Anti-inflammatory and antioxidant properties of *Piper* species: A perspective from screening to molecular mechanisms

Sarvesh Kumar[a,b], Shashwat Malhotra[c], Ashok K. Prasad[c], Erik V. Van der Eycken[d], Marc E. Bracke[e], William G. Stetler-Stevenson[a], Virinder S. Parmar[c,d,e] and Balaram Ghosh[b]*

[a]Radiation Oncology Branch, Center for Cancer Research, National Cancer Institute, Advanced Technology Center, 8717 Grovemont Circle, Gaithersburg, MD-20892, USA.

[b]Molecular Immunogenetics Laboratory, CSIR-Institute of Genomics and Integrative Biology, Mall Road, Delhi –110 007, India.

[c]Bioorganic Laboratory, Department of Chemistry, University of Delhi, Delhi-110 007, India.

[d]Laboratory for Organic & Microwave-Assisted Chemistry (LOMAC), Department of Chemistry, University of Leuven (KU Leuven), Celestijnenlaan 200F, B-3001 Leuven, Belgium.

[e]Laboratory of Experimental Cancer Research, University Hospital, University of Gent, DePintelaan 185, B-9000 Gent, Belgium.

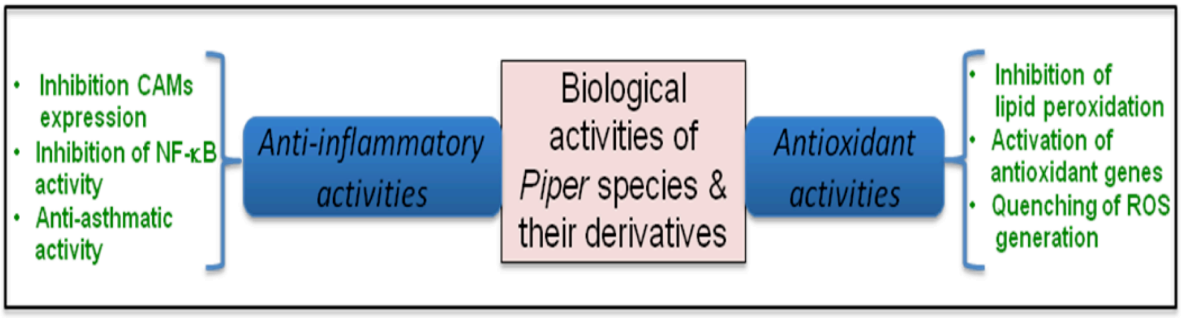
[*]To whom all correspondence should be addressed:
Dr. B. Ghosh
Molecular Immunogenetics Laboratory,
CSIR-Institute of Genomics and Integrative Biology
Mall Road, Delhi 110 007, India.
Tel.: 91-11-2766-2580
Fax: 91-11-2766-7471
E-mail: bghosh@igib.res.in

Running title: Anti-inflammatory and antioxidant properties of *Piper* species

Abstract

In recent years, considerable emphasis has been given to identify novel therapeutic agents from the natural sources, which could be useful for human beings. *Piper* species are highly important - commercially, economically and medicinally. Our groups have been working for more than two decades on the identification and characterization of novel therapeutic lead molecules from *Piper* species. We have extensively studied the biological activities of various extracts of *Piper longum* and *Piper galeatum* and identified and characterized novel molecules from these species. Using synthetic chemistry, various functional groups of the lead molecules were modified and structure activity relationship (SAR) studies identified synthetic molecules with better efficacy and lower IC_{50} values. Moreover, the mechanisms of actions of some these molecules were studied at the molecular level. The objective of this review is to summarize experimental data published from our laboratories and others on antioxidant and anti-inflammatory potentials of *Piper* species and their chemical constituents.

Graphical abstract



Keywords

Piper longum, *Piper galeatum*, Anti-inflammatory, antioxidant, cell adhesion molecules, endothelial cells.

Introduction

Plants have been the great source of medicines since thousands of years. Several medicinal herbs have been shown to augment specific cellular and humoral immune responses [1]. Species of the genus *Piper* are among the important medicinal plants used in various systems of medicine. *Piper longum* L., *Piper nigrum* L. and *Piper galeatum* L. (Piperaceae), commonly known as "long pepper", "black pepper" and "helmet pepper" respectively, are widely distributed in the tropical and subtropical regions of the world, throughout the Indian subcontinent, Sri Lanka, Middle Eastern countries and the Americas [2-4]. It is an important component of Indian traditional medicine reported to be used as a remedy for treating gonorrhea, menstrual pain, tuberculosis, sleeping problems, respiratory tract infection, chronic gut related pain and arthritic conditions [5-7].

Inflammation is the hallmark in the pathogenesis of various inflammatory diseases such as asthma, chronic obstructive pulmonary disease (COPD), cancer and cardiovascular diseases [8]. Cell adhesion molecules play critical roles in the recruitment and migration of inflammatory cells to the sites of inflammation [9]. A promising therapeutic approach to diminish aberrant leukocyte adhesion is, therefore, to inhibit cytokine-induced expression of cell adhesion molecules [10-12]. TNF-induces free radical generation like H_2O_2 which activates inflammatory signaling pathways, including NF-κB in vascular cells [13-15]. An excess production or decreased scavenging of reactive oxygen species (ROS) has been implicated in the pathogenesis of diverse metabolic disorders such as diabetes, cancer, atherosclerosis and neurodegeneration. Hence the antioxidant therapy has gained an utmost importance in the treatment of such diseases linked to free radicals [16]. Normally the antioxidant property of a compound is attributed to its (a) oxygen radical scavenging ability, (b) the ability to inhibit cellular microsomal P-450 linked Mixed Function Oxidases (MFOS) and the ability to suppress the formation of reactive oxygen species (ROS) or (c) inducing the expression of antioxidant genes like NQO1, HO1 and GCLM, etc. by activating a major transcription factor, Nrf2 [17]. In search of novel small molecules from natural sources, we have been studying the genus *Piper* for many years and have reported their antioxidant and anti-inflammatory activities. We identified many novel compounds from *Piper* species and synthesized their derivatives to study the structure activity relationships [18-20]. Herein, we will summarize the anti-inflammatory and antioxidant activities of *Piper* species and their constituents from our laboratories and independent groups across the world.

Biological activities of Piper species

Inflammation is caused by soluble antigen, live organisms and chemical or mechanical stress upon tissue, which serves to destroy and/or dilute the injurious materials, and remove the injured tissues. The migration of the leukocytes to the site of inflammation is regulated in part by the expression of cell adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin [15, 21]. These cell-adhesion molecules are induced on endothelial cells by various pro-inflammatory cytokines like IL-1and TNF-α and also by bacterial LPS [22-24]. Various synthetic drugs have been demonstrated to inhibit the expression of these molecules but they have been reported to have many side effects. Therefore, there is a growing need to develop a remedy that is safer and has fewer or negligible side effects. We have reported the effect of ethanol, chloroform and hexane extracts of *P. longum* and *P. galeatum* on TNF-α induced expression of ICAM-1 on human umbilical vein endothelial cells [2, 25]. ICAM-1 was expressed at low levels on unstimulated endothelial cells, and its expression was induced over five- fold by stimulation of endothelial cells with TNF-α. It was observed that the chloroform extract of *P. longum* exhibits 70 % inhibition of TNF-α induced ICAM-1 expression on endothelial cells, followed by hexane and ethanol extracts, which showed about 40 % inhibition [2]. The hexane and the chloroform extracts of *P. galeatum* exhibited 35 and 65 % inhibition of TNF-α induced ICAM-1 expression on endothelial cells, respectively, which is a little less than the activity of hexane and chloroform extracts of *P. longum* [2]. However, the inhibitory activity of ethanolic extract of *P. galeatum* extract is little higher than that of *P. longum*, it might have another active component present in it [25]. The most active *P. longum*'s chloroform extract (PlCE) was further studied in detail to elucidate the underlying molecular mechanism accountable for inhibition of TNF-α induced ICAM-1 expression on endothelial cells. To verify its functional consequences at the cellular level, we observed that *P. longum* chloroform extract (PlCE) significantly inhibits adhesion of neutrophils to endothelial cells monolayer. This inhibition is due to the ability of PlCE to block the TNF-α induced expression of cell adhesion molecules, i.e. ICAM-1 and VCAM-1 at 17.5 μg/ ml concentration and E-selectin at 15 μg/ ml concentration on human umbilical vein endothelial cells. By performing a series of *in-vitro* assays, we showed that the inhibition of ICAM-1, VCAM-1 and E-selectin by PlCE is mediated through inhibition of Nuclear factor-kappa B (NF-κB) in endothelial cells. NF-κB is knwon to be one of the major transcription factors involved in the transcriptional regulation of ICAM-1, VCAM-1 and E-selectin expression by

inflammatory cytokines or bacterial LPS. Further, we have demonstrated the antioxidant activity of PlCE; we have shown that the PlCE inhibited NADPH-catalysed rat liver microsomal lipid peroxidation significantly [2]. These results suggest a possible mechanism of anti-inflammatory as well as antioxidant activity of PlCE. Similarly, Kumar et al., studied the antiinflammatory activity of the oil of *P. longum* dried fruits in rats using the carrageenan-induced right hind paw edema method [26]. The activity was compared with that of standard drug ibuprofen. The oil of *P. longum* dried fruits inhibited carrageenan-induced rat paw edema. The results indicated that this oil produced significant ($p< 0.001$) antiinflammatory activity when compared with the standard or untreated control [26]. Ghosal et al. have reported that the ethanolic extract of fruits of *P. longum* and piperine, a pure compound, from this plant material cured 90 % and 40 % of rats with caecal amoebiasis, respectively [6, 27]. These biological activities of the extract of *P. longum* indicate that it has some active chemical constuents in it. Thus, we further studied the phytochemical analysis of *P. longum*'s extracts [19].

Bioactive compounds of Piper longum

P. longum fruit powder (100 g) was extracted with 50 % aqueous ethanol (150 mL). The supernatant (140 mL), collected by centrifugation at 14,000 rpm was dried under vacuum and designated as "ethanolic extract". This was treated with *n*-hexane (35 mL) and the hexane layer was dried and designated as "hexane fraction". The residual material was treated with chloroform (40 mL) and the chloroform solution was designated as "chloroform extract". The hexane and chloroform extracts of the fruits of *P. longum* were combined, because they exhibited almost similar spots on TLC examination. The combined extract was purified by column chromatography over silica gel using a gradient mixture of ethyl acetate and petroleum ether as the eluent; ethyl 3′,4′,5′-trimethoxycinnamate (ETMC) and piperine (**Figure 1 & Scheme- 1)**, were eluted with 5 and 17 % ethyl acetate/ petroleum ether solutions, respectively. The structures of ETMC and piperine were established on the basis of their spectral analysis (IR, ^{1}H NMR and ^{13}C NMR spectra and HRMS) and confirmed by comparison of spectral data and melting points with those reported in the literature [19].

ICAM-1 inhibition and SAR studies of identified compounds

We have tested the ability of isolated compounds in inhibiting the TNF-α induced expression of cell adhesion molecules on endothelial cells as a criterion of their anti-inflammatory activities. To elucidate their structure-function-activity relationship, we synthesized nine different analogues of ethyl 3',4',5'-trimethoxycinnamate (ETMC), and compared the ICAM-1 inhibitory activity of ETMC with those of its synthetic analogues esters (Scheme-1) as well as of the corresponding acids [19]. The pharmacological, biochemical and therapeutic properties of these cinnamates depend upon the pattern of substitution. Cinnamates have attracted intense interest in recent years because of their diverse pharmacological properties. Among these properties, their antioxidant effects have been extensively examined. The structure-activity studies indicate that the chain length of the alcohol moiety, substituents in the aromatic ring, and the presence of α,β-double bond of the cinnamic acid ester have significant effects on the inhibition of the TNF-α induced expression of ICAM-1 on endothelial cells [19]. Previously, we have reported that substitution of oxygen with sulfur enhances the ICAM-1 inhibitory activity of coumarins [28]. To test whether substitution of oxygen with sulfur in ETMC would increase its activity, we designed and synthesized novel analogs of cinnamates, thiocinnamates and thionocinnamates and evaluated the potencies of these analogs to inhibit TNF-α induced ICAM-1 expression on human endothelial cells. The initial screening data demonstrated that ethyl 3',4',5'-trimethoxythionocinnamate (ETMTC, **Figure-1 & Scheme-1**) is the most potent inhibitor of TNF-α induced ICAM-1, VCAM-1 and E-selectin expression [29]. Structure-activity relationship studies revealed the critical role of the chain-length of the alkyl group in the alcohol moiety, number of methoxy groups in the aromatic ring of the cinnamoyl moiety and the presence of α, β- C-C double bond in the thiocinnamates and thionocinnamates. The ICAM-1 inhibitory activity data revealed that activity of the cinnamates significantly decreases with; a) an increase in the length of the alkyl chain in the alcohol part, b) a decrease in the number of methoxy groups present in the benzene ring of cinnamates, c) a decrease in the number of hydroxy groups present in the benzene ring of cinnamates. It has been observed that the dihydroxy derivatives of cinnamates were less effective in inhibiting the TNF-α induced expression of ICAM-1 than trihydroxy derivatives [19, 29, 30]. After the structure-activity relationship study, we found that ethyl 3',4',5'-trimethoxythionocinnamate (ETMTC) is the most potent inhibitor of cell adhesion molecules, thus further preclinical and mechanstic study was focused on ETMTC [28].

Molecular mechanism of the inhibition of cell adhesion molecules

The functional consequences of inhibition of ETMTC at the cellular level revealed that it abrogates the TNF-α induced adhesion of neutrophils to the endothelial monolayer. Further, we have reported the molecular mechanism underlying the observed activity [30]. We also determined the status of NF-κB activation in ETMTC treated human endothelial cells. We found that ETMTC inhibited TNF-α induced nuclear translocation and activation of NF-κB by inhibiting phosphorylation and degradation of IκBα. The inhibition of IκBα phosphorylation and degradation by ETMTC was found to be due its ability to inhibit IκB kinase activity [31] .

Antioxidant activity of ETMTC

Emerging evidence suggests that reactive oxygen species contribute to diverse signaling pathways [32]. TNF-α induced oxidative stress activates inflammatory signaling pathways, including NF-κB in vascular cells [33]. As ETMTC inhibits TNF-α induced NF-κB activation, therefore, we examined the effect of ETMTC on TNF-α induced reactive oxygen species generation in endothelial cells. We found that ETMTC significantly inhibited TNF-α induced reactive oxygen species generation in endothelial cells. To further demonstrate its antioxidant potential, we measured the antioxidant gene expression in ETMTC treated bronchial epethelial cells. Nuclear factor (erythroid-derived 2)-like 2, also known as NFE2L2 or Nrf2, is a transcription factor, is encoded by the NFE2L2 gene in humans [34, 35]. It regulates expression of several detoxification or antioxidant enzymes and is therefore, capable of protecting oxidative stress-related injury and inflammatory disease in animals [36]. In response to antioxidants, xenobiotics, metals, and UV irradiation, Nrf2 protein binds strongly to antioxidant response elements (ARE) sequence and regulates ARE-mediated antioxidant enzyme gene expression and induction [37]. Nrf2 is negatively regulated by Kelch-like ECH-associated protein 1 (KEAP1) and positively regulated by DJ-1 [38]. The expression of Nrf2-regulated genes GCLM, HO1 and NQO1 in ETMTC treated epithelial cells was analyzed by qRT-PCR as a surrogate marker of antioxidant potential and Nrf2 activation activity in ETMTC treated human epithelial cells (0.01- 20 μM). We reported that ETMTC induces antioxidant gene expression in a concentration dependent manner [31]. Further, we reported that ETMTC is more potent than sulphoraphane at 10 μM concentration. ETMTC induces Nrf2-regulated anti-oxidant gene expression at protein level as confirmed by

western blots [30]. ETMTC modulates the expression of Nrf2 regulators, Keap1 and DJ-1. Further, we reported that ETMTC increases the levels of NQO1-ARE mediated luciferase reporter activity. NQO1-ARE luciferase activity was measured by using stably transfected Beas-2B cells after treatment with ETMTC or SFN as positive control or DMSO as solvent [17]. These findings clearly demonstrate the possible mechanism of cell adhesion molecules inhibition by ETMTC [31].

Anti-asthmatic activity in preclinical model

Asthma is a chronic inflammatory disease of airways and airway epithelial injury is the hallmark of respiratory diseases and therapeutic targeting of epithelial injury could be an effective strategy for controlling these diseases. We have already demonstrated that ethyl 3',4',5'-trimethoxythionocinnamate (ETMTC) was the most potent among various cinnamate derivatives in inhibiting inflammatory cell adhesion molecules (CAMs) expression. We further investigated the effects of ETMTC on the features of allergic asthma and epithelial injury in a murine model [18]. We found that ETMTC treatment to ovalbumin sensitized and challenged mice during ovalbumin challenge reduced airway hyperresponsiveness, and airway inflammation. This attenuation of asthma features was associated with the reduction in the expressions of various CAMs, NF-κB activation, Th2 cytokines, eotaxin and 8-isoprostane [18]. It increased activities of mitochondrial complexes I and IV in lung mitochondria and reduced cytochrome c and caspase 9 activities in lung cytosol. In addition, we have also reported that it reduced the levels of oxidative DNA damage marker in bronchoalveolar lavage fluid and DNA fragmentation of bronchial epithelia in lung sections. ETMTC not only increased the levels of 15-(S)-hydroxyeicosatetraenoic acid, suppressor of airway remodeling, but also inhibited goblet cell metaplasia and sub-epithelial fibrosis [18]. These results demonstrate that ETMTC reduces airway inflammation and thus shows its anti-inflammatory potential.

Biological activities of Piperine

Piperine, structure and synthesis as shown in **Figure 1 & Scheme-2**, is a major component of black (*Piper nigrum* Linn) and long (*P. longum* Linn) peppers, and is widely used as a traditional food and medicine [39]. It also exhibits a variety of biological activities, which includes antioxidant, anti-tumor and anti-pyretic properties [40]. Cell adhesion molecules play a critical role in the pathogenesis of inflammatory diseases [41, 42]. To elucidate the

anti-inflammatory potential of piperine, we determined its efficacy to inhibit the TNF-α induced expression of cell adhesion molecules on endothelial cells; we found that piperine inhibited the TNF-α induced expression of cell adhesion molecules by inhibiting nuclear factor-κB (NF-κB) activation in endothelial cells [20]. Further, we have reported that its ability to inhibit NF-κB activation is mediated via IκB kinase inhibition. Recently, independent groups around the globe have reported the pharmacological activities of piperine and shown that it possesses anti-inflammatory, cytoprotective and analgesic effects, anticonvulsant, anti-ulcer and antioxidant activities [35, 43].

Angiogenesis plays an important role in tumor progression and metastasis. It is regulated by a variety of pro-angiogenic genes and signaling molecules including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), platelet-derived growth factors, hypoxia-inducible factors, angiopoetin-1 and 2, and matrix metalloproteinases [44]. Thus, inhibition of angiogenesis by down regulating pro angiogenic factor or inducing various anti-metastatic proteins including tissue inhibitor metalloproteinases (TIMPs) could be novel therapy for cancer [45]. *Piper* leaf extract protects against carbon tetrachloride-induced liver fibrosis in rats [46]. Similarly, studies by Doucette et al. reported that piperine inhibits HUVEC proliferation and collagen-induced angiogenesis [47]. Piperine suppresses tumor growth and metastasis *in-vitro* and *in-vivo* model of breast cancer. It dose-dependently decreased PMA-induced COX-2 expression and PGE (2) production, as well as COX-2 promoter-driven luciferase activity. Transient transfections utilizing COX-2 promoter deletion constructs and COX-2 promoter constructs in which specific enhancer elements were mutated, revealed that the nuclear factor-κB (NF-κB), CCAAT/enhancer binding protein (C/EBP) and activator protein-1 (AP-1), were the predominant contributors to the effects of piperine. In addition, piperine inhibited PMA-induced NF-κB, C/EBP and c-Jun nuclear translocation [40]. Bae et al. have reported that administration of piperine reduced histologic damage and myeloperoxidase (MPO) activity in the pancreas and ameliorated many of the examined laboratory parameters, including the pancreatic weight (PW) to body weight (BW) ratio, as well as serum levels of amylase and lipase, and trypsin activity. Piperine pretreatment reduced the production of tumor necrosis factor (TNF)-alpha, interleukin (IL)-1beta, and IL-6 during cerulein-induced AP and reduced cell death, amylase and lipase activity, and cytokine production in isolated cerulein-treated pancreatic acinar cells via inhibiting the activation of mitogen-activated protein kinases (MAPKs) [48]. Oxygen radical injury and lipid peroxidation have been suggested as major

causes of atherosclerosis, cancer, liver disease and the aging process [49]. Piperine possesses many anti-inflammatory properties as reported by our group and others. It has been demonstrated in *in-vitro* experiments to protect against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species and hydroxyl radicals. The effect on lipid peroxidation was also reported. Piperine was found to act as a hydroxyl radical scavenger at low concentrations, but at higher concentrations, it activated the Fenton reaction resulting in increased generation of hydroxyl radicals [50]. Whereas it acts as a powerful superoxide scavenger (with IC_{50} of 1.82 mM), a 52 % inhibition of lipid peroxidation was observed at a dose of 1400 μM with an IC_{50} of 1.23 mM. The results depict that piperine possesses direct antioxidant activity against various free radicals [50]. Piperine exhibits antioxidant action in experimental conditions both *in vivo* as well as *in vitro* through its radical quenching effect and by preventing GSH depletion.

Bioactive compounds of P. galeatum

P. galeatum is native to India and grows wild in the forests of Wayanad and Kerala regions. Ten sesquiterpenes and monoterpenes have been reported to occur in the extracts of its fruits, namely β-elemene, δ-elemene, α-humulene, β-caryophyllene, α-copaene, α-ionone, 10-(acetylmethyl)-3-carene, dihydrocarvyl acetate, 1-p-menthen-8-yl acetate and linalyl acetate. We have isolated five compounds from the stems of P. galeatum, namely β-sitosterol as shown in (**Figure 1 & Scheme- 3**), cyclostachine-A, piperine, piperolein-B and a novel amide, viz. 1-(3′-hydroxy-5′-methoxycinnamoyl)-piperidine. The crude extracts as well as the isolated pure compounds were screened for their activity to inhibit the TNF-α induced expression of cell adhesion molecule ICAM-1 on the surface of human endothelial cells. Among all, β-sitosterol was found to be the most active compound, which was taken for further studies [25].

Anti-inflammatory activity of β-sitosterol

Cell adhesion molecules are expressed on various immune cells and play pivotal role during any inflammatory event. Inhibition of these molecules by any pharmacological molecule is being considered as surrogative marker of the anti-inflammatory property of the molecule [42, 51, 52]. We have reported that β-sitosterol significantly inhibits the TNF-α induced expression of ICAM-1, VCAM-1 and E-selectin. The functional correlation of cell adhesion molecules inhibition was assessed by cell adhesion assay using human neutrophils. We have also reported that β-sitosterol significantly blocks the adhesion of neutrophils to endothelial monolayer. We investigated the status of nuclear transcription factor-κB (NF-κB) and were able to establish that β-sitosterol significantly blocks the TNF-α induced activation of NF-κB [25]. In an independent study, Loizou et al. reported similar activity of β-sitosterol in human arterial endothelial cells (HAECs) [53]. They reported that b-sitosterol inhibits significantly

vascular adhesion molecule 1 and intracellular adhesion molecule 1 expression in TNF-α-stimulated HAEC as well as the binding of U937 cells to TNF-α-stimulated HAEC and attenuates the phosphorylation of nuclear factor-kB p65 [53]. b-Sitosterol isolated from leaves of Nyctanthes arbortristis Linn. (Oleaceae) has been reported to posses' analgesic activity and anti-inflammatory activities. Analgesic activity has been shown by hot plate test and acetic acid-induced writhings and anti-inflammatory activity by carrageenan-induced hind paw edema method [54]. Recently Han et al., investigated the effect of β -sitosterol in 2,4-Dinitrofluorobenzene (DNFB)-induced AD-like skin lesions in NC/Nga mice. They reported that infiltration of inflammatory cells and number of scratching were clearly reduced in the b-sitosterol treated group compared with the DNFB-treated group. b-Sitosterol significantly reduced the levels of inflammation-related mRNA and protein in the AD skin lesions. It significantly reduced the levels of histamine, IgE, and interleukin-4 in the serum of DNFB-treated NC/Nga mice [55]. They further reported that the activation of mast cell-derived caspase-1 was decreased by treatment with β-sitosterol in the AD skin lesions. b-Sitosterol also significantly decreased the production of tumor necrosis factor-alpha from the stimulated splenocytes. b-sitosterol inhibited the production and mRNA expression of TSLP through blocking of caspase-1 and nuclear factor-kappaB signal pathways in the stimulated HMC-1 cells [55]

Anti-oxidant activity of β-sitosterol

Uncontrolled production of reactive oxygen species contributes to the pathogenesis of diseases, such as cancer and cardiovascular disorders. β-Sitosterol has been associated with cardiovascular protection, exerting its effect mainly through increasing the antioxidant defense system and effectively lowering the serum cholesterol levels in humans. Study results showed that β-sitosterol reverts the impairment of the glutathione/oxidized glutathione ratio induced by phorbol esters in RAW 264.7 macrophage cultures [56]. Further, it has been reported that β-sitosterol increases manganese superoxide dismutase and glutathione peroxidase activities and decrease in the catalase activities, possibly associated with estrogen receptor (ER)-mediated phosphatidyl inositol 3-kinase (PL3K)/glycogen synthase kinase 3 (GSK3beta) signaling [57]. Antidiabetic and antioxidant potential of β-sitosterol has been reported in streptozotocin-induced experimental hyperglycemia [58]. In another study, β-sitosterol has been reported to protect rats from 1,2-dimethylhydrazine-induced colon cancer by preventing lipid peroxidation and improving antioxidant status. These data can be correlated with the increase in manganese superoxide dismutase and glutathione peroxidase activities and the decrease in catalase activity. They further demonstrated that the effects of β-sitosterol on antioxidant enzymes depend on the estrogen/phosphatidylinositol 3-kinase pathway [58, 59]. These studies extend existing data regarding the antioxidant potential of β-sitosterol and provide new insights into understanding the molecular mechanism underlying the beneficial effect of β-sitosterol.

Conclusion

This review opens new avenues for researcher to identify and characterize novel lead therapeutic molecules from *Piper* species and investigates their efficacy in preclinical animal models. The *in-vitro* and in-vivo studies from our group and others clearly demonstrated the anti-inflammatory and antioxidant potential of *P. longum* extract or its constituents. Thus, these findings led us to propose that use of *P. longum* extract or its constituents as a dietary supplement may be useful in the prevention of many diseases like asthma, COPD, cancer and cardiovascular diseases.

List of abbreviations

AP-1: Activator protein-1
ARE: Antioxidant response elements
AD: Alzheimer disease
bFGF : Basic fibroblast growth factor
CAMs: Cell adhesion molecules
(C/EBP) : CCAAT/enhancer binding protein
COPD: Chronic obstructive pulmonary disease
DMSO: Dimethyl sulfoxide
DNFB: 2,4-Dinitrofluorobenzene
EGF: Epidermal growth factor
ETMC: Ethyl 3′,4′,5′-trimethoxycinnamate
ETMTC: Ethyl 3',4',5'-trimethoxythionocinnamate
GSH: Glutathione
GCLM: Glutamate-cysteine ligase, modifier subunit
HO1: Heme oxygenase 1
H2O2: Hydrogen peroxide
HAECs: Human arterial endothelial cells
HUVEC: Human umbilical cord vein endothelial cells
ICAM-1: Intercellular adhesion molecule-1
IL-1β: Interleukin 1 beta
KEAP1: Kelch-like ECH-associated protein 1
IκBα: Inhibitory kappa B alpha
LPS: Lipopolysaccharides
μM: MicromolarmM: Millimolar
MFOS: Mixed function oxidases
MPO: Myeloperoxidase
NF-κB: Nuclear factor –kappa B
Nrf2: Nuclear factor (erythroid-derived 2)-like 2
NQO1: NAD(P)H dehydrogenase, quinone 1
PlCE: P. longum's chloroform extract
SAR: Structure activity relationship
TIMPs: Tissue inhibitor metalloproteinases

TNF-α: Tumor necrosis factor-alpha
TSLP: Thymic stromal lymphopoietin
VCAM-1: Vascular cell adhesion molecule-1
VEGF: Vascular endothelial growth factor Conflict of interest

Conflict of interest

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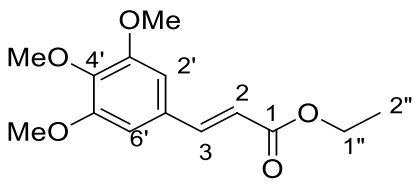
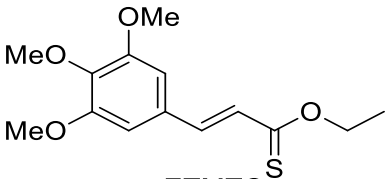
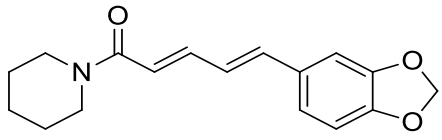
Figure 1: *Structures of identified potent inhibitors of cell adhesion molecules (CAMs):*



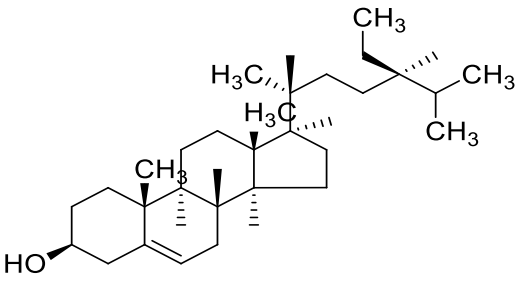
ETMC



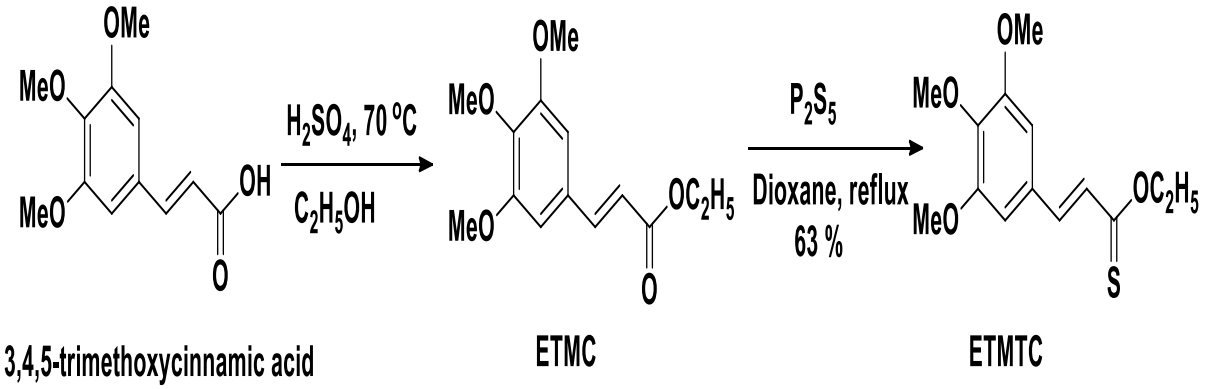
ETMTC



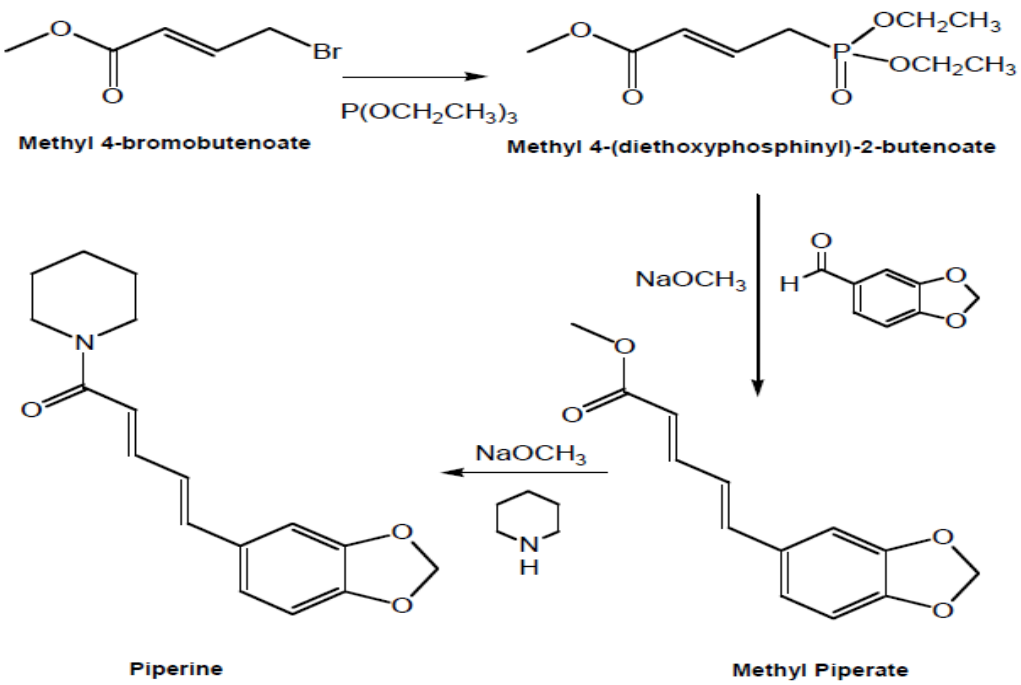
Piperine



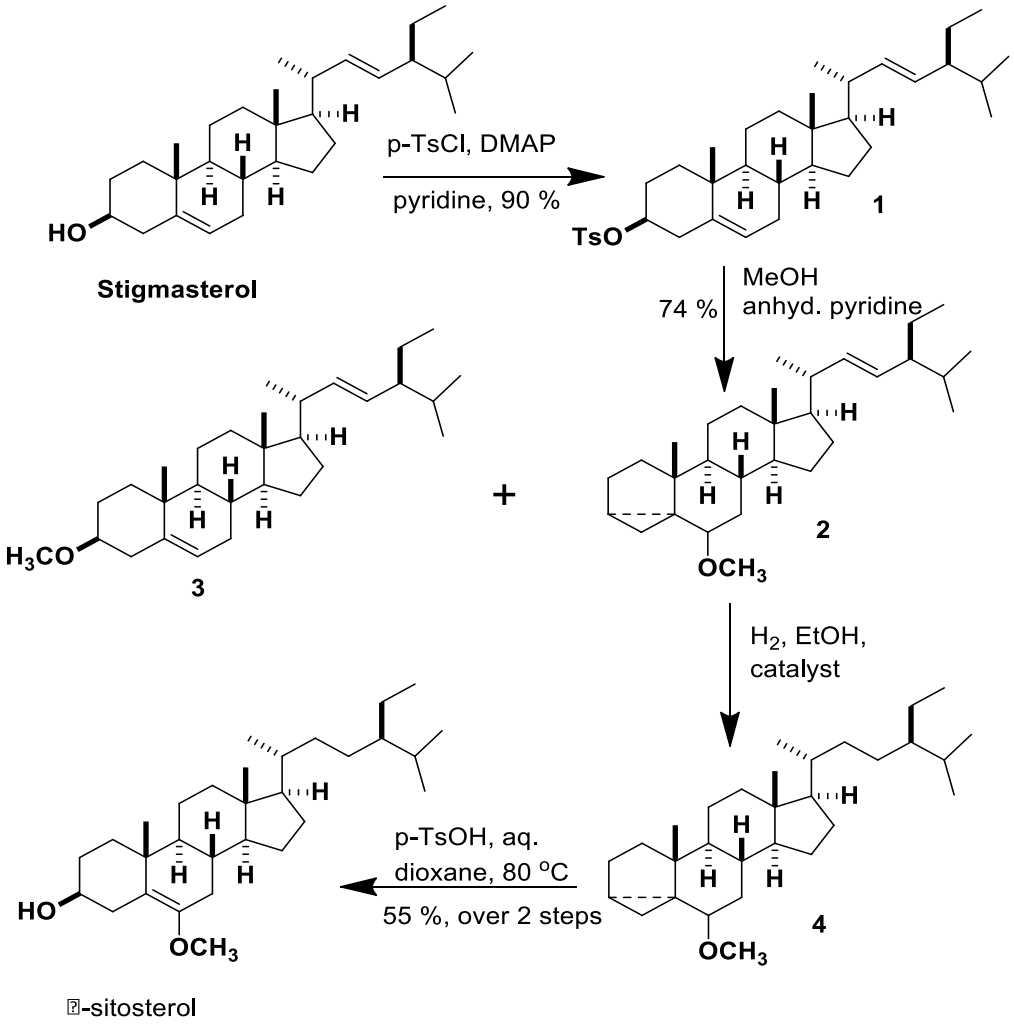
-sitosterol



Scheme-1: *Synthesis of ETMC and ETMTC.*



Scheme-2: *Synthesis of Piperine*



Scheme-3: Synthesis of *β-sitosterol*

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