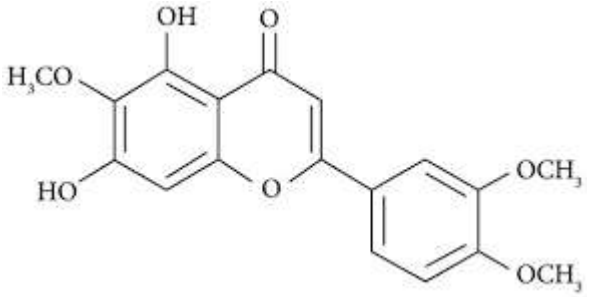

Figure 04: Chemical Structure of Eupatilin Biological activities of eupatilin and their mechanisms of action

The biologically active compound "eupatilin" has been demonstrated for its broad spectrum of pharmacological properties such as anti-cancer, anti-inflammatory, anti-oxidant, neuroprotective, anti-allergic, and cardioprotective. Various in vivo and in vitro investigations on eupatilin have elucidated its medicinal characteristics and mechanism of actions **(figure 01)**.

Anti-microbial activity

Kim et al. (1996) fractioned an extract of A. asiatica into hexane, chloroform, butanol, and aqueous fractions, and among these fractions, the hexane fraction exerted the highest anti-microbial activity and inhibited the growth of microorganisms including Bacillus subtilis, Escherichia coli, Staphylococcus aureus, and Lactobacillus plantarum at doses of 250, 500, and 750 μg/ml, although the inhibitory potency of this plant was weak (Kim et al., 1996). Essential oil isolated from A. asiatica in the flowering stage with main constituents including 1,8-cineol and selin-11-en-4a-ol exhibited anti-microbial and anti-fungal activities against Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Rhodotorula rubra, and Aspergillus fumigatus with a minimum inhibitory concentration ranging from 1–3 ml/ml (Kalemba et al., 2002). Furthermore, the monoterpene alcohol fractions showed the highest anti-microbial activity (Kalemba et al., 2002). Compared to other plant-derived extracts or antibiotics such as Oroxylum indicum, Camellia sinensis, and gentamicin (Sharma et al., 2012; Sithisarn et al., 2016), however, it is suggested that A. asiatica does not have strong anti-microbial property compared to other biological activities such as gastroprotective effect.

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Anti-cancer activity

Phytochemicals, bioactive constituents of medicinal plants, such as polyphenols (flavonoids, terpenoids and carotenoids) have potent competency for the cure of cancer and thus, are anti-cancerous in nature. Several studies on eupatilin have explained its anti-cancer property due to its promising capacity to prompt apoptosis in different cancer cell lines encompassing gastric cancer (AGS, MKN-1), leukemia cancer cells (HL-60), renal carcinoma (786-O), hepatocellular cancerous cells (Huh-BAT), osteosarcoma cancer cells (U-2), glioma cancerous cells (U87MG, LN229), and melanoma cancer cells (A375). Researches have concluded that eupatilin generally shows synergetic effects on cell cycle arrest, apoptosis, and numerous cell signaling pathways in distinct cancer types.

Eupatilin and apoptosis

Naturally occurring flavonoids have been reported as lead constituents in the development of several chemo preventive and chemotherapeutic agents. Compiled data by the researchers recommend that induction of apoptosis can result from varied chemotherapeutic and chemo preventive agents. Eupatilin has capability to stimulate apoptosis in human AGS cancerous cells by a reduction in the ratio of Bax and Bcl-2, along with the cleavage of (poly (ADP-ribose) polymerase) PARP and caspase-3. It has been recently approved as a cytoprotective agent by preventing H2O2-induced apoptosis dose-dependently in gastric cancer cells. The anti-metastatic effect of eupatilin, isolated from A. asiatica, was also studied in MKN-1 gastric cells. Results concluded that eupatilin effectively triggers apoptosis by enhanced expression of caspase-3 pursued by down-regulation of pro-inflammatory cytokine-interceded metalloproteinase (MMPs) levels in a dose- and time-related manner.[16]

Eupatilin, a novel anti-cancer compound, prompted apoptosis and upgraded the creation of reactive oxygen species (ROS) in renal 786-O cancer cells. The anti-proliferative potential of eupatilin in promyelocytic HL-60 leukemia cultured cells can be dedicated to its apoptosis inducing potential as described by distinctive nuclear condensation, release of cytochrome c, cleavage of PARP, and proteolytic activation of caspase-3/-7/-9, respectively. According to a study on Huh-BAT hepatocellular cancer cells, eupatilin notably decreased bile-acid stimulated hepatocytic apoptosis by debilitating caspase-8 cleavage in the bile duct-ligated rats. Eupatilin has potent competency to inhibit osteosarcoma (U-2) cancer cell growth by stimulating apoptosis and activating mitochondrial intrinsic pathway that is characterized by

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increased Bax/Bcl-2 proportion, decline in mitochondrial membrane potential, release of cytochrome c, activation of caspase-3/-9, and cleavage of PARP. Treatment with eupatilin specifically prohibited cellular proliferation in A375 human melanoma cells via inducing apoptosis through morphological innovations of DNA fragmentation in a dose-dependent manner. Eupatilin also displayed inhibitory response on glioma cancerous cells (U87MG, LN229) by induction of apoptosis via suppressing of Notch-1 signaling pathway.[17]

Eupatilin and cell cycle arrest

Eupatilin, a natural flavone, has proficiency to prompt G2/M cell cycle phase arrest in endometrial KLE and Hec1A cancerous cell lines in a time- and dose-dependent manner. It eloquently prohibited mutant p53 via up-regulating p21 expression, along with the activation of Checkpoint Chk2/ATM/Cdc2/Cdc25C pathway. Eupatilin treatment effectively increased the expression levels of p53 and p21 cell cycle regulators and induced G1 phase arresting in gastric AGS cancer cells. Moreover, eupatilin medication also caused G2/M cell cycle phase arresting associated with apoptotic cell death in U-2 and A375 cancer cell lines. Eupatilin has been demonstrated as powerful chemo preventive agent in inhibiting EGF-stimulated JB6 skin cell proliferation by targeting PI3K and promoting G0/G1 cell cycle arresting through decreased expression of cyclin D1 in a dose-related manner. In MCF10A-ras carcinoma cells, eupatilin forbided the expression of important cell cycle organizers cyclin B1, Cdk2, Cdc2, and cyclin D1. It has the capacity to up-regulate p53, p27Kip1 (Cdk inhibitors) and down-regulate p21waf1/Cip1 expressions in a time- and concentration-dependent manner. It can be concluded that eupatilin arrests cell cycle at G2/M phase but whether in G2 or M phase should be investigated. Furthermore, it would be interesting to investigate the mechanism by which eupatilin arrested cell cycle in breast cancer cells and melanomas at G0/G1 and G1/S phases as in case of other cancer cells it is generally arresting cells at G2/M phase. So, extensive studies are yet mandatory to fully understand molecular mechanism by which eupatilin regulates cell cycle or induces cell cycle arrest.[18-20]

NF-κB/STAT3 signaling pathways and eupatilin

Flavonoids, phenyl-substituted chromones, have drawn developing consideration as effective anti- cancer agents against several cancer cell lines. Eupatilin, a pharmacologically active flavone has been reported to acquire anti-cancer effects via suppressing NF-κB cell signaling pathways. Several investigations have revealed the fact that eupatilin actively participates in

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the prevention of gastric cancer (MKN-1, AGS) by decreasing NF-κB activity pursued by down-regulation of metalloproteinase (MMP-2, MMP-9) and pro-inflammatory cytokines. Furthermore, in MKN45 gastric cancerous cells, eupatilin significantly prohibited STAT3-induced VEGF expression most apparently under hypoxic situations, indicating its therapeutic potential to cure gastric cancer. Though several studies have reported that eupatilin induces apoptosis via inhibition of NF-κB but whether eupatilin has direct effect on NF-κB signaling pathway or via its up-stream signaling pathway JAK or STAT3 still needs to be investigated by researchers.[21-23]

Targeting PI3K/AKT & MAPK pathways with eupatilin

Eupatilin as a pharmacological active flavone derived from various Artemisia species has a potential to stimulate apoptosis via MAPK and AKT pathways in many cancer types. Eupatilin has strong ability to induce apoptosis in renal cancerous cells via reactive oxygen species (ROS)- mediated phosphorylation of p38 MAPK, ERK1/2, JNK, and inhibition of AKT/PI3K signaling cascade in a concentration-dependent manner. Thus, eupatilin can serve as persuasive therapeutic avenue leading to the cure of cancer. Demonstration of eupatilin as anti-cancer agent on human gastric cancer cells has declared the evidence that it works as an inhibitory agent for the activation of AKT and ERK that are crucial constituents of cellular survival cascades. According to a report, AKT inactivation or/and activation of ERK1/2 may have assisted in the activation and phosphorylation of Chk2/Cdc2 checkpoint proteins causing cell cycle arrest in eupatilin treated endometrial cancer cells. Studies on anti-proliferative potential of eupatilin against MCF10A-ras cancer cells have uncovered the fact that blockage of cell cycle advancement turns out to be preferable to prohibit ERK1/2 activation. 50 μM of eupatilin effectually receded phosphorylation of AKT and ERK/MAPK resulting in induction of apoptosis in MKN-1 cells. However, to precisely figure out this mechanism, further experimental investigations are recommended. In addition, there is lack of data regarding to multiple splice variants of JNKs such as whether eupatilin targets JNK1 or JNK2 which should be addressed by researchers.[2426]

Anti-inflammatory activity

Eupatilin, a bioactive flavone, was reported to possess anti-inflammatory potential. Oral administration of extract inclusive of eupatilin and quercetin-3-β-D-glucuronopyranoside meliorated the colonic injury and inflammatory response in dose-dependent manner

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by reducing the neutrophil activation and oxidative stress. Treated animals exhibit decreased expression level of mucosal myeloperoxidase, tumor necrosis factor-α, nitric oxide, and malondialdehyde and enhanced glutathione levels. Findings by researchers declared eupatilin as a suppressor of inflammatory response and it acts via inhibiting the inflammatory modulators and NF-κB activation in endotoxin-stimulated macrophages and carrageenan-induced inflammation in mice. Molecular mechanism underlying eupatilin associated extenuation of inflammatory response in TNF-α-induced bronchial epithelial inflammation lies in the inhibition of eotaxin expression and decline in the activities of NF-κB and IκBα kinase signaling.[27-28]

Characterization of molecular mechanism by eupatilin in Bacteroides fragilis enterotoxin-induced inflammatory response suggests that eupatilin can uncouple the association between Hsp90 and IKK-gamma in NF-kappa B pathway, thus, this bioactive flavone may have the ability to reduce inflammatory response. Eupatilin is an auspicious therapeutic agent against ischemia-reperfusion-induced liver injuries in mice as it can reduce apoptosis and inflammation by up-regulating B-cell lymphoma 2 protein, heat shock protein, and down-regulating the cleaved caspase-3 levels. Eupatilin may attune angiogenesis route, a part of anti-inflammatory response in gastric cancer cell line by inhibition of the vascular endothelial growth factor (VEGF) and STAT3 expression. Eupatilin caused inhibition of adherence of inflammatory cells to epithelial cells via NF-kappa B and AKT-dependent pathways as well as targeting adhesion molecule expression. Eupatilin, a lipophilic flavonoid, showed notable in vivo anti-inflammatory property in a dose-dependent way, qualitatively comparable to hydrocortisone and intervening in conditions of efficacy amid to those of steroids and non-steroidal anti-inflammatory drugs. Eupatilin, along with other natural compounds specifically inhibited 5-lipoxygenase: a potent mediator of inflammatory response and also cause the suppression of leukotriene D4 and C4 in cultured mastocytoma cells. Eupatilin has pronounced capability to inhibit TNF-α-induced inflammatory response via ROS/MAPK-NF-κB signaling pathway suppression in human umbilical vein endothelial cells.[29]

Antioxidant activity

Eupatilin has potential to activate sestrin-2-dependent autophagy via inhibition of oxidative stress stimulated by arachidonic acid (AA+) iron in HepG2, H4IIE, and Hepa-1c1c7 cells. It remarkably upgraded cell viability against AA+ iron in a concentration-related manner and also prohibited mitochondrial dysfunction and production of ROS.

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In human MCF-10A epithelial cells, eupatilin blocked TNF-α-mediated invasion and intracellular ROS formation, along with suppressed urokinase-type plasminogen activator (uPA) and β-catenin expression. Eupatilin may serve as potential therapeutic target against acute ischemia- stimulated. kidney injury through reduced neutrophil gelatinase-correlated lipocalin and renal damage molecule-1 expressions, serum creatinine levels, and blood urea nitrogen level. It also enhanced the expression of Hsp70 and Bcl-2 via debilitating iNOS, caspase-3, and Bax levels. The renoprotective effects of A. asiatica extract and eupatilin were observed in LLC-PK1 renal epithelial cells. Results disclosed that A. asiatica and eupatilin cotreatment has the ability to cure cisplatin-induced kidney damage via down-regulating phosphorylated JNK and p38 protein levels. Pretreatment with eupatilin actively blocked H2O2-mediated cell damage, ROS generation, and apoptosis via decreasing Bax and caspase-3 expressions with increasing Bcl-2 and phosphorylated PI3K/AKT levels in ARPE-19 retinal pigment epithelial cells.[30-31]

It also suppressed hypoxia/re-oxygenation (H/R)-stimulated cardio-myocyte oxidative injury in H9c2 rat cells via down-regulating the release of lactate dehydrogenase (LDL) and activating AKT/GSK-3β signaling pathway. In an investigation, wound healing potential of eupatilin was examined through inhibiting. H2O2- and FeSO4-induced cell injury in AGS epithelial cells in a dose-dependent manner. After effects terminated that eupatilin therapy decreased the expression of genes responsible for oxidative damage in H2O2-treated cells such as PLAUR, HO-1 and TNFRSF10A.[32]

Neuroprotective activity

The multifarious array of bioactive compounds abundantly found in natural products plays a crucial role in the treatment and prevention of several neurodegenerative ailments such as Parkinson's, Alzheimer's, and other neuronal dysfunctions. Within the recent decades, a variety of flavonoids have been recommended to be valuable for the cure of brain-related injuries incorporating depression and dementia.[33]

Eupatilin, a bioactive flavonoid from many Artemisia species, has been shown to be neuroprotective against global cerebral ischemia-stimulated neuronal damage in mice by reducing a number of deteriorating neuronal cells via enhanced expression of AKT phosphorylation. Eupatilin administration also exerted neuro defensive effects against focal cerebral ischemia in tMCAO mice in vivo through lessening microglial activation and number

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of lba1-immunopositive cells across the ischemic brain and suppressing NF-κB signaling cascade functions. Stillen and its major constituent, eupatilin has anti-depressant potential to overcome depression by up-regulating mRNA interpretation of estrogen β-receptor and down-regulating TNFα, IL-6, and IL-1β levels in the hippocampus, respectively.[34-36]

Eupatilin and its other biological activities

Among other biological activities, eupatilin actively plays the role of an anti-diabetic functional constituent in A. princeps and its mechanism of action proceeds by increasing liver and blood glucose metabolism, along with enhanced secretion of insulin in type 2 diabetic mice. Eupatilin was proved to be effective for inhibiting skin aging (IC50 = 1.18 μM) by mobilizing peroxisome proliferator-activated receptor alpha (PPARα). It considerably blocked TNFα-mediated matrix metalloproteinase (MMP-2/-9) expression by reducing NF-κB p65 nuclear translocation and down-regulating MAPK-AP-1 signaling pathway in human HaCaT epidermal keratinocytes. Eupatilin is an adjuvant topical avenue against atopic dermatitis (AD)-alike skin lesions and it acts via reducing interleukin-4/-19, TNF-α, and thymic stromal lymphopoietin expressions.[37-38]

Eupatilin has capability to reform cell viability via suppressing cardiomyocyte apoptosis and triggering AKT/GSK-3β signaling pathway in H9c2 cells in vitro. The molecular mechanism underlying this impact of eupatilin on vascular smooth muscle contractility suggests that it has compelling relaxation effect on agonist-activated vascular contraction via inhibiting fluoride-induced up-regulation in pMYPTI expressions despite endothelial function. Eupatilin is a powerful anti-atherogenic agent and its mechanism of action proceeds via prohibiting PDGF-BB-mediated growth and migration in human aortic smooth muscle cells (HASMCs), along with aortic sprouting that is expectedly stimulated through the debilitation of MKK3/6-p38 MKK4-JNK, MAPK, and PI3 K-activated pathways.[39-40]

In another investigation, eupatilin eloquently stimulated relaxation effect in human corpus cavernosal smooth muscle cells (CCSMC) through NO-independent pathways and decreased phosphorylation of myosin phosphate targeting subunit 1 at Thr853 of MLCP. It may act as an efficacious versatile curative intervention for osteoporosis via prohibition of actin rearrangement of pathogenic multinucleated cells (MNCs) and transcriptional down-regulation of NFATc1 and c-Fos of distinguishing osteoclasts (OC). Eupatilin can be useful for treating osteoarthritis (OA) with conspicuous antinociceptive and

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chondroprotective properties. Eupatilin medication reciprocally increased extracellular matrix formation in particular chondrocytes and suppressed oxidative injury as well as decreased IL-1β, IL-6, iNOS, and nitrotyrosine in the cartilage.[41-43]

Eupatilin may serve as an agent for the cure of various allergic infections. In guinea pig lung cells, eupatilin initially inhibited phosphorylation of Syk tyrosine and then blocked downstream multisignal pathways as well as Ca^{2+} influx during mast cell stimulation. Eupatilin was proved to be convenient for protection from passive cutaneous anaphylaxis (PCA) reaction and itching behaviors stimulated by IgE-antigen complex with IC50 of 3.4 μM. Eupatilin is a well-known gastroprotective agent against gastric mucosal lacerations and lowers gastrointestinal motility by inhibiting enhanced bowel segments of human ileum and colon. It was also reported as anti-ulcer agent during investigation on rat plasma, urine, bile, and liver but accurate mechanism of action is not fully understood.[44]

Eupatilin showed effective anti-xanthine oxidase (XO) activity with a Ki value of 0.13 μg/ ml and IC50 value ranging from 3.3 to 6.8 μM. It strongly exerted anti-mutagenic effects via prohibiting 3-amino-1-methyl-5H-pyrido [4,3-b] indole (Trp-P-2) metabolic activity and heterocyclic amines when treated with Salmonella typhimurium TA98 strain. Clarification of eupatilin as an inhibitory agent on the functions of cytochrome P450 enzymes in human liver microsomes has affirmed the documentation that it vigorously inhibits CYP1A2-organized phenacetin O-deethylation and CYP2C9-catalyzed diclofenac 4-hydroxylation. Kinetic studies on liver microsomes have reported eupatilin as a competitive inhibitor of CYP1A2 [K(i) of 2.3 μM] and a mix-type inhibitor of CYP2C9 [K(i) of 1.6 μM], correspondingly. It also has a potential to prevent thrombosis via blocking arachidonic acid (AA)-stimulated platelet aggregation and production of serotonin and thromboxane A2 (TXA2) as well as enhancing mobilized partial prothrombin time (PT) and thromboplastin time (aPTT) in vitro, thus, acts an important anti-platelet and anti-coagulant agent.[45]

Conclusions and future perspectives

Eupatilin, a flavonoid compound, has been found to possess various pharmacological properties, including anti-cancer, anti-inflammatory, anti-oxidant, neuroprotective, anti-allergic, and cardioprotective activities. Isolated from various plants, including Artemisia species, eupatilin has been shown to regulate several cell signaling pathways associated with

 ISSN:2582-6433

proliferation, inflammation, and other ailments. The compound has been found to be an important constituent in the seeds, flowers, leaves, and stems of varied plants, making it a therapeutic representative.

Accumulated data from multitudinous studies have provided evidence for the role of eupatilin in various types of cancer, regulating cell signaling pathways associated with proliferation, inflammation, and other ailments. Although eupatilin has been reported to have several pharmacological applications, further studies are needed to uncover its safety dosage, efficacy, and potential toxicity. Structure-activity relationship studies and preclinical trials are necessary to fully understand its biological and pharmacological applications.

Furthermore, studies related to the identification of genotoxicity, reproductive toxicity, nephrotoxicity, and hepatotoxicity need to be investigated by researchers. It would also be worthwhile to investigate the structure-activity relationship of eupatilin in several activities, such as anti-cancer and anti-inflammatory. Eupatilin may act as a template for the design and synthesis of many new drugs to cure various human ailments, but further experimentation, along with medicinal chemistry approaches and preclinical trials, is obligatory to uncover the knowledge of its biological and pharmacological applications and their associated mechanisms of action for the treatment and prevention of several diseases. Overall, eupatilin appears to be a promising compound with potential applications in the prevention and treatment of various diseases.

Acknowledgement

The authors would like to express their sincere gratitude to all the researchers, scientists, and experts whose work has contributed to the understanding of eupatilin's pharmacological properties and therapeutic potential. We also acknowledge the support and resources provided by our institutions and laboratories, which have enabled us to conduct this review and share our findings with the scientific community. Additionally, we would like to thank our colleagues and peers for their valuable feedback, suggestions, and encouragement. We would like to extend our special thanks to our supervisors for their guidance, mentorship, and unwavering support. Their expertise, constructive feedback, and encouragement have been invaluable to us, and we are grateful for the opportunity to work under their supervision.

 ISSN:2582-6433

Conflicts of Interest

The authors declare no conflicts of interest.

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