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(Review)

Boldine Alkaloid: Holistic Overview on Potential Health Benefits and Medicinal Merits a comprehensive review

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ABSTRACT

Alkaloids are considered as an important group of phytochemicals that are widely distributed in natural sources. Alkaloids are characterized by their myriad health benefits and medicinal merits being important to human health. Boldine is one of aporphine alkaloid which is widely distributed in several plant species, commonly in boldo tree (*Peumus boldus*). Boldine alkaloid plays a pivotal role in human health owing to its myriad pharmacological activities. It exhibited several biological activities which posed it for the treatment of several ailments. The current review aims to introduce a holistic highlight on available natural sources, and potential health benefits of boldine and further capitulates on previous pharmacological studies of boldine both in vivo and in vitro (2000-2022). Several databases were searched for a collection of the articles on boldine such as ScienceDirect, PubMed, Springer, Elsevier, Wiley Online Library, Taylor & Francis and EKB. Moreover, the valorization of boldine as a valuable natural compound of important value for the production of pharmaceutical preparations was also addressed. Ultimately, boldine is an extremely important alkaloid with diverse pharmacological actions such as hepatoprotective, antioxidant, anti-inflammatory, and anticancer.

Keywords: Alkaloids; Boldo; Boldine; Anticancer; Hepatoprotective

1-Introduction

Boldine (2,9-dihydroxy-1,10-dimethoxy-aporphine) Fig. 1, is an aporphine alkaloid, isolated for the first time since by Bourgoin and Verne , while it was prepared in a pure state in 1922 in Merck's laboratory [1, 2]. The main natural sources of boldine alkaloid are the leaves and bark of *Peumus boldus* Molina, family Monimiaceae, which is native to central and southern of Chile [3]. Boldo infusion has been used traditionally for the treatment of digestive, hepatobiliary disorders earache, headache, rheumatism, and nasal congestion[3]. Furthermore, it has been recognized as an herbal remedy in several pharmacopeias in South America and Europe [1]. Boldine is widely distributed in several plant species, Fig. 2. Boldine constitute about 75% of total alkaloids of boldo, responsible for the majority of the health benefits of the boldo extract [4].Boldine was also isolated from Lindera aggregate [5], which is native to China, Japan and Southeast Asia. Lindera aggregate leaves tea was used in Chinese traditional medicine for the prevention and treatment of hyperlipidemia [6]. Moreover, Actinodaphne pruinosa [2] distributed in Indonesia and Malaysia is considered as a source of boldine. Litsea cubeba is predominant in tropical and subtropical regions of India, Southeast Asia, southern China, Taiwan, and Japan[7], which is utilized in traditional Chinese medicines for treatment of various aliments [7] Fig. 2. In addition, it was identified as the major alkaloid in the bark of a local tree of Phoebe grandis cultivated in the Northern part of Peninsular Malaysia [8]. Boldine alkaloid was reported to exhibit a variety of biological activities viz, hepato-protective, anti-tumor, relief headache, anti-rheumatism, dyspepsia, urinary tract infections, sleep disturbances, anti-inflammatory, antipyretic and antiplatelet aggregation and antioxidant[9]. This review aims to present a holistic overview on the major sources of boldine alkaloid and its myriad biological importance and highlighting its structure activity relationships (SAR).

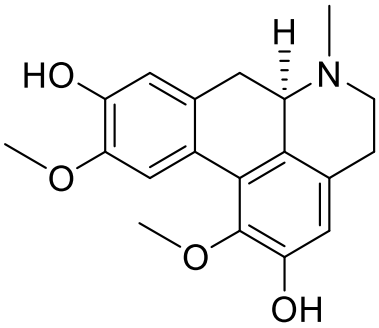

Figure 1. Chemical structure of Boldine

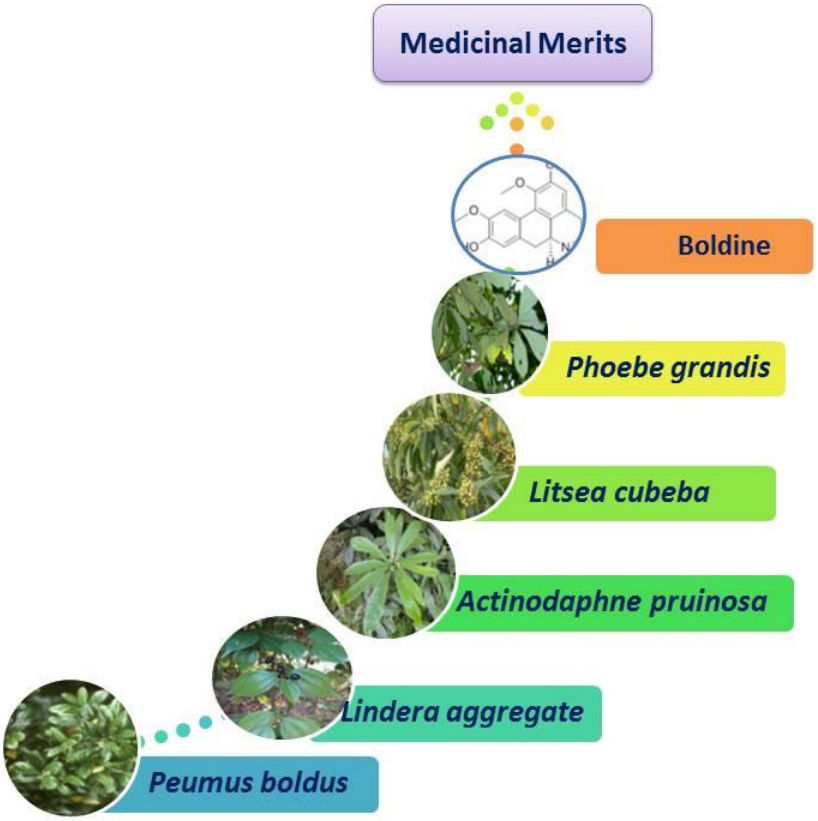

Figure 2. Natural plant sources of boldine

2. Health benefits

Several potential biological activities has been reported for boldine such as: antioxidant activity, anti-inflammatory activity, improve endothelial function in hypertensive and diabetic patient, nootropic effect, kidney protective effect, anti-cancer activity, acetylcholinesterase and butyrylcholinesterase inhibitor activity, cytoprotective activity, choleretic effect , anticonvulsant activity, cerebrovascular effect, antileishmanial

activity, anti-alzheimer's, treatment of GIT disorder, anti-osteoporotic effect, hepatoprotective activity, and neuroprotective effect.

2.1. Antioxidant activity

The antioxidant capacity of boldine was studied by Konrath, Santin et al. [10] using hippocampal slices from Wistar rats, which were exposed to oxygen and glucose deprivation (OGD) followed by reoxygenation, to imitate an ischemic condition. Results revealed that the addition of boldine during and after OGD exposure significantly increased cellular viability and reduced cell damage. Furthermore, it prevented the increase in lipoperoxidation levels. Boldine showed three-time activity compared to Trolox using a total reactive antioxidant potential level. In another study, boldine was able to elevate the expression of adiponectin and its regulators in 3T3-L1 cells, as there is a negative correlation between oxidative stress and adiponectin levels [11]. Furthermore, boldine exhibited potent scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) compared to ascorbic acid in a study performed by Klimaczewski, de Aquino Saraiva et al. [12]. The synergistic effect of boldine and salmon gelatin cause quenching free radical over 80% using DPPH free radical assay was studied by (López, Márquez et al. [13]. In another *in vivo* study, boldine revealed an antioxidant activity by increasing superoxide dismutase (SOD) and glutathione (GSH) levels on asthmatic animals, while it restrained the reactive oxygen species (ROS) and malonaldehyde (MDA) levels in bronchoalveolar lavage fluid (BALF) of ovalbumin-induced mice [14].

2.2. Anti-inflammatory activity

The anti-inflammatory activity of boldine alkaloid was studied *in vivo*. Boldine was evaluated against dextran sodium sulfate (DSS)-induced ulcerative colitis in mice. Results showed that colon damage was reduced, with significant reductions in both extent and the severity of the inflammation. Moreover, leukocyte infiltration in the mucosa by reduction in myeloperoxidase enzyme was observed [15]. In another study, boldine was evaluated against ankle edema swelling. Boldine showed a reduction in edema and ankle swelling, in addition, there was a decrease in the alleviated pathological damage and considerable prevention of bone destruction in collagen-induced arthritis in rats via inhibition of osteoclastogenesis [16]. In a further report, the anti-inflammatory activity of boldine was evaluated in xylene-induced ear edema and carrageenan-induced paw edema

in mice and rats. Results revealed that administration of boldine significantly mitigated ear weight in mice and decreased paw volume in rats. Additionally, it reduced the infiltration of neutrophil leukocytes in rat paw tissue [17]. Furthermore, the boldine effect on broncho-alveolar lavage fluid (BALF) of ovalbumin-induced mice was studied *in vivo*. Boldine showed an anti-inflammatory effect by inhibition of eosinophil, lymphocyte, macrophage, and neutrophil. Moreover, the immunoglobulin E levels significantly appeared in the serum of boldine-treated animals [14].

2.3. Hepatoprotective activity

Boldine was reported to be used as hepatoprotective. The effect of boldine in bile duct ligated (BDL) rats as a model of cholestasis and cirrhosis was evaluated. The results revealed that boldine restored the BDL-induced depletion of (GSH) content and tissue antioxidant capacity. Histopathological changes and collagen deposition were markedly attenuated. Such effects might be associated with the anti-fibrotic properties via antioxidant activities [18]. Boldine was evaluated against diethylnitrosamine-induced hepatocarcinogenesis in Wistar rats. Results showed that boldine-treated liver tissues exhibited regeneration of hepatocytes and restored the normal liver function tests. Moreover, liver enzymes level (AST, ALT, GGT, and LDH) and mRNA expression of Bcl2 protein was reduced. Moreover, it increased the mRNA expression of Bax protein and apoptosis executioner caspase 3 and initiated apoptosis [19]. In another study boldine–phospholipid complex compared to boldine free drug were evaluated against carbon tetrachloride-induced hepatotoxicity model in albino Wistar rats. Results showed that boldine–phospholipid complex has a significant hepatoprotective activity compared to free boldine by the reduction of elevated enzyme levels (AST, ALT, and ALP). Moreover, histopathological results showed a complete regeneration of liver cells and they were nearly normal [20]. Moreover, boldine was evaluated against paracetamol induce toxicity in male Swiss albino mice. Results showed a considerable reduction in liver enzymes (AST and ALT). Moreover, it prevents a rise in lipid peroxidation, and halted the downward of antioxidant enzymes (SOD, and CAT), and prevented the fall in liver GSH content. It showed a prominent decrease in the expression of proinflammatory markers in liver tissue TNF-α, IL-1β, and IL-6 [21]. In another study, the hepatoprotective activity of boldine in male Wistar rats was investigated using

methylprednisolone (MPL) to induce toxicity. Results showed that rats treated with MPL and boldine have a decrease in liver enzymes (ALT and AST) compared to rats treated with MPL alone. Moreover, oxidative stress markers such as lipid peroxidation (LPO) and nitric oxide (NOx) levels were increased in treated groups with MPL, while GSH levels were significantly decreased. On contrast the treated groups with boldine and MPL caused a reduction in oxidative stress parameters. Also, treatment with boldine significantly increased the levels of SOD, GSHPx, and GR, in contrast to treated groups with MPL. Also cytochrome c protein expressed in treated rats with MPL, meanwhile it was reduced with rats treated with boldine and MPL [22]

2.4. Improvement of endothelial function in the hypertensive and diabetic patient

Endothelial dysfunction is either an imbalance of numerous relaxant and constrictor substances produced from the endothelium or a change in the metabolism of the available nitric oxide (NO) [23]. Boldine effect on endothelial dysfunction in hypertension was investigated using a spontaneously hypertensive rat [24] model. Results showed that boldine administration lowered the systolic blood pressure in SHR. Besides, it enhanced the maximal relaxation of acetylcholine enhanced sensitivity. Additionally, it lowered aortic superoxide and peroxynitrite production. All of the previous results explained the boldine protective effect in hypertension, at least partially through the inhibition of NADPH-mediated superoxide production [25]. Another study was performed to investigate the effects on endothelium in a range of diabetes *in vitro* and *in vivo*. Results showed enhancement of endothelium-dependent aortic relaxations and reversed impaired relaxations induced by high glucose or angiotensin II in non-diabetic mouse aortas. In addition, boldine reduced the overproduction of reactive oxygen species (ROS) and increased phosphorylation of endothelial nitric oxide synthase (eNOS) in mice aortas. Also, it reduced oxidative stress and improved endothelium-dependent relaxation in aortas of diabetic mice through inhibiting ROS overproduction [26]. Moreover, boldine was evaluated against high glucose-induced oxidative stress in rat aortic endothelial cells (RAEC). Results revealed a reduction of the elevated ROS, nitrotyrosine formation, and preservation of nitric oxide (NO) production. As well, it improved endothelium-dependent relaxation in the aortas and normalized ROS over-production in the diabetic group [27].

2.5. Nootropic effect

Nootropic agents are responsible for the improvement of mental performance through raising mental functions (memory, motivation, concentration, and attention). They increase blood circulation to the brain and provide the human brain with important nutrients, energy, and oxygen through vasodilation of the brain's small arteries and veins [28]. The effect of boldine on the learning and memory of the Swiss albino male young and aged mice was investigated using Morris's water maze as a behavioral model. The results showed that boldine improved the learning and memory of young as well as aged mice. Additionally, boldine significantly reversed scopolamine, sodium nitrite, and aging-induced amnesia in mice. Moreover, it attenuated oxidative stress, by decreasing in brain malondialdehyde as well as brain nitrite levels. Furthermore, a significant increase in brain GSH levels of young as well as aged mice was detected. Brain acetylcholinesterase activity was also considerably inhibited by boldine in young and aged mice [29].

2.6. Kidney protective effect

The kidney protective effect of boldine was evaluated against 5/6 nephrectomized rats to induce chronic renal failure. Kidney parameters were decreased as follows: urinary protein/creatinine ratio and oxidative stress measuring Thio-barbituric acid reactive substances (TBARS), ED-1(a marker of inflammation), Col III (a marker of kidney damage) and this were done using Western blot and immunohistochemistry which propose its protective effect [30]. In another study on diabetic rats, boldine prevented elevation of the blood sugar level, blood pressure, renal thiobarbituric acid reactive substances and the urinary protein/creatinine ratio. Besides, boldine reduced the alterations in matrix proteins and markers of renal damage. Moreover, boldine prevented the elevation in oxidative stress in mesangial cells suggesting a protective effect against tissue damage in the kidney [31]. Moreover, by using the 2K1C hypertension model, boldine reduced the UProt/UCrea ratio, plasma thiobarbituric acid reactive substances, and slightly reduced SBP. Additionally, levels of α-SMA, Col III (markers of kidney damage), ED-1, and OPN (markers of inflammation) were decreased. Whereas, it prevented the increase in ACE-1 and TGF-β in 2K1C rats [32]. The protective effect of boldine, against cisplatin-induced nephrotoxicity in male Wistar albinos was investigated. Results showed that boldine protected renal function through lowering renal damage

(measured using blood urea nitrogen and creatinine levels), antioxidant activities (superoxide dismutase, glutathione peroxidase), lipid peroxidation (measured using thio-barbituric acid reactive substances), and tumor necrosis factor alpha (TNF-α) levels, at all doses. Pretreatment with boldine protected the renal function at both doses. Histopathological findings supported biochemical findings [33].

2.7. Anticancer activity

The anticancer activities of boldine were investigated *in vivo* revealing a reduction in tumor on implanted glioma model and histological characteristics indicating a less invasive and proliferative tumor. Furthermore, no vascular endothelial growth factor expression, which is a major angiogenic factor in glioma [34]. Furthermore, *in vitro* study revealed a reduction in cell viability and cell proliferation through apoptosis in urinary bladder cancer. Such results are linked to the inactivation of extracellular signal-regulated kinase protein. Additionally, the efficacy of boldine in apoptosis-induced in T24 cells is correlated with modulation of AKT (inactivation) and glycogen synthase kinase-3β (activation) proteins [35]. Further, *in vivo* and *in vitro* studies on breast cancer, boldine showed a cytotoxic effect and induced apoptosis in breast cancer cells. Additionally, it induced activation of caspase-9 and caspase-3/7 and inhibited nuclear factor kappa B activation, a key molecule in tumor progression and metastasis. Moreover, boldine was associated with disruption of the mitochondrial membrane potential and release of cytochrome C in the breast cancer cell. While *in vivo* study showed that intraperitoneal injection of boldine significantly reduced tumor size [36]. Furthermore, boldine inhibited telomerase in cells treated with sub-cytotoxic concentrations. The targeted cancer cell (breast cancer cell lines (MCF-7 and MDA-MB-231), human embryonic kidney 293 cells (HEK293) were evaluated using a modified quantitative real-time telomere repeat amplification protocol (q-TRAP). Telomerase inhibition occurs *via* down-regulation of human telomerase reverse transcriptase (hTERT), the catalytic subunit of the enzyme [37]. Moreover, boldine exhibited cytotoxicity against hepatocarcinoma (HepG-2) cells and induced apoptosis concurrently with increasing the expression of bax/bcl2 and p21 genes [38]. While another *in vivo* study against mouse mammary carcinoma showed that intraperitoneal injections of boldine repeatedly slowed tumor growth. In addition, *in vitro* study against human MCF-7 breast cancer cells

revealed that boldine was cytotoxic to MCF-7 cells and reduced cell viability and proliferation [39].

The effect of boldine as a telomerase-targeted anti-cancer on human colon carcinoma (HCT) 116 cell line was studied. Results showed that, boldine has a considerable inhibition of cell growth in a time- and dose-dependent, with an increasing percentage of inhibition to 94.4% after 72 hours using sulphorhodamine B assay. Moreover, boldine with concentration (22.4μg/mL) for 72 hours showed a down-regulation of mRNA expression for both hTERT and hTERC in Real-Time PCR (qRT-PCR). The down-regulation of hTERT protein expression correlated with the reduced hTERC mRNA expression in qRT-PCR [40].

2.8. Acetylcholinesterase and Butyrylcholinesterase inhibitor activity

Boldine showed acetylcholinesterase (AChE) inhibition activity compare to that of standard huperzine using Ellman's method [41]. Investigation of AChE and butyrylcholinesterase (BChE) inhibition activity of boldine using acetyl (butyryl) thiocholine and Ellman's reagents were studied. Results showed that, boldine exhibited a good activity as AChE of IC_{50} equal to 372μmol/L and a similar level to BChE, 321 μmol/L. The activity of boldine may be due to noncompetitive inhibition as revealed by Dixon plot which is the most common inhibition mechanism for cholinesterase [42].

2.9. Cytoprotective activity

Administration of poly-lactic-co-glycolide (PLGA)-loaded nano boldine (NBol) was reported to have a cytoprotective activity which was evaluated by using MTT-cell viability assay. Additionally, co-administration of a small dose of boldine with cisplatin showed a reduction of cisplatin-induced cytotoxicity in normal liver cells (WRL-68) which concluded by increased viability up to 75.6 %. However, it did not have any protective effect on the cancer cells (HepG2) [43]. On the other hand, boldine was compared to that of (PLGA)-nanoparticles loaded boldine against cisplatin-induced cytotoxicity in normal tissue. Results showed that the expression pattern of apoptotic genes like Bax, Top II, Bcl-2, p53, cytochrome c , and caspase-3 suggested greater cytoprotective potentials of NBol (29% approximately) in normal tissues compared to boldine [44].

2.10. Choleretic effect

Boldine was reported to support bile production as studied *in vivo* by administration of boldine which revealed increase in bile production by direct osmotic activity but should be administered in sufficient doses to attain the bile concentration. Despite of short-term action of boldine and long term pretreatment with doses of 50 mg/kg/day may induce sustained mild chlorosis on the basis of Farnesoid X receptor - mediated up-regulation of bile salt export pump with consequent stimulation of bile acids biliary secretion [38]. Another study was performed to clarify the effect of high sucrose diet (HSD) on bile formation in rats with hereditary hypertriglyceridemia (HHTg). Administration of HSD to HHTg rats led to increased triglyceride deposition in the liver. Boldine showed to the partially attenuated cholestatic effect of HSD by promotion of biliary secretion which increased in the biliary secretion of GSH as a consequence of its increased hepatic disposition [45].

2. 11. Anticonvulsant activity:

Boldine showed potent anticonvulsant activity against three experimental models of seizure including intravenous and intraperitoneal pentylenetetrazole (PTZ) injection and electroshock-induced seizures. The action was explained by increasing the colonic seizure threshold in the intravenous PTZ model and increasing the time latencies to the onset of myoclonic and colonic seizures induced by intraperitoneal PTZ model. Additionally, decreased tonic hind limb extension duration in the electroshock-induced seizure model [46].

2. 12. Cerebrovascular Effect

Boldine showed a cerebrovascular protective effect against neural apoptosis induced by traumatic brain injury (TBI) using immuno-histochemistry and Western blotting assay. As it significantly reduced the apoptotic index; however, the level of caspase-3 was greatly decreased. Moreover, it caused a significant reduction in the level of mitochondrial malondialdehyde together with an increase in superoxide dismutase activity [47].

2. 13. Antileishmanial activity:

The antileishmanial activity of boldine was assessed *in vitro* against *Leishmania amazonensis* murine cell infection. MTT viability assay was used for testing boldine cytotoxicity and intracellular parasite destruction. It showed that, reduction of parasite

infection in treated cells by 81% using 100 μg/ml of boldine. Besides, a concentration-dependent decrease in macrophage infection culminated with a 96% of reduction when cells were incubated with 600 μg/ml [48].

2.14. Anti-Alzheimer's:

A study by Yi, C. et al, 2017 [49] to investigate the neuroprotective activity of boldine was investigated on glial hemichannels in a murine model of Alzheimer's disease (AD). Results revealed that boldine inhibited hemichannel activity in astrocytes and microglia without affecting gap junctional communication in culture and acute hippocampal slices. While long-term oral administration of boldine in AD mice revealed preventing the increase in glial hemichannel activity, astrocytic Ca^{+2} signal, ATP, glutamate release, and alleviated hippocampal neuronal suffering. Another study was performed to investigate the neuroprotective effect of boldine in cellular models (primary hippocampal neurons and HT22 hippocampal derived cell line treated with amyloid beta peptide (Ab) oligomers) of Alzheimer's disease [50]. Results showed that boldine interacts with Ab *in silico*, preventing its aggregation and defending hippocampus neurons against synaptic failure brought on by AbO. Additionally, it restored variations in intracellular Ca^{2+} levels linked to mitochondria or the endoplasmic reticulum in HT22 cells that had received AbO treatment. It reversed the AbO-induced decrease in mitochondrial respiration in HT22 hippocampus cells, fully recovered the decline in mitochondrial membrane potential, and decreased the rise in mitochondrial reactive oxygen species [50].

2.15. GIT disorder

Boldine was reported to inhibit 5-HT-induced activation of 5-HT3 receptors which is responsible for the pathogenesis of functional gastrointestinal disorders. Boldine played an important role to relieve functional gastrointestinal disorders, in addition, it is valuable to relieve the effect of chemotherapy-induced nausea and vomiting and irritable bowel syndrome treatment. Its mechanism of action is explained by its competitive antagonists at 5-HT3A- vs 5-HT3AB receptors [51].

2.16. Anti-osteoporotic effect

Boldine was evaluated for estrogen deficiency-induced osteoporosis in mice through the Micro-CT and histomorphometry assays. The activity was explained by inhibiting bone resorption without affecting bone formation *in vivo*. additionally, it can

inhibit receptor activator of nuclear factor-κB ligand (RANKL)-induced osteoclast formation [52]. The effect of boldine in periodontitis induced by ligature in mice was studied. Results showed that boldine inhibits the alveolar bone resorption by decreasing in the osteoclast number and RANKL/OPG ratio in periodontal lesions, reduction in reduced Th17-lymphocyte detection and increasing the Treg-lymphocyte detection and response in periodontitis-affected tissues [53].

2.17. Neuroprotective effect

Boldine showed a neuroprotective effect on neuron inflammation and memory deficits induced by permanent middle cerebral artery occlusion (pMCAO) in mice. The mechanism of action of boldine was through a significant decreasing in the infarct area, improving neurological scores, and increasing cell viability. Additionally, myeloperoxidase activity and glial fibrillary acidic protein, tumor necrosis factor-alpha (TNF-α), and inducible nitric oxide synthase (iNOS) immunoreactivity were decreased. The vertical exploratory activity and aversive, spatial, object recognition, and working memory deficits induced by pMCAO were prevented. [54].

2.18. Erectile dysfunction effect.

Erectile function in penile tissue is mediated with an expression of nitric oxide from endothelial nitric oxide synthase (eNOS). Boldine at a dose of 40 mg/kg/day showed an enhancement in erectile function in metabolic syndrome rat model owing to improvement of eNOS activation in penile tissue and intracavernous pressure/ mean arterial pressure ratio in boldine-treated rats models [55]

2.19. Vasodilator activity

The vasodilation effect of boldine was investigated in male Wistar rats. Results showed that boldine involved in nitric oxide and small-conductance Ca^{2+} activated K^{+} channel activation. This action was confirmed by using glibenclamide, iberiotoxin, and charybdotoxin which did not alter the vasodilatory action of boldine. However, apamin (selective small-conductance Ca^{2+} activated K^{+} channel blocker) prohibited the vasodilatory action of boldine at all doses [56].

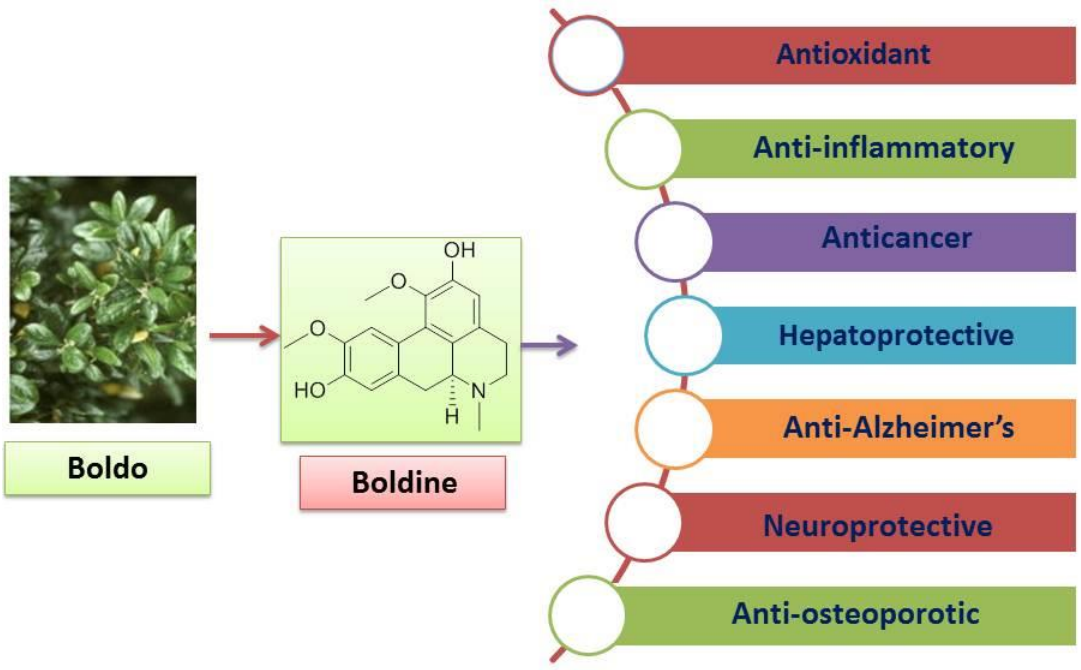

Figure 3: Major Potential health benefits of boldine

3. Structure-activity relationship (SAR) of boldine

The activity of boldine alkaloid is directly related to its structure as illustrated in **Fig. 4**. The secondary ammonium has a positive effect on the anticancer activity compared to the quaternary ammonium group which has negative effect on activity. Moreover, free carbon 7 at ring C has also a positive effect on enhancing boldine activity rather than being replaced by another functional group [57]. Additionally, the substitution at ring A with methyl and ethyl group has an important influence on the interaction between boldine structure and its target receptor[58]**.**

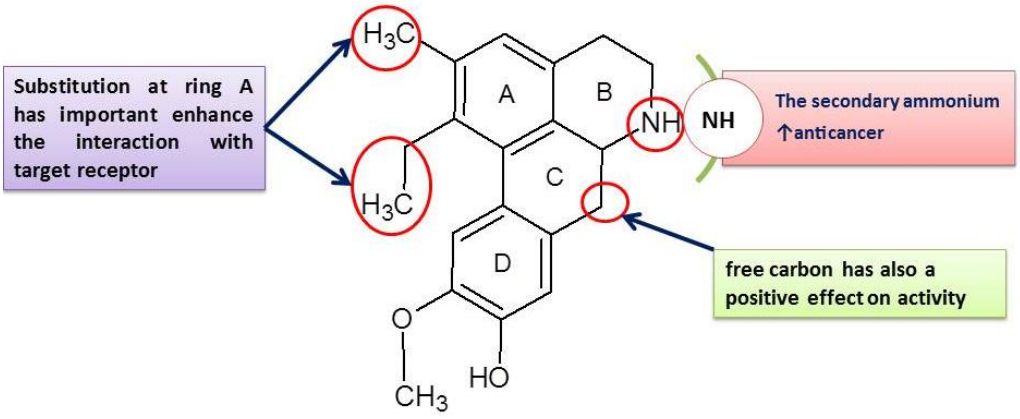

Figure 4. SAR of boldine alkaloid

Table 1: Pharmacological activities of Boldine

Method and/or animals	Dose	Results and pharmacological effect	References
Antioxidant activity			
Hippocampal slices were exposed to oxygen and glucose deprivation followed by reoxygenation, from Wistar rats and Trolox was used as standard antioxidant	10 μM	↑ cellular viability with no effect on cell damage and prevent the increase in lipoperoxidation levels.	[10]
2, 2-diphenyl-1-picrylhydrazyl (DPPH).	3.27 μg/mL	It exhibited potent scavenging activity against (DPPH and) moreover, a modest effect on lipid peroxidation and Fenton reaction.	[13]
Anti-inflammatory activity			
Dextran sulfate sodium (DSS)-induced Ulcerative colitis in mice	50 mg/kg / 7 days	↓ in tumor necrosis factor production, Interleukin 17, IL-26, and signal transducer	[59]
Collagen-induced arthritis [35] rats	4 mg/ kg, daily/16 day	↓edema, ankle swelling, and alleviated pathological damage and considerably prevented bone destruction in collagen-induced arthritis in rats.	[16]

Xylene-induced ear edema and carrageenan-induced paw edema in mice and rats	1.0 mg/kg	It mitigated ear weight in mice, decreased paw volume in rats, reduced the infiltration of neutrophil leukocytes in rat paw tissue and decreased of inflammatory cytokine mRNA expression levels, including levels of TNF-α and IL-6 in foot tissues	[17]
Improve endothelial function in hypertensive and diabetic patient			
Spontaneously hypertensive rat [24] model	20 mg/kg per day, for seven days	↓Systolic blood pressure in SHR, and aortic superoxide and peroxynitrite production and down-regulated p47phox protein expression, furthermore it enhanced the maximal relaxation of acetylcholine enhanced sensitivity.	[25]
Diabetes mice models	*In vitro* (using 1μmol L^{-1}, 12 h) and *in vivo* oral treatment (20 mg/kg/day , 7 days)	It enhanced of endothelium-dependent aortic relaxations, reversed impaired relaxations induced by high glucose or angiotensin II in non-diabetic mouse aortas, reduced the overproduction of reactive oxygen species (ROS) and increased phosphorylation of endothelial nitric oxide synthase (eNOS) in *db/db* mouse aortas.	[26]
High glucose-induced oxidative stress in rat aortic endothelial cells	Acute (1 mM, 30 min) and chronic (20	↓The elevated ROS and nitrotyrosine formation preserved nitric oxide production and improved endothelium-dependent relaxation in the aortas.	[28]

	mg/kg/daily, i.p., 7 days)		
Nootropic effect			
Morris water maze as a behavioral model.	1.5, 3 and 6 mg/kg, per oral for 7 days	It improved learning and memory of young and aged mice Moreover, it attenuated oxidative stress, by decreasing in brain malondialdehyde, as well as brain nitrite levels Furthermore, significant it increased Glutathione (GSH) levels in the brain of young as well as aged mice.	[29].
Kidney protective effect			
Nephrectomized rats induced Chronic renal failure	50mg/kg/day, gavage for 28 days	It decreased urinary protein/creatinine ratio, and oxidative stress measuring thiobarbituric acid reactive substances (TBARS), also reduced ED-1(a marker of inflammation), Col III (a marker of kidney damage) levels-.	[30]
Diabetic rats	50 mg/kg/day-for ten weeks	It prevented the elevation of hyperglycemia, blood pressure, renal thiobarbituric acid reactive substances, the urinary protein/creatinine ratio, as well as reduced the alterations in matrix proteins and markers of renal damage.	[31]
2K1C hypertensive rat model	50 mg/kg/day, gavage, 42 days	It reduced the UProt/UCrea ratio, plasma thiobarbituric acid reactive substances, and slightly reduced SBP. Additionally, levels of α-SMA, Col III, ED-1, and OPN,	[32]

		were decreased. It also prevented the increase in ACE-1 and TGF-β in 2K1C rats	
Anticancer and anti-proliferative activity			
Implanted glioma model	50 mg/kg intraperitoneal for 10 days	It reduced Tumor size in the treated group	[34]
Urinary bladder cancer *in vitro*	10–500 μM	It reduced cell viability and cell proliferation	[35]
Breast cancer *in vivo*	50- 100 mg/kg	It reduced tumor size	[36]
Breast cancer cell lines (MCF-7 and MDA-MB-231), human embryonic kidney 293 cells (HEK293)	10-160 μM under 48 hours	It inhibited telomerase in cells treated with sub-cytotoxic concentrations. Telomerase inhibition occurs via down-regulation of hTERT, the catalytic subunit of the enzyme	[37]
Hepatocarcinoma (HepG-2) cell line	(170 μM under 48 hours) (50 μg/mL)	It exhibited cytotoxicity-induced apoptosis	[38]
Human Colon Carcinoma (HCT) 116 cell line.	(100 μg/mL)	A considerable inhibition of cell growth in a time- and dose-dependent using Sulphorhodamine B assay. Moreover, boldine with concentration (22.4 μg/mL) for 72 hour showed a down-regulation of mRNA expression for	[40]

		both hTERT and hTERC in Real-Time PCR (qRT-PCR). The downregulation of hTERT protein expression correlated with the reduced hTERC mRNA expression in qRT-PCR.	
Acetylcholinesterase and Butyrylcholinesterase inhibition activity			
Ellman's method	IC_{50} equal to 8.5 μmol/l	Showed activity	[41].
Acetyl (butyryl) thiocholine and Ellman's reagents	AChE of IC_{50} equal to 372 μmol/l and a similar level to BChE, 321 μmol/l.	Showed activity	[42].
Cytoprotective activity			
Cell viability assay by MTT	63µg/ml	↓of cisplatin-induced cytotoxicity in normal liver cells (WRL-68)	[43]
Cisplatin-induced cytotoxicity in normal tissue	10 mg/kg nano boldine	It exhibited greater cytoprotective potentials as nano boldine in normal tissues	[43]

Choleretic effect			
Female Wistar rats, Mrp2 deficient Lewis TR^- (transport-deficient) rats	50 mg/kg/day	↑bile flow 1.4-fold in healthy rats, as well in animals with Mrp2 deficiency or ethinylestradiol-induced cholestasis	[60].
Rats with hereditary hypertriglyceridemia	50 mg/kg/day intraperitoneal , for 14 days	It attenuated the cholestatic effect of HSD by promotion of biliary secretion of bile acid through up-regulation of Bsep and Ntcp, and by an increase in the biliary secretion of glutathione because of its increased hepatic disposition	[45]
Anticonvulsant activity			
Intravenous and intraperitoneal PTZ injection and electroshock-induced seizures	1, 5, 10, 25, 50 and 75 mg/kg	↑clonic seizure threshold, time latencies to the onset of a myoclonic and clonic seizure. In addition, it decreased tonic hind limb extension duration in the electroshock-induced seizure model	[46].
Cerebrovascular Protective Effect			
Neural apoptosis induced by traumatic brain injury using immuno- histochemistry and Western blotting assay	10, 20, and 30 mg/kg i.p after 30 minute of trauma.	↓the apoptotic index; however, the level of caspase-3 was greatly decreased. Moreover, it showed significant reduction in the level of mitochondrial Malondialdehyde together with increase in Superoxide dismutase activity	[47].
Antileishmanial activity			

Leishmania amazonensis murine cell infection using MTT viability assay	100-600 µg/ml	↓parasite infection in treated cells	[48].
Alzheimer's disease effect			
Glial hemichannels in a murine model of Alzheimer's disease		It inhibited hemichannel activity in astrocytes and microglia without affecting gap junctional communication in culture and acute hippocampal slices. Long-term oral administration of boldine in AD mice prevented the increase in glial hemichannel activity, astrocytic Ca21 signal, and ATP and glutamate release and alleviated hippocampal neuronal suffering. Reduce neuronal damage associated with neurodegenerative situations	[49].
Primary hippocampal neurons and HT22 hippocampal derived cell line treated with Amyloid beta peptide (Ab) oligomers	1–10 mM	It interacts with Ab *in silico*, preventing its aggregation and defending hippocampus neurons against synaptic failure brought on by AbO. Additionally, it restored variations in intracellular Ca^{2+} levels linked to mitochondria or the endoplasmic reticulum in HT22 cells that had received AbO treatment. It reversed the AbO-induced decrease in mitochondrial respiration in HT22 hippocampus cells, fully	[50]

		recovered the decline in mitochondrial membrane potential, and decreased the rise in mitochondrial reactive oxygen species.	
Anti-osteoporotic effect			
Estrogen deficiency-induced osteoporosis in mice	(20 mg/kg/d) for 6 weeks.	It prevented osteoporosis induced by estrogen deficiency by inhibiting osteoclastogenesis.	[52]
Hepatoprotective activity			
Bile duct ligated (BDL) rats	5, 10, and 20 mg/kg/day, oral	It restored the bile duct ligated-induced depletion of glutathione content and tissue antioxidant capacity. Histopathological changes and collagen deposition were markedly attenuated.	[18]
Carbon tetrachloride-induced hepatotoxicity model in albino Wistar rats.	20 mg/kg of boldine p.o for 14 days	boldine–phospholipid complex showed significant hepatoprotective activity compared to free boldine drug by the reduction of elevated enzyme levels (AST, ALT, and ALP). Moreover, histopathological results showed that complete regeneration of liver cells was nearly normal.	[20]
Paracetamol induce toxicity in male Swiss albino mice.	20 mg/kg, b.w. p.o.	↓of liver enzymes (AST and ALT). Moreover, it prevents a rise in lipid peroxidation and halted the downward of antioxidant enzymes (SOD, and CAT). It prevented the fall in liver GSH content. It showed a prominent decrease in	[21]

		the expression of proinflammatory markers in liver tissue TNF-α, IL-1β, and IL-6.	
Using Methylprednisolone induced hepatotoxicity Male Wistar Rats.	50 mg/kg	Rats treated with MPL and boldine have a decrease in liver enzymes (ALT and AST) compared to rats treated with MPL alone. Moreover, oxidative stress markers were increased in treated groups with MPL, and reduced in boldine treated. Boldine significantly increased the levels of SOD, GSHPx, and GR.	[22]
Neuroprotective effect			
Neuroinflammation and memory deficits induced by permanent middle cerebral artery occlusion in mice	25 mg/kg, intraperitoneal for 5 days	It improved neurological scores, and myeloperoxidase activity and glial fibrillary acidic protein, tumor necrosis factor-alpha (TNF-α), and inducible nitric oxide synthase (iNOS) immunoreactivity.	[54].
Vasodilator activity			
Perfused Rat Kidney	30, 100, and 300 nmol	It showed a vasodilator activity through nitric oxide and small-conductance Ca^{2+}- activated K^{+} channel activation	[56]
Inhibition the alveolar bone resorption			
Periodontitis induced by ligature in mice	10, 20, or 40 mg/kg	It showed inhibition the alveolar bone resorption by decreasing in the osteoclast number and RANKL/OPG ratio in periodontal lesions, reduction in reduced the Th17-	[53]

		lymphocyte detection and increasing the Treg-lymphocyte detection and response in periodontitis-affected tissues.	
Nephroprotective effect			
Cisplatin-induced nephrotoxicity in male Wistar albino	(20 or 40 mg/kg/day), intraperitoneal for 14 days	boldine protected renal function by lowering renal damage, antioxidant activities, lipid peroxidation, and tumor necrosis factor-alpha (TNF-α) levels, at all doses. Pretreatment with boldine protected the renal function at both doses.	[33]

Conclusion:

The current review provides a comprehensive display of the distinct health benefits, therapeutic potential and different plant sources of boldine alkaloid. Large library of pharmacological activities have been reported on boldine *viz*, antioxidant, anti-inflammatory, anticancer, cytoprotective, choleretic , anticonvulsant, Antileishmanial, Anti-Alzheimer's, Anti-osteoporotic, hepatoprotective and Neuroprotective. Such review recapitulates on the valorization of boldine as a bioactive metabolite as well as its utilization as potential therapeutic agent. The improvement of boldine effects for enhancing its physiological functions and bioavailability and hence its therapeutic efficacy, appears to be very limited except for a modification of its particle size *via* micronization. This opens new insights towards the advantages of employing other processing and formulation technologies such as nanoparticles, nanoemulsion, microencapsulation, extrusion, and high-pressure homogenization to improve the physiological functions. The design of new pharmaceutical formulations based on boldine as health promoting phytochemical is presented as future potential products endeavors.

Conflict of Interest

The authors declare that no conflict of interest

5. References

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