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Hepatoprotective effect of boldine in a bile duct ligated rat model of cholestasis/cirrhosis

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Thiobarbituric acid (PubChem CID: 2723628)
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ABSTRACT

Liver fibrosis is a debilitating disease associated with chronic liver injury. Oxidative stress is known as a pivotal mechanism in the initiation and propagation of liver fibrosis. Boldine is a potent antioxidant molecule with several pharmacological effects. The current investigation aimed to evaluate the effect of boldine in bile duct ligated (BDL) rats as a model of cholestasis and cirrhosis. BDL animals received boldine (5, 10, and 20 mg/kg/day, oral) for 14 days (Cholestatic rats) and 28 days (Cirrhotic rats). The serum biomarkers of liver injury were drastically increased in the BDL group. Moreover, the level of oxidative stress markers was significantly increased in BDL animals. Severe bridging fibrosis, tissue necrosis, and inflammation were also detected in BDL rats. It was found that boldine (5, 10, and 20 mg/kg/day, oral) restored the BDL-induced depletion of glutathione content and tissue antioxidant capacity. Moreover, histopathological changes and collagen deposition were markedly attenuated by the boldine treatment. The beneficial effects of boldine administration in cholestasis/cirrhosis might be associated with anti-fibrotic properties via antioxidant activities.

1. Introduction

Boldine (1, 10-dimethoxy-2, 9-dihydroxyaporphine; Fig. 1) is the major alkaloid in the bark and leaves of the boldo tree (*Peumus boldus*) [1,2]. This alkaloid is believed to be responsible for the majority of the health-promoting properties of the boldo extract [1,2]. A wide range of pharmacological properties is attributed to boldine [1,2]. Boldine has been shown to have cytoprotective, antitumor, anti-inflammatory, immunomodulatory, hepatoprotective, and antipyretic properties [1,3–6]. Moreover, this alkaloid is known as a potent antioxidant and radical scavenging molecule [1,7].

Several chronic diseases, such as viral hepatitis and alcoholism, might finally lead to liver fibrosis and hepatic failure [8]. Currently, the only effective available therapeutic option for liver fibrosis, cirrhosis, and liver failure is liver transplantation. However, many factors limit the impact of liver transplantation and mention the importance of effective hepatoprotective and antifibrotic therapies [9–11].

The mechanisms involved in the development of liver fibrosis have been established [12]. It has been found that free radicals and oxidative stress are key players in the initiation and propagation of liver injury and fibrosis [13,14]. Hence, the administration of potent antioxidant molecules such as boldine could be a potential therapeutic approach in preventing liver injury. The antioxidant and radical scavenging properties of other aporphine structures also have been reported [1]. On the other hand, structure–activity relationship studies have provided data which indicate boldine as a potent antioxidant and radical scavenging molecule among aporphine-based alkaloids [1].

Bile duct ligation (BDL) is a very reproducible animal model of chronic liver injury, cholestasis, and cirrhosis [15,16]. This model induces a high yield of cirrhosis in animals obstructed for one month or longer [15]. The morphological changes in the liver of BDL animals are similar to human cholestasis/cirrhosis [15,16].

The antifibrotic and hepatoprotective effect of boldine in chronic liver injury has not been investigated so far. Hence, this study aimed to examine the potential protective effects of boldine in a rat model of cholestasis/cirrhosis induced by BDL. Changes in serum biomarkers of liver injury, parameters of oxidative stress in liver tissue, liver hydroxyproline content, and liver fibrotic changes were monitored to evaluate whether boldine has any hepatoprotective properties in BDL rats as an animal model of chronic liver injury.

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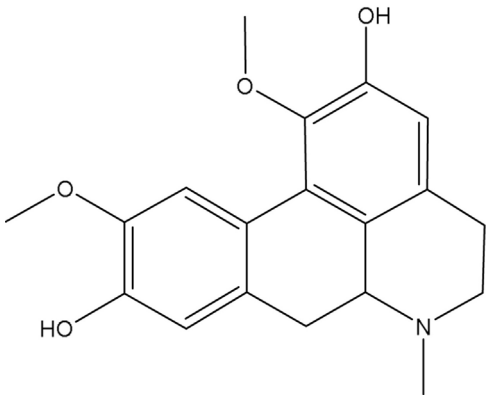

Fig. 1. Boldine chemical structure.

2. Material and methods

2.1. Chemicals

N-chloro tosylamide (Chloramine-T), Trichloroacetic acid (TCA), Sodium acetate, Citric acid, *n*-Propanol, *p*-Dimethyl amino benzaldehyde, 5,5′-Dithionitrobenzoic acid (DTNB), Dithiothreitol (DTT), Sucrose, 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), Thiobarbituric acid (TBA), Sodium citrate, Ethylenediamine tetra-acetic acid (EDTA), Phosphoric acid, 2-amino-2-hydroxymethyl-propane-1,3-diol-Hydrochloride (Tris-HCl), were obtained from Merck (Darmstadt, Germany). Boldine was purchased from Sigma-Aldrich (St. Louis, MO, USA). Kits for evaluating biomarkers of liver injury, including ALT, LDH, AST, ALP, γ-glutamyl transpeptidase (γ-GT), and bilirubin, were obtained from Pars Azmun® (Tehran, Iran). All salts used for preparing buffer solutions were of analytical grade and obtained from Merck (Darmstadt, Germany).

2.2. Animals

Male Sprague-Dawley rats (n = 60; 200–250 g weight) were obtained from the Center of Comparative and Experimental Medicine, Shiraz University of Medical Sciences, Shiraz, Iran. Animals were housed in plastic cages over hardwood bedding. There was an environmental temperature of 23 ± 1 °C and a 12L: 12D photoschedule along with a 40% of relative humidity. The rats were allowed free access to a normal standard chow diet and tap water. Animals received humane care and all the experiments were performed in conformity with the guidelines for care and use of experimental animals approved by an ethic committee in Shiraz University of Medical Sciences, Shiraz, Iran (#94-01-36-10649).

2.3. Surgery

Animals were anesthetized (10 mg/kg of xylazine and 70 mg/kg of ketamine, i.p), a midline incision was made and the common bile duct was localized, doubly ligated, and cut between these two ligatures (Day = 0) [17]. The sham operation consisted of laparotomy and bile duct identification and manipulation without ligation.

2.4. Experimental setup

Animals (n = 60) were equally allotted into five groups containing 12 rats in each. Rats were treated as follows: 1) Sham-operated (Vehicle-treated); 2) BDL; 3) BDL + Boldine (5 mg/kg/day, oral, started from day 1 after BDL operation); 4) BDL + Boldine (10 mg/kg/day, oral, started from day 1 after BDL operation); 5) BDL + Boldine (20 mg/kg/day, oral, started from day 1 after BDL operation). Six animals in each group were evaluated 14 days after BDL operation (Cholestasis model), and six animals were assessed 28 days after BDL operation (Cirrhosis model) [17,18]. Boldine doses were selected based on the previous investigations [19–21].

2.5. Serum biochemistry

A Mindray BS-200® auto analyzer and standard kits were used to measure serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (γ-GT), and bilirubin [22].

2.6. Liver histopathology

For histopathological assessments, samples of liver were fixed in buffered formalin solution (0.4% sodium phosphate monobasic, NaH_2PO_4, 0.64% sodium phosphate dibasic, Na_2HPO_4, and 10% formaldehyde in distilled water). Paraffin-embedded sections of tissue (5 μm) were prepared and stained with hematoxylin and eosin (H & E) before light microscope viewing. Liver fibrotic changes was determined by Masson's trichrome staining in BDL rats. The Ishak system which uses a six-point scale for fibrosis stage (0–6) was applied for scoring liver fibrosis in the current investigation [23,24]. Samples were analyzed by a pathologist in a blind fashion.

2.7. Reactive oxygen species (ROS) formation

Reactive oxygen species formation in liver was estimated by a previously described method [25,26]. Briefly, liver tissue (200 mg) was homogenized in 5 ml of ice-cooled Tris-HCl buffer (40 mM, pH = 7.4). Samples of the resulted tissue homogenate (100 μl) were mixed with Tris-HCl buffer (1 ml) and 2′, 7′-dichlorofluorescein diacetate (Final concentration 10 μM). The mixture was incubated at 37 °C (30 min, in dark). Finally, the fluorescence intensity of the samples was assessed using a FLUOstar Omega® multifunctional microplate reader with $\lambda_{excitation}$ = 485 nm and $\lambda_{emission}$ = 525 nm [25,27].

2.8. Lipid peroxidation

The thiobarbituric acid reactive substances (TBARS) were measured as an index of lipid peroxidation in liver tissue [28]. The reaction mixture was consisted of 500 μl of tissue homogenate (10% w/v in KCl, 1.15% w/v), 1 ml of thiobarbituric acid (0.375%, w/v), and 3 ml of phosphoric acid (1% w/v, pH = 2). Samples were mixed well and heated in boiling water (100 °C) for 45 min. After the incubation period, the mixture was cooled, and then 2 ml of *n*-butanol was added. Samples were vigorously vortexed and centrifuged (10,000*g* for 10 min) [26]. Finally, the absorbance of developed color in *n*-butanol phase was measured at 532 nm using an Ultrospec 2000®UV spectrophotometer (Pharmacia Biotech, Uppsala, Sweden) [28].

2.9. Hepatic glutathione content

Liver samples (200 mg) were homogenized in 8 ml of ice-cooled EDTA solution (40 mM). Then, 5 ml of the prepared homogenate were added to 4 ml of distilled water (4 °C) and 1 ml of trichloroacetic acid (50%; w/v). The mixture was vortexed and centrifuged (10,000*g*, 4 °C, 15 min). Then, 2 ml of the supernatant was mixed with 4 ml of Tris-HCl buffer (40 mM, pH = 8.9), and 100 μl of DTNB (0.01 M in methanol) [26]. The absorbance of the developed color was measured at 412 nm using an Ultrospec 2000®UV spectrophotometer (Pharmacia Biotech, Uppsala, Sweden) [28].

2.10. Protein carbonylation in liver tissue

Total protein carbonyl content of liver tissue was measured by a spectrophotometric assay after derivatizing the protein carbonyl