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Original Article

Study of the Protective Effect of Livergol against Liver Toxicity Caused by Bromobenzene in Mice

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Abstract

Liver is a major organ of the body which can be exposed to various chemicals, drugs, and many other xenobiotics such as bromobenzene. Bromobenzene must be converted to its active metabolites to produce liver and kidney toxicity. Livergol is an herbal product which contains silymarin. The objective of this study was to find out the protective effect of livergol against liver toxicity induced by bromobenzene in mice. In this study, doses: 50, 100, 200, 300 mg/kg of livergol were administrated to mice orally 2 hours after bromobenzene(460 mg/kg) administration for 7 days (test groups). The negative control group received normal saline. The positive control group received 460 mg/kg of bromobenzene orally. 24 hours after the last administration animals were sacrificed; their blood was collected to determine serum enzyme activities of aspartate aminotransferase (AST) , alanine aminotransferase (ALT) and alkaline phosphatase (ALP). The livers were removed for histological examination.The results showed that livergol at doses 200 and 300 mg/kg cause significant reduction in the level of enzymes ($p > 0.05$). The histopathological study of liver tissues showed that doses of 200 and 300 mg/kg are more effectively restore tissue damage to the normal state. Our finding indicated that livergol in the high doses (200 and 300 mg/kg) have protective effects and cause significant improvement in the liver tissue and biochemical markers in bromobenzene intoxicated mice.

Keywords: bromobenzene, hepatoprotective, hepatotoxicity, liver, livergol, mice.

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1. Introduction

The use of natural products with therapeutic features is as age-old as human culture and, for a long time, inorganic, plant and animal products were the major sources of drugs[1]. Plants have formed the basis of traditional medicine systems that have been in

existence for thousands of years and continue to provide humankind with new treatment [2].*Silybum marianum* L. (Milk thistle), a member of *Carduus marianum* family, is an ancient medicinal plant which has been used for centuries for treatment of different diseases such as liver and gallbladder disorders, protecting liver against snake bite and insect stings, mushroom poisoning, and alcohol abuse[3].In the 1st century A.D., Dioskurides used this plant as emetic as well as a general medicinal herb. It became a favored medicine for hepatobiliary diseases in 16th century and the drug was revived again in 1960 in central Europe [4].

This plant can be found in Kashmir, North America, Canada, and Mexico with large leaves and a reddish-purple flower that are all thorny and the medicinal part of the plant is either the seeds or fruits[5].*Silybum marianum* extract has been called silymarin and consist of silybin, silychristin, silydianin and isosilybin[6]. Currently standardized extracts from the *Silybum marianum* marketing are silymarin and silybinin capsules and tablets with an improved bioavailability under the trade names like Livergol, Silipide and Legalon[7].

Bromobenzene (BB) is a colorless liquid with a characteristic aromatic odour. Exposure with (BB) may occur during its production as well as its use as a solvent in the chemical industry and as a chemical intermediates in organic synthesis [8-10]. BB is subjected to biotransformation in the liver. The metabolites of BB are highly hepatotoxic and nephrotoxic [11].

The present study was conducted to investigate the hepatoprotective effects of Livergol on bromobenzene -induced hepatotoxicity in mice.

2. Materials and Methods

2.1. Animals

Male Swiss albino mice (6–8 weeks old, 25–30 g), were used in this experiment, obtained from Animal house of Ahvaz Jundishapur University of Medical Science, Iran. The mice were maintained in 12/12 h light/dark cycles and with free access to regular chow food and drinking water ad libitum. The animals were acclimated to the environment for a minimum of one week prior to inclusion in experiment. All animal care and use procedures were in accordance with the guidelines of the Institutional Animal Care and Use Committee at AJUMS University.

2.2. Chemicals

All chemicals used in this study were of analytical grade and obtained through commercial sources. bromobenzene were purchased from Roche chemical company (Germany).The Livergol powder was purchased from Goldaru Company (Iran).

2.3. Study Design

The animals were divided into six groups, each group consist of 8 mice. Group 1 as negative control group, received normal saline for 7 days; group 2 received BB (460 mg/kg orally) as positive control for 7 days; groups 3-6 received Livergol orally in doses of 50, 100, 200, and 300 mg/kg respectively, 2 hour after bromobenzene (460 mg/kg orally) administration during 7 days.

Then on the day 8^{th}, 24 hours after the last administration, animals were sacrificed. Blood was withdrawn by cardiac puncture to determine aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). Liver was removed for histological examination. All experiments were performed in compliance with the relevant laws and institutional guidelines, also the institutional committee have approved the experiments.

2.4. Biochemical Assays

The blood samples were allowed to clot for 40 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 25°C for 10 min. for evaluating hepatic function, activities of aspartate aminotransferase (AST) , alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were assayed according to methods of Reitman and Frankel, King[12, 13].

2.5. Histopathological Assessments

For the histological examination, the livers were fixed in 10% formalin for at least 24 h. then liver tissues were dehydrated with a sequence of ethanol solutions, embedded in paraffin, cut into 5 µm sections and stained with hematoxylin &eosin dye (H&E stain).

2.6. Statistical Analysis

Statistical analysis was performed using the statistical package SPSS 16.0 for Windows. All results were expressed as the mean ± standard deviation (SD).The data were obtained by one-way ANOVA followed by Tukey, s post-test. The level of significance was set at $p < 0.05$.

3. Results and Discussion

The hepatoprotective effects of Livergol on BB-induced hepatotoxicity in mice are shown in Table 1.hepatotoxicity and liver damages is usually associated with marked increase in serum ALT, AST and ALP levels. Data showed that bromobenzene administration in mice developed sever hepatotoxicity, that reflected by a significant increase in the levels of mentioned parameters in positive control group ($p < 0.05$). Groups 3-6 that treated with Livergol for 7 consecutive days showed decrease in the level of enzymes activities in all doses in comparison with positive control group. Also Low doses of Livergol (50 and 100 mg/kg) partially prevented the elevation of ALT, AST and ALP serum levels, whereas the most significant reduction in the elevated

Table 1. Effects of post-treatment with Livergol on the serum activities of AST, ALT and ALP in bromobenzene -induced hepatotoxicity.

Groups	SGPT (U/l)	SGOT (U/l)	ALP (U/l)
1- normal saline	103.125± 7.524 [b]	308.375 ± 1.742 [b]	205.625 ± 5.762 [b]
2- bromobenzene	178.375 ± 2.104 [a]	550.250± 11.634 [a]	359.500± 10.946 [a]
3- bromobenze+livergol (50 mg/kg)	135.375 ± 6.580 [a]	350.625± 3.812 [a]	270.750± 7.859 [a]
4- bromobenze+livergol (100 mg/kg)	118.625 ± 4.954 [b]	318.750± 7.971 b	219.250± 1.925 [b]
5- bromobenze+livergol (200 mg/kg)	110.625 ± 1.889 [b]	310.250 ± 4.859 [b]	210.750± 2.534 [b]
6- bromobenze+livergol (300 mg/kg)	102.750 ± 1.578 [b]	307.750± 7.186 [b]	204.875± 1.777 [b]

Mice in group 1 received normal saline solution, while group 2 received bromobenzene (500 mg/Kg) for 7 days. The mice in groups 3, 4, 5 and 6 were pre-treated with livergol (50,100, 200 and 300 mg/Kg, p.o, respectively) once daily for seven consecutive days. One hour after the final treatment, the mice were treated with bromobenzene(500 mg/Kg, p.o). Hepatotoxicity was determined 24 h later by quantifying the serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as well as .alkaline phosphatase (ALP). Each value represents the mean ± SD for eight mice

.a: Significantly different from the control group at $p < 0.05$

.b: Significantly different from bromobenzene group at $p < 0.05$

serum activities was seen in doses of 200 and 300 mg/kg. Additionally, the hepatoprotective effect of the Livergol was confirmed by the histological examination of the liver.

The histopathological study of liver in the negative control group showed normal hepatic architecture. The hepatocytes are within normal limits and separated by narrow blood sinusoids. The structure of liver lobules was preserved and no cell necrosis, fatty change, and inflammation were seen (Figure 1 A). In the BB-intoxicated group Severe congestion, tissue necrosis in the lobules, excessive accumulation of lymphocytes in the portal, and fatty change in hepatocytes were seen (Figure 1 B). Treatment with 50 mg/kg of Livergol showed mild improvement and Congestion, diffuse necrosis, accumulation of lymphocytes in the portal as well as fatty changes observed in hepatocytes (Figure 1C). Administration of

100 mg/kg *of* Livergol showed mild to moderate Congestion, moderate lymphocytic aggregation in portal, sporadic and brief fatty change in hepatocytes, whereas tissue necrosis was not seen(Figure 1D). In the group treated with the 200 mg/kg of Livergol, Slight congestion, mild lymphocytic accumulation, fatty changes were seen very briefly (Figure 1E). Administration of 300 mg/kg of Livergol resulted in light congestion. Moreover, necrosis and lymphocyte accumulation and fatty changes were not seen (Figure 1F).

Liver is one of the largest organs in human body and the major site for metabolism and excretion. It has a wide range of functions, including detoxification, protein synthesis, and production of biochemical's necessary for digestion[14, 15]. The liver is a major target organ for various chemicals, drugs, and many other toxic compounds[16].

The classical hepatotoxin BB has been shown previously to be toxic to liver. BB undergo cytochrome P450-mediated phase I metabolism to reactive epoxide intermediates, bromobenzene-3,4-oxide, which can bind with GSH, thereby depleting GSH pool and impairs protection against reactive oxygen species (ROS)[17]. This may lead to a number of secondary events like arylation of critical cellular macromolecules, lipid peroxidation, and altered intracellular calcium levels that damage the cell[18, 19].

Since hepatic damage induced by Bromobenzene is mediated by its free radical metabolites , antioxidant activity or inhibition of the generation of free radicals is important in the protection against Bromobenzene -induced liver injury[20]. Previous studies in cell culture and animal models clearly showed hepatoprotective property of *Silybum marianum* extract against carbon tetrachloride, paracetamol and Amanita phalloide toxin[21]. Furthermore, another studies has been reported that *Silybum marianum* possess profound antioxidant activity, and also is capable of scavenging both free radicals and reactive oxygen species[22].These properties motivate us to study its hepatoprotective effects in BB -induced liver toxicity.

Aminotransferases (GOT and GPT) are the first enzymes to be used in diagnostic enzymology when liver damage has occurred. Because, these are normally located in the cytosol, and toxicity affects the liver with subsequent breakdown in membrane architecture of the cells leading to their spillage into plasma, and their concentration rises in the latter [23, 24].

Our results showed significant increases in the liver parameter in BB -intoxicated mice, and Livergol administration during this priod, was very efficient at reversing the BB-induced liver damage. These protective effects were dose-dependent, and best results were observed in doses of 200 and 300 mg/kg (Table 1). Moreover, the histopathological observation of liver confirmed the protective effects of Livergol against the BB -induced liver damage as it was evident by the reversal of centrilobular necrosis, fatty changes (steatosis) and scattered lymphocytes infiltrate in hepatic parenchyma by Livergol

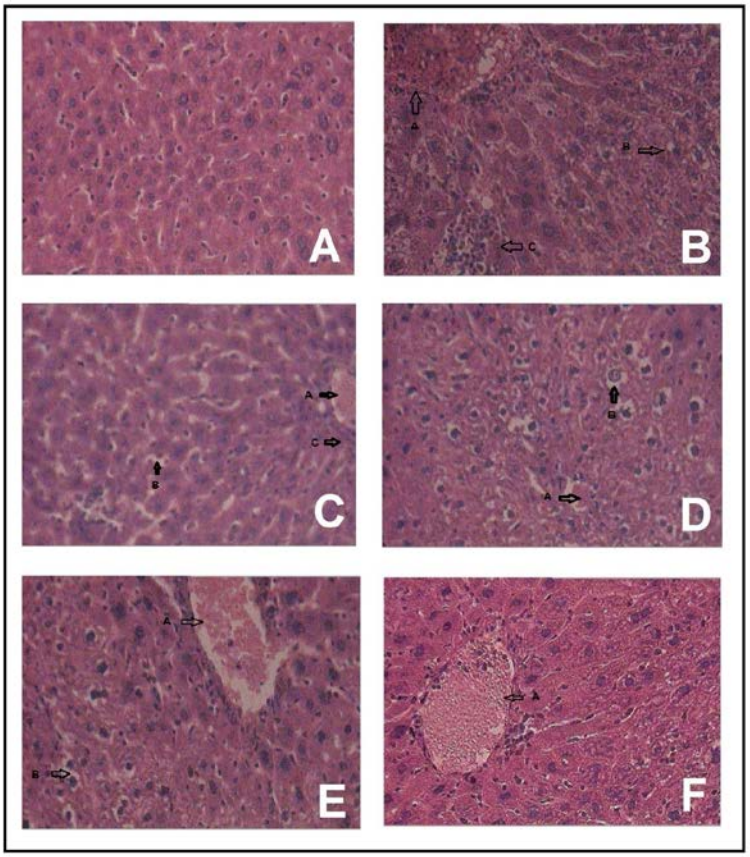

Figure 1. Histopathological observations (liver sections stained with Hematoxylin and Eosin, magnification x 400) showing the effects of Livergol on bromobenzene-induced histopathological changes in mouse liver. (A) control group, shows normal hepatic architecture with distinct hepatic cells, sinusoidal spaces and a central vein; (B) bromobenzene-treated group shows severe centrilobular hepatic necrosis, fatty changes, ballooning degeneration, and infiltrating lymphocytes; (C), (D), (E) and (F) are bromobenzene groups pre-treated with 50, 100,200 and 300 mg/Kg of Livergol, respectively. Picture D shows milder degree of hepatocyte necrosis, fatty changes, ballooning degeneration, and infiltrating lymphocytes. In pictures E and F, only mild inflammation and lymphocyte infiltration are observed.

administration. Thus, as shown in Figures 1E and 1F, only mild inflammation and lymphocyte infiltration were observed.

The mechanism which Livergol exert its protective effects is thought by: (1) preventing entry of various toxins, e.g., alcohol, carbon tetracholoride and heavy metals, into hepatocytes; (2) stimulating protein synthesis with hepatocyte regeneration; (3) acting as a free-radical scavenger and antioxidant; and (4) modulating the immune response[25, 26].

Many other studies investigated natural compound in BB intoxication. In similar study Hamed *et al* investigated the Effects of black seed oil on resolution of hepato-renal toxicity induced by BB in rats. Their evaluation was done through measuring liver oxidative stress markers such as reduced glutathione(GSH),

superoxide dismutase(SOD) and malondialdehyde (MDA), succinate dehydrogenase (SDH), lactate dehydrogenases (LDH) and glucose-6- phosphatase , Serum aspartate and alanine aminotransferases (AST, ALT) and alkaline phosphatase. Their findings, demonstrated treatment with black seed oil alleviated the elevation of GSH, SDH, LDH, G-6- Pase, and attenuated MDA, SOD, AST, ALT and ALP. Furthermore, Diminution of collagen content and improvement in liver and kidney architectures were observed[27].

In another study performed by Madani et al, the protective effects of polyphenolic extracts of *Silybum marianum* on thioacethamide induced hepatotoxicity in rat was investigated.

The extracts were injected to the rats, at a dose of 25 mg kgG1 body weight together with thioacetamide at a dose of 50 mg kgG1 body weight. To assess the affectivity of extracts, against thioacetamide, the activity of aminotransferases (SGOT and SGPT), alkalin phosphatase, bilirubin, Na+ and K+ were measured. Significant decrease in the activity of aminotransferases, alkalin phosphatase, and bilirubin were observed in the groups treated with extracts and thioacetamide compared with the group that was treated only with thioacetamide [28].

Our finding showed that Livergol reduces BB hepatotoxicity in the dose dependent manner, and significant protection can only be expected from a high dose of this drug.

4. Conclusion

Our findings indicated that under the present experimental conditions, Livergol showed hepatoprotective effects against BB induced liver damage in mice. The hepatoprotective action may be mediated through antioxidants activity of Livergol. Furthermore, silymarin has been accepted as a safe herbal product, since using the physiological doses of silymarin is not toxic unless the improper administration of therapeutic dosages[29, 30].

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