Boldine Ameliorates Vascular Oxidative Stress and Endothelial Dysfunction: Therapeutic Implication for Hypertension and Diabetes

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Abstract: Epidemiological and clinical studies have demonstrated that a growing list of natural products, as components of the daily diet or phytomedical preparations, are a rich source of antioxidants. Boldine ([S]-2,9-dihydroxy-1,10-dimethoxy-2,9-dihydroxy-1,10-dimethoxy-aporphine), an aporphine alkaloid, is a potent antioxidant found in the leaves and bark of the Chilean boldo tree. Boldine has been extensively reported as a potent “natural” antioxidant and possesses several health-promoting properties like anti-inflammatory, antitumor promoting, antiabetic, and cytoprotective. Boldine exhibited significant endothelial protective effect in animal models of hypertension and diabetes mellitus. In isolated thoracic aorta of spontaneously hypertensive rats, streptozotocin-induced diabetic rats, and db/db mice, repeated treatment of boldine significantly improved the attenuated acetylcholine-induced endothelium-dependent relaxations. The endothelial protective role of boldine correlated with increased nitric oxide levels and reduction of vascular reactive oxygen species via inhibition of the nicotinamide adenine dinucleotide phosphate oxidase subunits, p47phox and nicotinamide adenine dinucleotide phosphate oxidase 2, and angiotensin II–induced bone morphogenetic protein-4 oxidative stress cascade with downregulation of angiotensin II type 1 receptor and bone morphogenetic protein-4 expression. Taken together, it seems that boldine may exert protective effects on the endothelium via several mechanisms, including protecting nitric oxide from degradation by reactive oxygen species as in oxidative stress–related diseases. The present review supports a complimentary therapeutic role of the phytochemical, boldine, against endothelial dysfunctions associated with hypertension and diabetes mellitus by interfering with the oxidative stress–mediated signaling pathway.

Key Words: boldine, hypertension, diabetes mellitus, oxidative stress, antioxidant, endothelial function

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INTRODUCTION

Free radical–induced oxidative stress is involved in the pathogenesis of a number of diseases, such as diabetes mellitus, hypertension, and atherosclerosis. Endothelial dysfunction, usually assessed as decreased endothelium-dependent vasodilation to vasoactive factors, such as acetylcholine (ACh), and precedes clinical symptoms and obvious vascular pathologies, is widely recognized as a major predictor of disease progression. Such endothelial dysfunction is observed early in the development of the pathology and is due, at least in part, to an excessive vascular formation of reactive oxygen species (ROS) in particular superoxide anion (O2•−), which is a known scavenger of nitric oxide (NO).

Boldine ([S]-2,9-dihydroxy-1,10-dimethoxy-aporphine) is an aporphine alkaloid found abundantly in the leaves and bark of Chilean boldo tree (Peumus boldus molina), a widely distributed tree native to central region of Chile.1 Over the past decade, boldine was also found as one of the major alkaloids in the bark of a local tree in northern part of Peninsular Malaysia.2 Boldine-containing herbal teas are gaining popularity in South America, and its usage has been extended to some European countries for further pharmaceutical processing into boldine-containing concentrates. Boldine has been extensively reported earlier as a potent “natural” antioxidant and possesses several health-promoting properties like anti-inflammatory, antitumor promoting, antiabetic, and cytoprotective. These activities of boldine are attributed to its ability to scavenge reactive free radicals.1

Boldine given in drinking water (100 mg/kg) prevented oxidative mitochondrial damage and possesses antiabetic effect.3 Boldine [20 mg/kg, 7 days, intraperitoneal (ip)] also exhibited both free radical scavenging and antinociceptive activities in mice.4 Boldine has been indicated to have low toxicity as relatively high doses are required to cause side effects, toxicity, or lethality in several mammalian species, for example, doses of 500 and 1000 mg/kg were required to cause death of mice and guinea pigs, respectively.1,5

Epidemiological studies reported that dietary and supplementary intake of antioxidants may reduce coronary artery diseases or cardiovascular events.6,7 The present article reviews the therapeutic potential of the “natural” antioxidant boldine, as a potential endothelial protective agent against the damaging effects of free radicals in oxidative stress–related diseases, such as hypertension and diabetes mellitus.
ENDOTHELIAL DYSFUNCTION AND OXIDATIVE STRESS

Under normal physiological conditions, the healthy endothelial cells maintain the balance between vasodilation and vasoconstriction. NO is the most important vasodilator released by the endothelium.16,17 Conversely, in diseased conditions, endothelium-dependent vasoconstriction becomes more prominent and endothelial dysfunction is exacerbated in the presence of various vasoconstrictors, including endothelins, angiotensin (Ang) II, cyclooxygenase-derived prostanoids, and ROS, particularly $O_2^-$.18 Typically, the hallmark of endothelial dysfunction is impaired endothelium-dependent vasodilation because of attenuated NO release and increases in EDCFs.18-20

ROS are highly bioactive molecules or chemical species formed by incomplete reduction of oxygen, for example, $O_2^-$, OH, peroxyl radical, and alkoxy radical. These molecules possess varying oxidizing potencies. Other molecules like hydrogen peroxide ($H_2O_2$), hypochlorous acid, ozone, and singlet oxygen also possess oxidizing capability.21 In the past few decades, $O_2^-$ has been identified as a “primary” ROS, which can further interact with other molecules to generate “secondary” ROS such as $H_2O_2$ or react rapidly with NO to form peroxynitrite ($ONOO^-$).22 Eventually, excessive ROS production stimulates a series of oxidative cascade or oxidative stress and reduces the NO bioavailability in the blood vessel walls, leading to impairment of endothelium-dependent relaxations.23

Oxidative stress is defined as overproduction of pro-oxidant molecules, such as ROS and nitrogen species, which can cause oxidative damage to biomolecules (lipids, proteins, DNA, RNA) and organs.24 An increase in oxidative stress is typically attributed to either an excessive overproduction of ROS or decreased endogenous antioxidant activity. Such an imbalance plays a critical contributory role in the pathophysiology of endothelial dysfunction. Additionally, ROS has direct vasoconstrictive effect on vascular smooth muscle cells (VSMCs) or indirectly activates the release of EDCFs to the VSMCs through arachidonic acid metabolism. In addition, EDCFs may activate...
endoperoxides–thromboxane A₂ prostanoid receptors in VSMCs to evoke vasoconstriction (Fig. 1).²³

**Nicotinamide Adenine Dinucleotide Phosphate Oxidase: Physiological Sources of ROS**

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase or NOX is a multicomponent enzyme that comprises a backbone, membrane-bound cytochrome b558, which consists of 2 subunits p22phox and gp91phox (NOX2) and other regulatory cytosolic proteins (p47phox, p67phox, p40phox, and Rac).²⁵ There are additional 6 NOX isoforms (NOX1, NOX3, NOX4, NOX5, DUOX1, and DUOX2) that have been identified, and NOX1, 2, 4, and 5 have been found in membranes of vascular cells, including endothelial cells, VSMCs, and fibroblasts.²⁵–²⁸ NADPH oxidase seems to be the primary source of O₂⁻ production system in the endothelial cells by transferring one electron from NADPH to molecular oxygen. Furthermore, it can potentially influence the generation of ROS by other oxidant enzymes like xanthine oxidase and mitochondrial enzymes and promotes NO synthase (NOS) uncoupling.²⁹ An increase of oxidative stress is associated with the upregulation of oxidant enzymes, which later contribute to cardiovascular complication in hypertension and diabetes mellitus.

**Endothelial Dysfunction in Hypertension**

Hypertension defined as elevated blood pressure can result when total peripheral vascular resistance is increased.³⁰,³¹ The risk factors that contribute to the increase of blood pressure include obesity, insulin resistance, high alcohol intake, high salt intake (in salt-sensitive patients), aging, and stress.³¹ There are 2 forms of hypertension: primary hypertension and secondary hypertension. Primary or essential hypertension is seen in almost 90% of patients presenting with hypertension with an unknown cause. It is classified as secondary hypertension if the increase in blood pressure is secondary to renal disease, endocrine disorders, or other identifiable causes.³² Impaired endothelial-dependent relaxation has been well documented in several cardiovascular diseases, such as hypertension and atherosclerosis.³³–³⁵ Similarly, endothelial dysfunction is also a common feature in various animal models of hypertension, including spontaneously hypertensive rats (SHRs), salt-induced hypertension, and renovascular hypertension.³⁴–³⁶

De Champlain et al.³⁷ demonstrated a greater sensitivity of the vascular tissue of SHRs to oxidative stress is attributed to the excess production of vascular ROS, such as O₂⁻. Of note, almost all experimental models of hypertension have been associated with the excessive production of oxidative stress. For example, Ang II– and diet-induced animal models and genetically modified hypertensive rat models have been reported to exhibit increased vascular oxidative stress via the activation of NADPH oxidase.³⁸,³⁹ Moreover, an upregulation of NADPH subunit (p22phox or p47phox) has been demonstrated in aortas of hypertensive rat models, whereas NADPH oxidase knockout animal attenuated the development of hypertension, suggesting that NADPH oxidase is probably one of the major enzymatic sources of O₂⁻.³⁸–⁴⁰ Hypertensive patients are also reported to have reduced antioxidant capacity with lower expression of superoxide dismutase (SOD), catalase, and glutathione peroxidase in the whole blood and peripheral mononuclear cells.⁴¹ Elevated blood pressure has also been reported in animal model in which glutathione synthesis was inhibited and extracellular SOD gene deleted.⁴²

Excess O₂⁻ leads to greater vasoconstriction by altering the cellular signal transduction system characterized by an enhanced production of inositol trisphosphate and a reduced cGMP.⁴³ It is worth noting that apart from reduced endothelium-dependent relaxation, increased endothelium-dependent contraction also significantly contributes to the development of endothelial dysfunctions in hypertension.¹¹

**Endothelial Dysfunction in Diabetes Mellitus**

Diabetes mellitus presents a growing health and socioeconomic problem especially in developing countries like Malaysia, and it is one of the most common cardiovascular disease worldwide, with estimated over 285 million people are affected with diabetes mellitus in 2010, at an annual growth of 2.2%.⁴⁴,⁴⁵ The hyperglycemia in diabetes may result from poor glucose utilization or defective insulin secretion, insulin resistance, or both.⁴⁶ There are 2 main types of diabetes mellitus: type 1 and type 2. Type 1 diabetes is caused by pancreatic β-islet cell failure with resulting insulin deficiency, encompasses 5%–10% of diabetes diagnosed, whereas type 2 diabetes, accounting for almost 90% of diabetics, is characterized by insulin resistance.⁴⁷ It is noteworthy that an increased risk of developing type 2 diabetes in women has also been reported to be associated with the incidence of gestational diabetes during pregnancy. Gestational diabetes mellitus is defined as glucose intolerance that is first recognized during pregnancy and is of greater risk for the subsequent development of type 2 diabetes mellitus, metabolic syndrome, and cardiovascular diseases.⁴⁸,⁴⁹ ROS generated by hyperglycemia are implicated in the microvascular and macrovascular complications in diabetic patients. These microvascular complications include nephropathy and retinopathy, whereas macrovascular complications include atherosclerotic cardiovascular diseases.⁵⁰

Endothelial dysfunction has been demonstrated in both type 1 and type 2 diabetes, which is associated with decreased level of EDRFs after increased destruction by ROS.⁵¹ Glucose oxidation is believed to be a main source of ROS generation in diabetes followed by glycation and protein kinase C activation.⁵²,⁵³ Similar to hypertension, excessive oxidative stress has been associated with the increased production of O₂⁻ via NADPH oxidase and accounts for the endothelial dysfunction in the early stage of the disease.⁵⁴ Impaired endothelium-dependent vasodilations had been observed in several animal models of type 1 and type 2 diabetes, including streptozotocin (STZ)-induced diabetes, db/db mice, Otsuka Long-Evans Tokushima Fatty rats, and Goto-Kakizaki rats.⁵⁵–⁶⁰

There are increasing evidences showing that increased production of ROS interferes with NO-cGMP signaling.⁵⁴,⁶¹ In both diabetic patients and animal models, elevated generation of oxidative stress has been linked to the upregulation or activation of oxidant enzymes, such as NADPH oxidase, and impaired antioxidant enzyme system, such as SOD, catalase, and glutathione peroxidase, leading to excess ROS activity.⁵⁸,⁵⁹,⁶²,⁶³ NADPH-induced O₂⁻ reacts
Boldine improves endothelial function

The degree of oxidative stress is dependent on the fine balances between the generation of ROS and the antioxidant systems. As reducing agents, antioxidants play a crucial role in protecting the cells against the excessive ROS formation by reacting with the ROS and therefore minimizing their action. Boldine has been reported to protect the red blood cells against free radical–induced hemolytic damage, even in micromolar concentrations; inhibit spontaneous autoxidation of brain membrane lipids and prevent lipid peroxidation in human liver microsomes; protect lysozyme inactivation against 2,2-azobis(2-aminopropane) dihydrochloride–derived peroxyl radical damage; and scavenge hydroxyl radicals. Additionally, boldo extracts (P. boldus) containing boldine were found to effectively inhibit lipid peroxidation in erythrocytes. Boldine has been shown in several studies to have anti-inflammatory properties via its ability in interfering with the free radical generation.

The important enzymatic antioxidants include (1) catalase, responsible to degrade H₂O₂ into H₂O; (2) glutathione peroxidase, protects membrane lipids, proteins, and nucleic acids from oxidation by reducing H₂O₂ into H₂O; and (3) SOD, converts O²⁻ into O₂ and H₂O₂. In low-density lipoprotein (LDL) receptor knockout (LDLR⁻/⁻) mice, treatment with boldine for 12 weeks decreased atherogenic lesion formation and inhibited oxidation of LDL without altering plasma cholesterol, triglycerides, and LDL and high-density lipoprotein levels. In the STZ-induced diabetic models, elevated manganese-SOD and glutathione peroxidase activities in pancreas, liver, or kidney have been demonstrated together with increased production of O²⁻. The increased antioxidant status may be because of the activation of the first line of defense system in the host tissues against excessive levels of ROS, suggesting that excessive O²⁻ production may trigger the activation of SOD, catalase, and glutathione peroxidase, which convert O₂⁻ to H₂O₂ and finally to H₂O. Treatment with boldine in drinking water (100 mg/kg, daily) for 8 weeks normalized the elevated manganese-SOD and glutathione peroxidase activity in pancreatic mitochondria, suggesting that the apomorphine alkaloid may normalize the enzyme activities through its ROS scavenging actions. In addition, treatment with boldine decreased malondialdehyde level, a reactive lipid peroxidation by-product, and carbonyls in liver, kidney, and pancreatic mitochondria in STZ-induced animals and thus implying that the beneficial effect of boldine is likely to be attributed to its antioxidant property.

Several studies in cell cultures, animal models, and human vessels have shown that oxidative stress is the single most important mechanism implicated in endothelial dysfunction. One of the major ROS strongly implicated in the pathogenesis of endothelial dysfunction is the O₂⁻. There are 2 important enzymatic sources of O₂⁻: (1) NADPH oxidase enzyme complex, which catalyzes the reduction of molecular oxygen using NADPH substrate as an electron donor, and (2) uncoupling of NO, resulting in O₂⁻ formation instead of NO because of the deficiency of the substrate, L-arginine, and cofactor tetrahydrobiopterin. Increased O₂⁻ could potentially inactivate NO released by the endothelial cells and in turn form a highly reactive molecule, ONOO⁻. In our previous study, we have shown that boldine effectively reduced vascular O₂⁻ and ONOO⁻ in SHR and diabetic aorta. Similarly, increased ROS production by treatment with high glucose in rat/mouse aortic endothelial cells for 48 hours was reduced by cotreatment with tempol. Furthermore, upregulated protein expression of nitrotyrosine, a molecular marker of ONOO⁻, was ameliorated by boldine treatment after high glucose stimulation in rat endothelial cells and in db/db diabetic aorta. The dual antioxidative and potent ROS scavenging actions of boldine may explain its therapeutic potential against oxidative stress–associated conditions.

Boldine and Vascular NADPH Oxidase

Similar to SOD, superoxide scavenger, boldine concentration dependently (0.01–1 μM) reversed the β-NADPH–induced impaired relaxation in Sprague-Dawley rat aorta. Treatment of boldine also reduced the expression of p47phox in SHR and type 1 diabetic rat aortas and prevented NOX2 and p47phox expression in rat aortic endothelial cells induced by high glucose for 48 hours. It is worth noting that polyphenols, such as flavonoids, apart from their well-known antioxidant activity, exert their protective effects by inhibiting NADPH oxidase. The polyphenols have also been demonstrated to improve endothelial function by enhancing NO signaling pathway in vascular endothelial cells in several human and rat arteries. Hence, the present finding further demonstrates that boldine not only exhibits potent antioxidant effects but also most likely acts through the inhibition of NADPH-dependent superoxide axis leading to increased bioavailability of NO and improved endothelium-dependent relaxation.

Boldine and Ang II–Induced Bone Morphogenetic Protein–dependent Endothelial Dysfunction

Ang II is a bioactive product of renin–Ang system and is a potent vasoconstrictor with pro-inflammatory, mitogenic, and profibrotic actions. There are 2 receptor subtypes for Ang II, denoted as Ang II type 1 receptor (AT₁R) and Ang II type
It is well documented that Ang II acts on the AT$_1$R, and this receptor subtype is found in both VSMCs and endothelial cells. There are also growing evidences indicating that AT$_1$R not only activates the classical receptor-coupled calcium signaling pathway but is also a potent stimulator of NADPH oxidase–dependent pathway. Ang II is one of the major regulators of vascular NADPH oxidase to produce O$_2^-$ upon stimulation of AT$_1$R, triggering the oxidative stress cascade in vascular endothelial cells. Furthermore, we and others have shown that treatment with losartan, an AT$_1$R blocker, significantly improved ACh-induced vasodilation and reduced the upregulation of NADPH oxidase and oxidative stress in hypertensive and diabetic animal models, supporting that Ang II induced NADPH-dependent ROS generation via AT$_1$R stimulation. Chronic treatment with boldine (20 mg/kg, 7 days, oral) reduced AT$_1$R expression in db/db mouse aorta and decreased ROS production, suggesting that reducing Ang II–dependent ROS generation plays a contributive role in the improvement of endothelial function in diabetic db/db mice. Pretreatment with boldine reversed the Ang II–induced impairment of endothelium-dependent relaxations by ACh in the aorta in vitro. The endothelial protective actions of boldine most likely involve inhibition of Ang II–dependent ROS production as tempol, a free radical scavenger, restored the ACh-induced relaxation of the aortic rings.

Ang II–induced overproduction of ROS in the blood vessels may be mediated by increased bone morphogenetic protein-4 (BMP4) level. BMP4 is multifunctional growth factor that belongs to the transforming growth factor-$eta$ superfamily and is found in calcified atherosclerotic plaques. BMP4 plays an important pathological role in vascular inflammation. BMP4 is upregulated after exposure to disturbed flow and oxidative condition in cultured endothelial cells triggering NADPH-dependent ROS generation and inflammatory responses. BMP4-mediated oxidative stress. Increased NADPH-induced O$_2^-$ production scavenges endothelium-derived NO, leading to reduced NO levels and increased formation of ONOO$^-$ which further diminishes the protective role of NO in the regulation of the vasculature. Repeated treatment of the SHRs with boldine (20 mg/kg, ip) for 7 days attenuated the elevated SBP and improved the endothelium-dependent relaxations to ACh in the isolated aorta from these rats (Table 1). The result provided further support that the endothelial protective effect of boldine in hypertensive rats is at least in part because of the inhibition of NADPH-mediated superoxide production. Protein expression studies denoted that the inhibitory effect of boldine on the NADPH oxidase is associated with downregulation of the membrane-bound regulatory cytosolic protein subunit, p47$^{phox}$. This novel finding of the cellular action of boldine provides further support to the antioxidant actions and therapeutic potential of the alkaloid in the regulation of vascular tone and the maintenance of vascular patency to preserve cardiovascular health.

Similarly, boldine exhibited potent antioxidant effects in both in vitro and animal models of type 1 and type 2 diabetes mellitus. As in hypertension, excessive generation of ROS interferes with NO signaling and plays a pathological role in the development of the vascular complications associated with diabetes. In hyperglycemia, glucose autooxidation increases
Boldine Improves Endothelial Function

TABLE 1. Comparison of pEC$_{50}$ and R$_{\text{max}}$ Values of ACh-induced Endothelium-dependent and SNP-induced Endothelium-independent Relaxation in Isolated Aorta of Hypertensive and Diabetes Animals Treated With Boldine

| Group                        | ACh       | SNP       |
|------------------------------|-----------|-----------|
|                              | pEC$_{50}$ (−log M) | R$_{\text{max}}$ (%) | pEC$_{50}$ (−log M) | R$_{\text{max}}$ (%) |
| Hypertensive group$^2$       |           |           |
| WKY                          | 6.52 ± 0.48 | 88.44 ± 1.85 | 8.01 ± 0.23 | 100.50 ± 4.90 |
| WKY + boldine                | 7.68 ± 0.21* | 90.38 ± 3.05 | 8.37 ± 0.07 | 109.67 ± 2.74 |
| SHR                          | 7.96 ± 0.12* | 40.60 ± 5.64* | 7.61 ± 0.55 | 102.00 ± 1.97 |
| SHR + boldine                | 8.28 ± 0.13† | 94.67 ± 5.28† | 8.47 ± 0.20 | 106.80 ± 4.00 |
| Type 1 diabetes mellitus$^3$ |           |           |
| SD                           | 6.53 ± 0.48 | 89.04 ± 1.23 | 8.38 ± 0.09 | 106.80 ± 1.55 |
| SD + boldine                 | 6.68 ± 0.08 | 90.60 ± 2.69 | 8.05 ± 0.25 | 106.50 ± 1.85 |
| Diabetes                     | 6.80 ± 0.17 | 63.75 ± 3.05‡ | 8.58 ± 0.16 | 103.30 ± 2.29 |
| Diabetes + boldine           | 6.70 ± 0.15 | 88.71 ± 2.08§ | 8.43 ± 0.21 | 102.00 ± 3.22 |
| Type 2 diabetes mellitus$^4$ |           |           |
| db/m$^+$                     | 6.87 ± 0.07 | 82.42 ± 2.02 | 7.34 ± 0.11 | 95.54 ± 1.92 |
| db/db                         | ND          | 26.33 ± 6.97# | 7.73 ± 0.08 | 96.40 ± 0.97 |
| db/db + boldine              | 6.83 ± 0.11 | 69.33 ± 4.12** | 7.54 ± 0.09 | 104.30 ± 4.12 |

Data are the mean ± SEM of 6 separate experiments.

*P < 0.05 compared with WKY.
†P < 0.05 compared with SHR.
‡P < 0.05 compared with SD.
§P < 0.05 compared with diabetes.
#P < 0.05 compared with db/m$^+$.  
**P < 0.05 compared with db/db.

ND, could not be derived.

production of free radicals leading to overproduction of ROS and subsequent inactivation of NO. In both STZ-induced diabetic rat (type 1 diabetes) and db/db mice (type 2 diabetes), endothelial dysfunctions were markedly observed by the impaired endothelium-dependent relaxations to ACH in isolated rat or mouse aorta. Acute (30 minutes pretreatment) and repeated treatment (7 days) of boldine significantly reversed the impaired endothelial function in aorta of both diabetic animals (Table 1). As in hypertensive vessels, the endothelial protective actions of boldine are strongly attributed to decreasing ROS formation and enhancing NO bioavailability via increased activity of eNOS in the endothelial cells lining the aortic wall of the diabetic animals. Incubation of rat and mouse aortic endothelial cells grown in high-glucose medium with boldine decreased the generation of O$_2^{-}$ in these cells, thus protecting NO against degradation. In type 1 diabetic rats, treatment with boldine normalized the overproduction of ROS and this correlated with the downregulation of the NADPH oxidase subunits NOX2 and p47$^{phox}$. Repeated treatment with boldine in the db/db mouse decreased protein expression of AT$_1$R and BMP4 in the vascular wall. This finding suggests that in the type 2 diabetic mice, boldine may additionally have reduced the generation of O$_2^{-}$ by inhibiting the AT$_1$R-BMP4-ROS axis and increasing eNOS phosphorylation.

On the other hand, treatment with boldine (20 mg/kg, ip) slightly decreased blood glucose levels in the type 1 diabetic rat but did not have a significant effect in the type 2 diabetic mice. Administration of STZ to rats damages pancreatic beta cells and resulted in diabetes, a mechanism involving increased formation of hydroxyl radicals and other ROS before generation and/or subsequent decay of highly reactive STZ carbonium radicals.$^{10}$ Jang et al$^3$ demonstrated that boldine treatment (100 mg·kg$^{-1}$·d$^{-1}$) in their drinking water for 8 weeks attenuated the development of hyperglycemia and weight loss induced by STZ. Another study also similarly showed that boldine treatment (50 mg·kg$^{-1}$·d$^{-1}$) for 10 weeks reduced the hyperglycemia of the type 1 diabetic animals.$^{10}$ The glucose-lowering effect by boldine may be strongly correlated with its cytoprotective action against oxidative damage in pancreatic β cells induced by cytotoxic effects of STZ.

Figure 2 and Table 2 summarize the vasculoprotective effect of boldine in animal models of hypertension and diabetes and the protective effects of boldine in animal models in vivo, respectively. Taken together, it seems that boldine may exert positive effects on the endothelium via several mechanisms and mainly by protecting NO from degradation via inhibiting the excessive production and scavenging of O$_2^{-}$ and additionally by increasing NO bioavailability by promoting eNOS phosphorylation. In addition, boldine attenuates development of STZ-induced diabetes in rats by inhibition of O$_2^{-}$ production and attenuation of peroxidation-induced product formation. The present results further support the potential use of boldine as a naturally effective antioxidant with protective actions against endothelial dysfunctions associated with oxidative stress as in chronic diseases, such as hypertension and diabetes mellitus.

PHARMACOKINETIC AND TOXICOLOGICAL STUDIES OF BOLDINE

The first reported studies on the biological disposition of boldine were performed by Jimenez and Speirs$^{105}$ in vitro using isolated rat hepatocytes and in vivo using Wistar rats.
respectively. The results showed that the extracellular and intracellular concentrations of boldine declined time dependently to respective concentrations of 20 and 344 µM at 60 minutes in isolated rat hepatocyte suspension from an initial concentration of 200 µM boldine. This declining concentration of boldine was attributed to the biotransformation process and a lowering of the net flow of boldine molecules into the cells. However, it is worth noting that the intracellular concentration of boldine after 60 minutes was still sufficient to warrant its antioxidant and hepatoprotective activities as it compares with the effective boldine concentration (10–30 µM) needed to protect rat liver microsomal membranes from lipid peroxidation. Such antioxidative and cytoprotective effects of boldine were predicted to be sustainable after 150 minutes and with the expected intracellular concentration of boldine of approximately 50 µM.

In the in vivo studies, boldine concentration declined rapidly in plasma after a single oral (50 or 75 mg/kg) or an intravenous

![FIGURE 2. Hyperglycemia and hypertension are common risk factors involved in pathophysiology of endothelial dysfunction that is due, at least in part, to an increased NADPH-dependent ROS production. Schematic diagram showing boldine exerts its vascular protection through inhibition of NADPH-dependent ROS production, Ang II–mediated BMP4-induced ROS, and decreased ONOO– level.](image)

| TABLE 2. Summary of Protective Effects, Therapeutic Doses, and Route of Administration of Boldine in Animal Models In Vivo |
|---|
| Animal Model | Dose and Duration | Route of Administration | Tissues | Effect | Reference |
| STZ-induced diabetic rats | 100 mg/kg daily for 8 wk | Drinking water | Liver, pancreas, kidney | Attenuation of hyperglycemia and weight loss, Decreased malondialdehyde and carbonyl level, Normalized manganese-SOD and glutathione peroxidase enzyme activities | Jang et al |
| STZ-induced diabetic rats | 50 mg/kg daily for 10 wk | Oral administration | Kidney | Attenuation of hyperglycemia and high blood pressure, Prevention of kidney damage and protection of renal parenchyma | Hernandez-Salinas et al |
| SHR | 20 mg/kg daily for 1 wk | ip Injection | Thoracic aorta | Improved endothelial function, Reduced oxidative stress, Downregulation of P47phox subunit expression | Lau et al |
| STZ-induced diabetic rats | 20 mg/kg daily for 1 wk | ip Injection | Thoracic aorta | Improved endothelial function | Lau et al |
| Diabetic db/db mice | 20 mg/kg daily for 1 wk | Oral administration | Thoracic aorta | Improved endothelial function, Reduced oxidative stress, Downregulation of BMP4, nitrotyrosine, and AT1R expression | Lau et al |
| LDL receptor knockout mice | 1 or 5 mg per mouse per day, 5 times a week for 12 wk | Oral administration | Aortic trunk | Decreased in atherosclerotic lesion formation in mouse aorta, Inhibited oxidation of LDL | Santanam et al |
(10 or 20 mg/kg) administration in rats. After its oral administration, absorption of boldine into the plasma was rapid with a first-order type of kinetics and the maximal plasma concentration was detected between first 15 and 30 minutes. Once absorbed, boldine concentration in the liver increases 3- to 4-fold higher than those detected in brain and almost 10-fold higher than those found in heart. Again, Jimenez and Speissey have also noted that the estimated maximal concentration of boldine that had reached the liver was 72–88 μM, which was 4- to 5-fold greater than the concentrations needed to exert its hepatoprotective activity in vitro.

Likewise, the boldine concentration found in the heart was approximately 7.2–8.8 μM, which was 7- to 8-fold higher than those used to protect endothelial function in vitro. The aorta is the most important blood vessel that carries the oxygen-rich blood from the heart to the entire body. These pharmacokinetic studies demonstrated that repeated oral or ip administration (20 mg/kg) of boldine for 7 days was sufficient to protect against endothelial dysfunction in SHR and diabetic aortas as concur with the effective endothelial protective concentrations (ie, 1 μM) in the isolated aortas.

Boldine is a relatively safe drug judging from the many animal studies and its widespread use as over-the-counter drug supplement. A relatively high boldine concentration is needed to induce lethality in laboratory animal tests. Kreitmair demonstrated that an oral administration of 500–1000 mg/kg of boldine was required to cause death in mice and guinea pigs, respectively. Another study showed that a lower dose of intravenous boldine administration (250 and 50 mg/kg) was needed to induce death in mice and guinea pigs, respectively. Similarly, the LD₅₀ of boldine by ip injection in mice was reported as 250 mg/kg, similar to that lethal dose of boldine administered intravenously.

LIMITATION OF THE STUDY AND FUTURE WORKS

Despite mixed outcomes from clinical trials of vitamins C and E, the good safety profile of boldine and its potent antioxidant action should provide new impetus to conduct preliminary investigations of the effectiveness of the alkaloid either for treatment or as prophylaxis in hypertensive and diabetic patients. Combining boldine with current treatments for hypertension and diabetes mellitus would be another area of study given that drug combinations have been shown to be successful for the treatment of hypertension. Nevertheless, randomized trial results from human population studied by Heart Protection Study Collaborative Group showed that the combined antioxidant regimen did not produce any significant effect on 5-year mortality from vascular and non-vascular diseases. Similarly, meta-analysis by several clinical trials demonstrated supplementation of beta-carotene and vitamin E did not demonstrate any benefit. Therefore, longer treatment durations of boldine should be studied to investigate its long-term effect. Finally, other studies with boldine should also examine other mechanisms, including potential anti-inflammatory and the central actions of the aporphine alkaloid.

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