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NATURAL PRODUCTS: FROM CHEMISTRY TO PHARMACOLOGY (Z-Y SU, SECTION EDITOR)

Mechanism-Based Physiological Effects of Piperine: A Review

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Abstract

Background Piperine is the piperidine alkaloid present in the fruits of Black pepper (*Piper nigrum*) and long pepper (*Piper longum*). It is a pungent constituent with remarkable pharmacological properties. Its appropriate use in some pathological conditions of CNS (central nervous system), CVS (cardiovascular system), GIT (gastro intestinal tract) dysfunctions, bone, and various other physiological activities like anti-asthmatic, anti-tumor immunomodulatory, cytoprotective, and hepatoprotective have been reported.

Purpose The review tries to emphasize the mechanisms that are involved in various physiological activities of piperine along with its toxicity assessment.

Study Design Different physiological and pharmacological characteristics of piperine were evaluated through a literature search from authorized manuscripts based on piperine studies, with more emphasis given to the dose and parameters assessed.

Results Literature search of piperine showed remarkable properties like antioxidant, anti-apoptotic, anti-inflammatory, and bioavailability enhancing abilities that fit its appropriate use in pathological conditions of CNS (central nervous system), CVS (cardiovascular system), GIT (gastro intestinal tract) dysfunctions, bone, and various other physiological activities like anti-asthmatic, anti-tumor and immunomodulatory, cytoprotective, and hepatoprotective.

Conclusion The basic properties which are responsible for most of the mechanistic approach of piperine in various diseases are its 'antioxidant, anti-inflammatory, anti-apoptotic, and bio-availability enhancing abilities' that provide the initial framework in managing and alleviating severe disease conditions.

Keywords Piperine · Physiological effects · Bioavailability enhancer · Toxicity

Introduction

Compound: Piperine

Chemical name: 1-piperoyl piperidine

Nature: Pungent constituent

Category: Piperidine alkaloid

Plant: Black pepper (*Piper nigrum* L.); long pepper (*Piper longum* L.).

Family: Piperaceae

Synonyms: Kali Mirch-Urdu and Hindi; Pippali-Sanskrit; Milagu-Tamil; Peppercorn, White pepper, Green pepper, Black pepper, Madagascar pepper-English.

Geographical consideration: Black pepper is grown in many tropical regions like Brazil, Indonesia, and India.

Description: Piperine is the vital and essential bioactive compound mostly present in black pepper species, namely, *Piper nigrum* L. which is recognized as a widely used spice

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in the world. The unripe fruits of pepper are slightly cooked and dried to obtain black pepper, whereas, white pepper is initially naked, dried, and ripe seeds pepper. Piperine can be procured from the oleoresin present in the peppercorns and constitutes about 5–7% of the peppercorns [1].

Chemistry: Piperine [1-[5-[1,3-benzodioxol-5-yl]-1-oxo-2,4,pentadienyl]piperidine] is the key pungent alkaloid present in the fruits of *P. nigrum* L., along with its stereoisomer (Fig. 1), Chavicine which gets slowly converted into piperine on storage and results in a loss in pungency [2]. The fundamental constituents present in peppers are alkaloids/alkamides, terpenes, flavones, steroids, and lignans [3]. The aerial parts of *P. nigrum* L. containan essential oil that constitutes some major components like globulol, β-caryophyllene, α-pinene, and α-terpinene [4]. The fruits of pepper contain 1.0–2.5% volatile oil and 5–9% of alkaloids, among which the vital ones include piperine, piperidine, piperetine, resins, and chavicine [5]. Piperine is the first amide that is isolated from piper species and resembles strongly the structure of natural carcinogens like- estragole, safrole, and methylengenol which are mostly contained in plant oils and spices [6].

Therapeutic use: The pharmacological activities of *P. nigrum* fruits are accredited to a piperidine alkaloid, piperine, in a considerable amount of 1.7–7.4% in fruits. Piperine acts as a 'bioavailability enhancer' and improves the bioavailability of certain drugs like tetracycline, sulfadiazine, streptomycin [7], isoniazid, rifampicin, ethambutol, pyrazinamide, and phenytoin [8]. Traditionally, piperine was also recruited for the treatment of bronchitis, asthma, dysentery, insomnia, and pyrexia [9]. The recently reported advancement of piperine reflects its therapeutic potential as immunomodulatory, anti-asthmatic, anti-carcinogenic, hepatoprotective, stimulatory, anti-inflammatory [1], and ulcer protective properties [10]. Piperine is now regarded as a biomarker due to its diverse pharmacological potential for the standardization of piper fruits and polyherbal formulations [5].

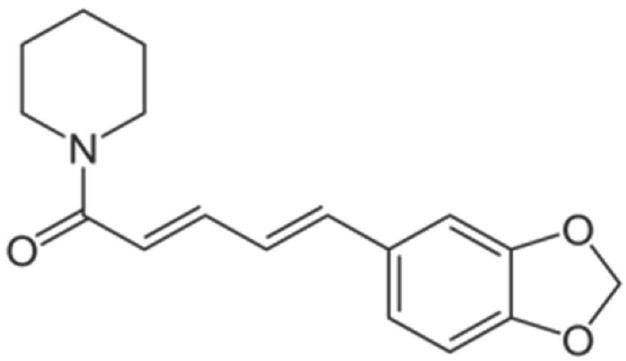

Fig. 1 Chemical structure of piperine

Pharmacokinetics of piperine

Bhat and Chandrashekhar (1986) demonstrated the absorption, tissue distribution, metabolism and urinary excretion of piperine [11].

Absorption: When rats were administered with 170 mg/kg piperine by gavage and 85 mg/kg intraperitoneally for a period of 5 days it was observed that only 3% of the dose was excreted unchanged in feces and the peak excretion occurred on the 1st and 3rd day after intraperitoneal and oral administration of piperine respectively thereby showing 97% absorption of piperine. Moreover piperine exerts similar absorption as piperonyl butoxide which is a structurally similar compound to piperine.

Tissue Distribution: Piperine attains its highest concentration in the stomach and small intestine in 6 h. Traces of piperine can be detected in the kidney, spleen, and serum from 30 min to 24 h. In comparison to the orally administered dose, i.p administration of piperine showed comparatively more piperine (about 1–2.5%) in the liver during 0.5–6 h.

Metabolism: Piperine does not undergo any metabolic change during absorption, since, incubation of everted sacs of rat intestine with 200–1000 µg of piperine showed only the presence of piperine in the serosal fluid and the intestinal tissue. Nevertheless, piperine undergoes later metabolism rapidly through other tissues wherein demethylenation of methylenedioxy group, glucuronidation, and sulphation of piperine are major metabolic reactions. Hence, efforts are still being made to strive the specific metabolites of piperine.

Urinary Excretion: Piperine either orally or intraperitoneally, was not excreted in the urine. Due to the demethylation of piperine, increased excretion of conjugated sulphates, conjugated uronic acids, and phenols were observed. Hence, sulphation and glucuronidation occur in the disposition of piperine in the rat.

Physiological Effect of Piperine on Central Nervous System (CNS)

Antidepressant Effect

The antidepressant activity of piperine was investigated by Li et al. [12] through chronic mild stress (CMS) induced model of depression. Piperine on repeated i.p. administration for 14 days at 2.5, 5, and 10 mg/kg doses produced reversal in CMS-induced changes like a decrease in plasma corticosterone level and an increase in sucrose consumption. According to him excessive cell death and

reduced neurogenesis in the hippocampus can be the cause of depressive disorders. Thus, Li et al. showed that Piperine treatment helped in improving decreased proliferation of hippocampal progenitor cells and upregulated the level of BDNF (brain-derived neurotrophic factor) in the hippocampus of CMS-stressed mice [12].

Mao et al. [13] also showed the anti-depressant effect of piperine in relation to the serotonergic system. The chronic unpredictable mild stress (CUMS) produced depression-like behavior in rats which is observed by a significant reduction in sucrose consumption and an increase in immobility time in the forced swim test and also decreased the serotonin and BDNF contents in the hippocampus and frontal cortex. The suppression of behavioral and biochemical changes by piperine (10 mg/kg; i.p.) induced by CUMS suggests that the anti-depressant-like effect of piperine is probably due to increased 5-HT (serotonin) and BDNF contents in selective brain tissues [13].

Neuroprotective Effect

Mao et al. [14] observed that neuroprotection can be the active mechanism in treating depression since hyperactivation of the HPA (hypothalamic–pituitary–adrenal) axis and the associated hippocampal atrophy was noticed in patients with depression. Thus, the protective effect of piperine treatment on corticosterone-induced neurotoxicity in cultured rat pheochromocytoma (PC12) cells were investigated, and results obtained shows that piperine (1 μM) co-treatment significantly reduced intracellular reactive oxygen species level, enhanced superoxide dismutase activity and total glutathione level in corticosterone-treated PC12 cells and also reversed the reduction in BDNF mRNA level caused by corticosterone in PC12 cells. These findings recommend the neuroprotective effect of piperine through the inhibition of oxidative stress and upregulation of BDNF mRNA expression [14].

Alzheimer's Disease

Chonpathompikunlert et al. [15] demonstrated the effect of piperine on memory performance and neurodegeneration in an animal model of Alzheimer's disease. Adult male Wistar rats (180–220 g) were orally administered with piperine at various doses ranging from 5, 10, and 20 mg/kg B.W. (body weight) for a period of 2 weeks before and 1 week after the bilateral administration of ethylcholine aziridinium ion (AF64A) intracerebro ventricularly. The results proved that piperine irrespective of all dosage ranges successfully improved memory impairments and neurodegeneration in the hippocampus due to decrease lipid peroxidation, inhibition of acetylcholinesterase enzyme activity, and increase in neuronal density [15].

Parkinson's Disease

Shrivastava et al. [16] illustrated the molecular mechanism of inhibition of neuronal cell apoptosis by piperine. Piperine (10 mg/kg B.W.) reduced 6-OHDA (hydroxydopamine)-induced lipid peroxidation and stimulated glutathione levels in the striatum of rats indicating its antioxidant effect. Piperine protected 6-OHDA-induced apoptosis by blocking the release of cytochrome-c, caspase-3, and caspase-9. Further, the narrow beam test and rota rod also showed improvement in motor coordination and balance behavior in rats and reduces contralateral rotations induced by apomorphine after treatment with Piperine. Depletion of inflammatory markers, TNF-α (tumor necrosis factor-alpha), and interlukin-1β (IL-1β) in 6-OHDA-induced Parkinson's rats depict its anti-inflammatory mechanism. Inhibition of poly (ADP-ribose) polymerase activation, pro-apoptotic Bax levels, and elevation of Bcl-2 levels by piperine show its anti-apoptotic mechanism in treating parkinsonism [16].

Seizures

Piperine at 40 and 80 mg/kg; p.o. significantly reduces the occurrence of Maximal Electroshock Seizures (MES)-induced tonic hind limb extension and delays the onset of generalized clonic seizures, and myoclonic jerks, and decreases the mortality as compared to the vehicle-treated animals [17].

Physiological Effect of Piperine on Cardio Vascular System (CVS)

In normotensive anesthetized rats, a dose-dependent decrease in mean arterial pressure (MAP) was observed after i.v. administration of piperine at 1–10 mg/kg doses. Thus, the calcium channel blocker ability of piperine accounts for its cardio-depressant and vasodilator activities which contribute to its blood pressure-lowering effect. The related vasoconstrictor effect of piperine causes a restricted fall in BP and a then small rise in BP followed by a decline after each dose [18].

Dyslipidemia

Shah et al. [19] determined the anti-dyslipidemic effect of piperine on obesity-induced dyslipidemia fed by a high-fat diet (HFD) for the first eight weeks. It was observed that piperine along with the HFD supplementation significantly reduced body weight, total cholesterol, triglyceride, fat mass, low-density lipoproteins (LDL), very low-density lipoproteins (VLDL), and increased high-density lipoproteins (HDL) levels without showing any change in food

intake. Hence, it was justified that piperine at 40 mg/kg dose acquires the potential to reduce fat and lowers lipid without altering food appetite [19]. Piperine exhibits thyrogenic activity by modulating apolipoprotein levels and insulin resistance in HFD-fed rats and thereby manages dyslipidemia by dietary supplementation with nutrients [20]. Piperine also modulates lipid metabolizing enzymes like Lecithin-cholesterol acyltransferase (LCAT) and Lipoprotein lipase (LPL). As a result, it inhibits lipid and lipoprotein accumulation [21].

Hypertension

Inhibition of nitric oxide synthase (NOS) produces vasoconstriction in rats and consequently develops hypertension. Therefore, Kumar et al. [22] investigated the effect of piperine on N^{ω}-Nitro-L-arginine methyl ester hydrochloride (L-NAME)-induced hypertension. On administration of 40 mg/kg, L-NAME in drinking water for 4 weeks in rats caused a sustained reduction in the nitrite/nitrate concentrations (NO_x) in plasma as compared to control rats. Through the study, it was observed that L-NAME-treated hypertensive rats restored the plasma NO metabolites concentration after administration of piperine (50 mg/kg/day). It was observed that piperine significantly restored enzymatic catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), non-enzymatic antioxidants (vitamin E, vitamin C, and reduced glutathione (GSH) and decreases the levels of lipid peroxidation markers as compared to the L-NAME-treated group indicating that piperine attenuates NO-deficit hypertension through antioxidant activity [22].

Attenuation of CVS Metabolic Changes

Diwan et al. (2013) through a study concluded that piperine was effective in reducing the symptoms of human metabolic syndrome induced by a high-carbohydrate, high-fat (HCHF) diet in rats by reducing oxidative stress, plasma liver enzymes, inflammation, and improving liver and heart structure and function as compared to corn starch (CS) diet. Supplementation with piperine (375 mg/kg food; approximately 30 mg/kg/day) in HCHF-fed rats was helpful in reversing impaired glucose tolerance along with abdominal obesity, liver fibrosis, and fat deposition [23].

Hyperlipidemia

Vijayakumar and Nalini [21] demonstrated the action of piperine on apolipoproteins and thyroid hormone in hyperlipidemic rats induced by HFD and antithyroid drugs. Male Wistar rats were categorized into control diet and HFD groups for 10 weeks. Piperine administration with HFD decreased the plasma lipids and lipoproteins level and remarkably elevated HDL level. Plasma levels of apo A-1, triiodo-thyronine (T3), tetraiodothyronine (T4), and testosterone were improved by piperine supplementation and this supplementation also reduced apo B, thyroid stimulating hormone (TSH), and insulin to a normal level, unlike carbimazole. Thus, piperine showed significant improvement in hormone profiles and the expression of major apolipoproteins induced by a high-fat diet, thereby markedly reducing plasma lipid levels and the risk of atherogenesis [21].

Myocardial Infarction (MI)

Ischemia which manifests in MI often precipitates into cardiovascular disorders. Therefore, Dhivya et al. [24] studied the protective effect of piperine in MI induced by isoproterenol (ISO). It was observed that on pre-treatment with orally administered piperine (20 mg/kg), the levels of serum markers, protein carbonyl content (PCC), and lipid peroxidation were significantly decreased with an increase in antioxidant status (GSH, SOD, CAT, GPx and glutathione S-transferase (GST) in the heart tissues unlike ISO treated rats which produced opposite effects. Moreover, piperine pre-treatment restored the increased levels of glycoprotein components in serum and decreased heart tissue levels to normal. Piperine also modulates membrane-bound ATPase and shows protection against ISO-induced changes in membrane fluidity. Thus, this study suggests the anti-ischemic effect of piperine on ISO-induced cardiotoxicity by MI [24].

Physiological Effect of Piperine on Gastro-Intestinal Tract (GIT)

Gastric Secretion

In white albino rats in comparison with the control basal acid secretion, piperine in a dose-dependent manner significantly increased gastric acid secretion from 20 mg/kg B.W. to 142 mg/kg B.W. Hence, it was noticed that increased gastric acid secretion induced by piperine was due to the stimulation of histamine H_2 receptors without any involvement of cholinergic receptors [25].

Gastric Emptying and Transmit (Anti-diarrheal)

Bajad et al. (2001) explored the anti-diarrhoeal effect of piperine by drawing the conclusion that piperine in the dose and time-dependent manner, inhibited gastric emptying (GE) of solids/liquids in rats and gastrointestinal (GT) transmit in mice. GT and GE of solids at 1 mg/kg; p.o. (per oral) in rats and 1.3 mg/kg; p.o. dose in mice which was extrapolated from humans was significantly inhibited by piperine but the same dose of piperine produced an insignificant effect on the

GE of liquids indicating that piperine shows more inhibitory effect on the GE of solids than liquids. The inhibitory effect of piperine on gastric emptying is independent of pepsin and gastric acid secretion [26]. The underlying mechanism involved in the reduced inhibitory effect on GE of liquids is probably due to the more prominent action of piperine on the peristaltic activity of antrum which mostly controls solid emptying [27].

Gastric Ulcer

The effect of piperine on gastric ulcers in rats and mice was demonstrated by Bai and Xu (2000) by experimentally inducing gastric ulcers through indomethacin (inhibits PG synthesis), stress (increase acid and pepsin secretion and damages mucosa), hydrochloric acid (an aggressive factor that induces ulcer), and pyloric ligation (stimulate acid output). It was observed that pre-treatment with piperine at 25, 50, and 100 mg/kg intragastrically produced significant protection against gastric ulceration in a dose-dependent manner. Thus, piperine shows a shielding effect against gastric ulceration by inhibiting gastric acidity, gastric juice and pepsin A activity [10].

Inflammatory Bowel Disease (IBD)

The therapeutic potential of piperine in relation to amelioration of IBD was studied experimentally in an animal model of ulcerative colitis induced by 150 µl of 5% acetic acid administered once intra-rectally along with the determination of Toll-Like Receptor (TLR4) role in the signaling pathway of inflammatory gene expression in ulcerative colitis. Pre-treatment of piperine at 5 and 10 mg/kg; p.o. administration for 7 days shows anti-inflammatory activity at colorectal sites probably due to suppression of expression and production of inflammatory mediators and also decreases TLR4-mediated inflammation induced by FFA (free fatty acid). The histological examination reflects a reduction in edema in sub-mucosa, hemorrhages, cellular infiltration, and ulceration as compared to the experimental animal model [28].

Gastro-Intestinal Tract (GIT) Motility Disorder

The therapeutic effect of piperine in constipation and diarrhoea was investigated by Mehmood et al. [29] using (3–300 µM) of piperine on isolated guinea pig ileum. Thus, piperine holds spasmodic activity mediated via muscarinic receptors along with the antispasmodic activity mediated via calcium channel blocker and opioid receptor activation indicate possible mechanisms for the therapeutic use of piperine in constipation, diarrhea, and indigestion [29].

Physiological effect of Piperine on Bone

Rheumatoid Arthritis

The therapeutic effect of piperine on arthritis was explored by Murunikkara et al. [30] in rats. The experimental model for rheumatoid arthritis was established by administering 0.1 ml of heat-killed *Mycobacterium tuberculosis* intradermally into the right hind paw of rats that produces an immune reaction that characteristically involves increased paw volume, lysosomal enzymes, glycoproteins, and tissue marker enzymes and decreased body weight. Hence, all these parameters were found to be reverted to near normal levels upon i.p. administration of 30 mg/kg B.W. of piperine. Moreover, piperine was equally effective in alleviating mononuclear infiltration and synovial hyperplasia in arthritic rats via mechanisms involving suppression of inflammation and cartilage destruction [30]. The decrease in body weight was due to reduced absorption of leucine and glucose in the rat intestine [31].

Gouty Arthritis

The anti-inflammatory effect of piperine was determined in an experimental model for gouty arthritis in mice induced by monosodium urate crystal in which a significant increase was observed in lipid peroxidation, paw volume, lysosomal enzymes, TNF-α and decrease in activities of antioxidant status. These changes were abrogated to near normal levels by 30 mg/kg B.W. of piperine administered intraperitoneally. Hence, it proved piperine's therapeutic efficacy in treating gouty arthritis [32].

Osteoporosis

Deepak et al. [33] investigated that osteoclast production in human CD14 + monocytes and murine RAW264.7 macrophages was inhibited by piperine induced by RANKL (Receptor activator of nuclear factor-K-b ligand) and breast cancer cells. The main transcription factors which are involved in osteoclastogenesis such as expression of c-Fos and nuclear factor of activated T cells, cytoplasmic1 (NFATc1) were strikingly inhibited by piperine. Furthermore, piperine disrupts bone resorption and ring structure of actin which are the distinctive feature of osteoclast and suggests that by suppressing the p38/NFATc1/c-Fos signaling axis, piperine can inhibit osteoclast differentiation [33].

Another work done on osteoporosis by Kim et al. [34] showed expressions of osteogenic genes such as runt-related transcription factor 2 (Runx2), distal-less homeobox 5(Dlx5), and inhibitor of DNA binding-1 (Id1) by

piperine. Apart from this, matrix mineralization and alkaline phosphatase (ALP) activity was also increased by piperine treatment. Hence, it was confirmed that osteoblast differentiation was enhanced by piperine through AMP-activated protein kinase (AMPK) phosphorylation in MC3T3-E1 cells [34].

Physiological Effect of Piperine on Reproductive System (Fig. 2)

Male

Administration of piperine at doses 10 and 100 mg/kg, B.W. orally in adult male rats for 30 days produced a significant decrease in antioxidant enzyme activities in the testis as compared to the control group. Piperine treatment elucidates dose-dependent increase in Fas protein and caspase-3 in testicular germ cells. Thus, the underlying etiology contributing to hampered reproductive functions by piperine includes triggering apoptosis due to induction of oxidative stress [35]. Another work performed on mature male albino rats at 5 and 10 mg/kg B.W; p.o. of piperine for 30 days depicts the anti-spermatogenic activity of piperine and highlights its potential usefulness in contraception (Fig. 2) [27].

Female

Piperine shows a controversial effect on female reproduction. It produces antifertility activity by inhibiting implantation in mice [36] and also enhances the percentage of fertilization after artificial insemination with spermatozoa from untreated male hamster (Fig. 2) [37].

Physiological Effect of Piperine on Hormones and Neurotransmitters

Catecholamine

Infusion of piperine at 650 nmol/kg; i.v. produced more potent catecholamine secretion, especially epinephrine, from the rat adrenal medulla and induce a warming action [38]. Mori et al. [39] demonstrated that 1 h after the intraperitoneal administration of 60 mg/kg of piperine in convulsion-induced E1 mice, produced no effect in the brain except in the hypothalamus region, where noticeably higher dopamine levels were observed which may be due to the inhibition of β-hydroxylase activity. Along with this, low norepinephrine level was observed in every part of the brain [39].

Thyroid Hormones

Panda and Kar [40] evaluated the efficacy of piperine in male Swiss albino mice according to its thyroid hormone and glucose regulatory activity. 2.5 mg/kg daily oral administration of piperine for 15 days lowered the serum levels of T_4 and T_3 hormones along with glucose concentrations and associated decrease in hepatic 5′D and glucose-6-phospatase (G-6-Pase) enzyme activity similar to propylthiouracil, a standard antithyroid drug. Thus, a higher dose of piperine may inhibit thyroid function and serum glucose concentration in euthyroid individuals [40].

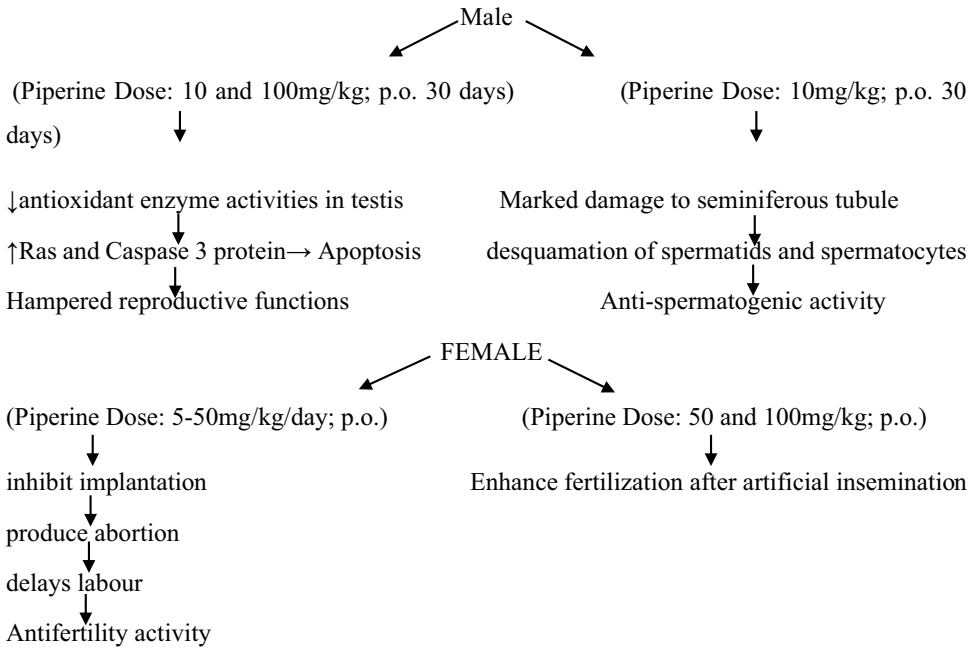

Fig. 2 Summary of physiological role of piperine on male and female reproductive system

Serotonin

Piperine at 60 mg/kg; i.p. was found to suppress the convulsions in E1 mice (low 5-HT levels) induced by "throwing" stimulation. The 5-HT level was significantly increased in the cerebral cortex than in the control mice after 1 h of piperine administration and this increase may be directly related to the mechanism involved in the inhibition of convulsions by piperine, since, high 5-HT levels increase the convulsive threshold [39].

Other Physiological Effects of Piperine

Inflammation

Anti-inflammatory effect of piperine in rats was demonstrated by different acute and chronic experimental models such as carrageenin-induced rat paw edema, croton oil-induced granuloma pouch, and cotton pellet granuloma. Significant changes in acute inflammatory processes and chronic granulative changes were observed by piperine [41].

Asthma

The therapeutic potential of piperine in asthma was demonstrated by evaluating the action of piperine in a murine model of asthma induced by ovalbumin sensitization and inhalation in Balb/c mice. Piperine at 4.5 and 2.25 mg/kg when administered 5 times a week, orally for 8 weeks causes alleviation of eosinophil infiltration, Th2 cytokines namely, IL-4 and IL-5, and considerable reduction of the thymus as well as activation regulated chemokine, eotaxin-2 in lung tissue. Apart from this, reduction of IL-4, IL-5, and eotaxin levels was also observed in bronchoalveolar lavage fluid along with immunoglobulin E synthesis in serum which is histamine and ovalbumin-specific. Hence, this study manifests the therapeutic mechanism by which piperine helps in treating asthma [42].

Cytoprotective Effect

The binding affinity of piperine with immune cell receptor was observed to determine its protective and ameliorative potential in Deltamethrin (DLM) induced immunotoxicity under in vitro conditions. Piperine at 1, 10, and 50 µg/ml was found to increase cell viability in a concentration-dependent manner in vitro. Enhanced reactive oxygen species (ROS) and caspase-3 activation along with phenotypic changes were significantly reduced by piperine. Piperine also restored GSH and cytokine levels (IFN γ, IL-2, and IL-4) in a dose-dependent manner. Therefore, these observations prominently reflect the anti-oxidative, anti-apoptotic and chemo-protective potential of piperine in thymic apoptosis induced by DLM [43].

Immunomodulatory and Anti-Tumor Effect

Administration of piperine (1.14 mg/dose/animal) and alcoholic extract of *Piper longum* (10 mg/dose/animal) inhibits the development of solid tumor in mice induced with Dalton's lymphoma ascites (DLA) cells and significantly increases the life span of mice tolerating Ehrlich ascites carcinoma tumor by increasing total white blood cells count in Balb/c mice along with bone marrow cellularity and α-esterase positive cells [44].

The immuno-protective potential of piperine was delineated in cadmium-induced apoptosis in mice where cadmium (cd) suppresses immune functions. To outline the anti-apoptotic role of piperine in vivo, cd was administered as $cdcl_2$ at a dose of 1.8 mg/kg; i.p. once at 4th day in piperine (2.5 mg/kg/day; oral, 7 days) treated Balb/C mice. Piperine-modified cadmium-induced alterations in apoptotic markers and oxidative stress. Moreover, inhibition of cell proliferative response, alterations in cytokine release, T and B cell phenotypes, and morphological changes were re-established to normal levels by piperine [45].

Hepatoprotective Effect

A single dose of acetaminophen (900 mg/kg B.W; i.p.) induces hepatotoxicity in mice and to abrogate this effect, equal doses of piperine and silymarin at 25 mg/kg B.W; i.p. were administered 30 min after a single administration of acetaminophen. Significant increase in the levels of liver marker enzymes, TNF-α, and lipid peroxidation along with depletion of antioxidant status were observed after acetaminophen induction but these changes were reverted back after piperine and silymarin treatment in acetaminophen-induced mice suggesting the promising hepatoprotective potential of piperine similar to the standard drug, silymarin [46].

The administration of normal doses of antitubercular drugs for 45 days does not produce any significant changes in the histopathology of the liver, instead, increases lipid peroxidation and decreases the reduced glutathione levels. Thus, co-administration of piperine with antitubercular drugs helps in abrogating the changes in antioxidant status [47].

Interaction of Piperine

Bioavailability Enhancer

Piperine is shown to possess the ability to enhance the bioavailability of various therapeutically and structurally diverse

drugs which may be credited to increased absorption due to alteration in membrane lipid dynamics and change in the enzyme conformation in the intestine. Due to the alteration in enzyme kinetics, the activity of Glycyl-glycine dipeptidase and Leucine amino peptidase was also stimulated by piperine. Hence, this indicates modulation of membrane dynamics due to the apolar nature of piperine which interacts with surrounding lipids and hydrophobic fragments in the protein domain and decreases the likelihood of membrane lipids acting as a stearic force to enzyme proteins and thereby modifying enzyme conformation. Ultra-structural studies showed induction in protein synthesis associated with cytoskeletal function which resulted in an increase in the absorptive surface of the small intestine, thus, providing efficient permeation via. epithelial barrier [48].

Inflammation

A combination of nimesulide and piperine in the writhing test showed a significantly lower effective dose (ED_{50}) value i.e., 1.5 mg/kg as compared to nimesulide alone, i.e., 11.2 mg/kg. Nimesulide–piperine combination in carrageenan-induced inflammatory tests showed dose-to-dose superiority over nimesulide alone. Thus, showing a better therapeutic index of nimesulide–piperine combination. Acute toxicity studies conducted on mice disclosed a fall in lethal dose (LD_{50}) of the combination (980 mg/kg) as compared to nimesulide (1500 mg/kg) alone. Hence, reducing the incidence of adverse effects related to only nimesulide [49].

Cardioprotective effect

Curcumin is a well-recognized cardioprotective agent but possesses poor bioavailability. Therefore, curcumin was combined with piperine as a bio-enhancer against cyclophosphamide (CP)-induced cardiotoxicity in rats to increase the therapeutic efficacy of curcumin. The combination of 50 mg/kg; p.o. of curcumin with 20 mg/kg; p.o. piperine showed the best effective results with a marked significant decrease in ECG an increase in serum biomarker level and a moderate significant decrease in antioxidant levels, lipid profile and histopathological score in comparison to the curcumin alone treated group [50].

Epilepsy

Pattanaik et al. [51] evaluated the activity of piperine in poorly managed epilepsy patients on carbamazepine monotherapy of 300 and 500 mg/kg based on the steady-state pharmacokinetics of a single dose of carbamazepine. The mean plasma concentrations of carbamazepine were significantly increased by 20 mg/kg; p.o. piperine. Thus, either by decreasing the elimination or by increasing the absorption of carbamazepine, piperine could significantly enhance its bioavailability and could also prevent a fall in carbamazepine levels [51].

Diabetes

A combination of glimepiride with piperine enhances the bioavailability of glimepiride by inhibiting the CYP2C9 enzyme, suggesting its usefulness as an adjuvant to glimepiride in diabetic patients [52].

Co-administration of piperine with antibiotics such as beta-lactam, cefotaxime sodium, and amoxicillin trihydrate produces significant enhancement of bioavailability in rats. The improved bioavailability is depicted in various pharmacokinetic parameters viz. t_{max}, C_{max} $t_{1/2}$, and area under the curve (AUC) of these antibiotics. The action of piperine on microsomal metabolizing enzymes or enzymes system attributes to its increased bioavailability [53].

Toxicity Study of Piperine

Acute and Subacute Toxicity

The LD_{50} values of piperine were found to be 15.1, 43, 200, 400, and 330 mg/kg B.W. when administered once through i.v, i.p., s.c., i.m., and i.g., respectively in adult male mice. A lethal dose produces respiratory paralysis within 3–17 min and causes the death of animals. Thus, piperine shows acute toxicity in mice, rats, and hamsters. The subacute toxicity studies caused the death of rats within 1–3 days after piperine treatment possibly due to multiple dysfunctions in their organs. Moreover, severe hemorrhagic necrosis along with edema in the gastrointestinal tract, adrenal glands, and urinary bladder was observed [37].

Sub-chronic Toxicity

The sub-chronic toxicity of piperine was determined in 30 male BALB/c mice which were divided into 5 groups namely the normal control group and other four treatment groups at a dose of 17.5 mg/kg, 35 mg/kg, 70 mg/kg, and 140 mg/kg body weight for a period of 21 days. The toxicity was based on the histological score of the liver, kidney, and lungs at day 22 of the dosing and it was observed that 35, 70, and 140 mg/kg dose of piperine was toxic to the liver and 140 mg/kg dose of piperine was toxic to the kidney and lung. Conclusively, 35 mg/kg or above dose of piperine should be avoided [54].

Immunotoxicity

The immune-toxicological effect of piperine gavaged at a dose of 1.12, 2.25, or 4.5 mg/kg was demonstrated in Swiss male mice for 5 consecutive days. Apparently, toxic effects were not produced by these dose levels and the liver gained weight normally. Higher doses of 2.25 and 4.5 mg/kg decrease primary antibody levels in serum and the number of primary antibodies (IgM) forming cells in the spleen and suppressing the cellular population of lymphoid organs. The lowest dose of 1.12 mg/kg B.W. of piperine is referred to as the "no observed adverse effect level (NOAEL)" dose and shows no immunotoxicity [55].

Reproductive Toxicity

The reproductive toxicity of piperine was conducted on Swiss albino mice using 10 and 20 mg/kg body weight whose oral and intrauterine administration during pre and post-mating showed interference with vital reproductive processes such as estrous cycle, fertilization, mating behavior, and implantation. Oral administration of piperine caused inhibition of implantation by 83.33% at both doses (10 and 20 mg/kg) possibly due to luteolysis of corpora lutea. The sperm shape abnormality test of piperine did not reflect any significant abnormalities up to 75 mg/kg possibly due to the non-mutagenic nature of piperine in male germ cells. Thus, piperine intrudes with several essential reproductive events in a mammalian model and specific recommendations regarding its safety in human consumption are uncertain [56].

Genotoxicity

The genotoxic potential of piperine was evaluated in Swiss albino mice. The non-mutagenic nature of piperine was assessed in the Ames test where six different doses of piperine, in the range of 0.005–10 µmol/plate did not induce his + revertants. Ten and 20 mg/kg B.W. of piperine was again found to be non-mutagenic in the bone marrow micronucleus test. Piperine at 10 and 50 mg/kg B.W. in the dominant lethal and sperm shape abnormality test failed to induce mutations in the male germ cells of mouse. Hence, piperine showed a non-genotoxic effect [57].

Oral administration of piperine at 25, 50, and 75 mg/kg causes a significant reduction in the micronuclei formation induced by benzopyrene and cyclophosphamide in mice [58].

Conclusion

Through this review, it is concluded that piperine shows valuable and effective properties in treating various physiological disorders. Recent advancement in piperine is depicted through its anti-cancer, anti-apoptotic, immunomodulatory, and anti-osteoporotic activity. The basic properties which are responsible for most of the mechanistic approaches of piperine in various diseases are its 'antioxidant, anti-inflammatory, anti-apoptotic, and bio-availability enhancing abilities. These four activities of piperine provide the initial framework for managing and alleviating severe disease conditions. Piperine also attributes to enhancing the bioavailability of synthetic as well as natural compounds. However, despite its several pharmacological activities, it shows some controversial responses regarding its safety and consumption but recent studies have proven its safety in animal studies. The study performed on piperine determines a lack of accuracy in drawing specific recommendations and conclusions regarding its doses since the work conducted by many authors on different doses of piperine lays its own pharmacological and pathological interpretations such as 5 to 10 mg/kg p.o. of Piperine induces anti-spermatogenic and ant implantation effects, while similar and higher dose has been suggested as protective in many diseases, e.g., CVS related.

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Data Availability All the data related to manuscript presented in the manuscript.

Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

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