Original Article

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Protective Effect of Bene (Pistacia Atlantica) on Busulfan-Induced Renal-Liver Injury in Laboratory Mice

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ABSTRACT

BACKGROUND AND OBJECTIVE: Busulfan is a chemotherapy drug that has side effects such as hepatic and renal injury. Pistacia atlantica from the family Anacardiaceae has a potent antioxidant agent due to phenolic compounds. The purpose of this study was to investigate the effects of Pistacia atlantica on histopathology and liver and kidney function indicators in busulfan-induced liver and kidney injuries in adult mice.

METHODS: A total of 28 BALB/c mice were randomly divided into four groups (control, busulfan, bene and bene+busulfan). The busulfan group received 10 mg/kg busulfan as a single dose and intraperitoneally. The bene group received plate containing 10% of bene for 35 days. The bene+busulfan group received 10 mg/kg busulfan+10% bene. Then, liver enzymes alanine amino transferase, aspartate amino transferase, urea, creatinine and histopathology of liver and kidney investigated.

FINDINGS: The mean urea (P= 0.009) and creatinine level (P= 0.02) in the busulfan group was 55.2±4.23 and 0.8±0.11 mg/dl that it was significantly higher than the bene + busulfan. A significant decrease in ALT level in the bene + busulfan group compared to the busulfan treated group (P= 0.03). In the bene treated group steatosis, necrosis and fibrosis in the liver parenchyma and glomerular sclerosis, inflammation and tubular atrophy in kidney tissue, was not observed.

CONCLUSION: The results of present study indicated that administration of 10% bene for 35 days improved histopathology of kidney and liver as well as functional index of liver and kidney after renal-liver injury.

KEY WORDS: *Pistacia atlantica, wild pistacia, mouse, liver, kidney, busulfan.*

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Introduction

The liver and kidney play an important role in metabolism and excretion of drugs and harmful substances as well as in maintaining homeostasis. Therefore, liver and kidney injuries can be very damaging. Kidney injuries occur for a variety of reasons, such as chemotherapy, shock, infection, surgery, or antibiotic use (1-3). Liver damage is also caused by the use of some drugs or toxic chemicals, etc. Oxidative stress has been shown to play an important role in causing liver damage, so antioxidants may be effective in healing injuries (3-5). Pistacia atlantica from the family Anacardiaceae is one of the wild pistachio species that has strong antioxidant properties due to phenolic compounds (phenolic acids, flavonoids and tannins) (6-9).

Pistacia is found on the Mediterranean coast, Turkey, Iran, Central and West Asian countries and Africa. This plant grows massively in Iran between Kurdistan province and Fars province. Pistacia atlantica is used in traditional medicine to treat eczema, throat infection, kidney stones, asthma, as well as in the treatment of hypertension, cough, gastric pain and jaundice (6-9). In addition, it has antibacterial, antifungal, and anti-parasitic properties (10-14). Toul et al reported that Pistacia atlantica has high tocopherol and high beta-carotene compounds that produce high antioxidant power (15). In addition, Rigane et al reported the presence of compounds such as steroids, terpenoids, anthocyanins, tannins, coumarin, proteins, amino acids, flavonoids, phytosterols, and carbohydrates in different parts of Pistacia atlantica (16). Gholami et al reported that administration of Pistacia atlantica improved intestinal histopathology and decreased levels of oxidative stress marker in ulcerative colitis model rats (17).

The results of a study by Heidarian et al also showed that the administration of Pistacia atlantica increased catalase, superoxide dismutase, vitamin c and improved histopathology of the kidney. However, the levels of urea, uric acid, creatinine and malondialdehyde decreased (18). Tolooei et al. revealed that Pistacia atlantica reduced the free radicals and prevented liver damage by decreasing the level of MDA and increasing the level of antioxidant superoxide dismutase and catalase (19). In addition, the levels of glutamate pyruvate transaminase (GPT), glutamic oxalacetate transaminase (GOT) and alkaline phosphatase (ALP) in the Pistacia atlantica-treated group decreased compared to the carbon tetrachloride group. Mahjoub et al reported that pharmacologically the major constituents of Pistacia atlantica fruits include alpha-pinene, camphor, terpineol, myrsene, and sabinen (20). Norasteh et al. reported that Pistacia atlantica administration improved testicular histopathology and reduced oxidative stress after injury with busulfan in male rats (21). Bagheri et al. reported the positive effects of Pistacia atlantica administration on diabetes and increased levels of glutathione peroxidase, superoxide dismutase, and catalase antioxidants (22). Shakarami et al. reported that Pistacia atlantica administration reduced inflammatory factors such as interleukin in asthmatic rats (23). Busulfan is a cytotoxic drug that is widely used in chemotherapy and is commonly used to treat chronic leukemia, ovarian cancer, and bone marrow transplantation in cancer patients (24,25). It has a wide range of adverse effects on the body and has adverse effects on many organs including the liver and kidney (24,25). In this study, busulfan was used to develop a model of liver-kidney injury. The aim of the present study was to investigate the effects of Pistacia atlantica administration on liver and kidney histopathology as well as serum levels of hepatic enzymes, urea and creatinine following busulfan-induced liver and kidney injury.

Methods

This experimental study was performed on 28 male Balb/c mice after being approved by the Ethics Committee of Mashhad University of Medical Sciences under code IR.MUMS.fm.REC.1397.96. The rats were kept under standard conditions at the Animal Laboratory of the Medical School (22 °C, twelve hours of light cycle, twelve hours of darkness). All the principles and rules of working with laboratory animals were respected. Animals were randomly divided into four groups of control, busulfan, bene and bene+ bosulfan. The control group received busulfan solvent (DMSO) once per day. The busulfan group received 10 mg/kg single dose intraperitoneally (24). The bene group received a diet containing 10% bene. This group was considered as a positive control to better compare the results in the study. The bene+ bosulfan group received 10 mg/kg bosulfan on the first day of the experiment. Then, they received a diet containing 10% bene for 35 days (25).

Evaluation of liver and kidney function indices: On day 35 of the experiment, blood was drawn from the ventricular cavity of the heart and transferred to the microtubes immediately after the abdomen was opened. The microtubes containing blood samples were then

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centrifuged at 20,000 rpm. After serum isolation, samples were stored in -20 ° C freezer and liver and urea and creatinine levels were measured by spectrophotometer. Quantitative detection kit (Pars Test Co., Iran) was used to evaluate urea and creatinine levels. In accordance with the kit protocol, the two-step procedure was performed. Urea optical absorption at 340 nm and creatinine optical absorption at 500 nm were read. Alanine amino transferase (ALT) and aspartate aminotransferase (AST) enzymes were also tested by Pars Co. at a wavelength of 340 nm. All measurements were performed in duplicate (26-28).

Histopathological examination: On day 35, after opening of abdominal cavity, liver and kidney were removed and placed on 10% formalin and dehydration and clearance procedures were performed by ascending alcohol and xylene, respectively. The tissues were then molded with melt paraffin and sectioned with a 5-micron microtome and stained with hematoxylin-eosin. The slides were examined by a pathologist after staining with a light microscope (29). Finally, the pathologist's report was presented qualitatively by examining the slides of 28 mice. Twenty-one slices from each group and a total of 84 slices were examined. Inflammation and necrosis of the liver were assessed using modified histological activity index-grading (30). Data were analyzed using SPSS software version 20, one-way ANOVA and Tukey post hoc test. $p<0.05$ was considered significant.

Results

Urea levels in the blood serum of the Bene treated group was 38.3±5.10 and in the busulfan group was 55.2±4.23 mg/dL, respectively. Urea levels in bene+bosulfan group were significantly lower than those of bosulfan group (p= 0.000). Also, the level of urea was significantly different in the groups that received bene+ bosulfan compared to bene (p= 0.01) (Fig. 1). In addition, the mean urea level in the busulfan group was 55.2±4.23 mg/dl and in the control group was 26.8±6.2 mg/dL (p= 0.000). The mean creatinine level was 0.5±0.14 mg/dl in the bene+ Bosulfan group and 0.8±0.11 mg/dl in the busulfan group, respectively. (p= 0.02) (Fig. 2). The mean creatinine level in the group treated with busulfan was 0.8 ± 0.11 and in the control group was 0.56±0.20 mg/dl which was significantly increased in creatinine level in the busulfan group compared to the control and bene groups (p=0.02 and p=0.004, respectively).

The level of ALT was 41±5.1 in bene+bosulfan group and 59±4 in busulfan group. Statistical analysis showed a significant decrease in the mean level of ALT in the bene+bosulfan group compared to the bosulfan treated group (p= 0.03). Significant increase in mean ALT was observed in busulfan group compared to control group (p= 0.013) (Fig. 3). In addition, the level of ALT enzyme was significantly lower in bene group compared to busulfan group (p= 0.007). The level of AST enzyme was 91.2± 5 in the bene+Busulfan group and 110.1±6 in the busulfan group. Statistical analysis showed a significant decrease in mean AST enzyme level in bene+bosulfan group compared to bosulfan group (p= 0.000). Significant increase in mean AST enzyme was observed in busulfan group compared to control (p= 0.013) and bene (p= 0.013) groups (Fig. 3). In addition, the level of AST enzyme was significantly lower in bene group compared to bene+ bosulfan group (p= 0.01).

Results of Histopathology of Liver and Kidney: Normal view of hepatocytes and port space was observed in liver sections of control group. In the group receiving busulfan, there was inflammation of the port space and small necrosis of the liver parenchyma with congestion. Liver slices of bene treated group did not show steatosis, hepatocyte necrosis and fibrosis in liver parenchyma. There was a slight sinusoidal dilatation in all the slides, a finding that was nonspecific (Fig. 4). The kidney slices in the control group had normal glomerular structure and renal tubules. In all sections of the group treated with busulfan, congestion and foci of interstitial nephritis were observed. In the treated group with cormorant glomerular sclerosis, there was no increase in glomerular cellularity, fibrosis and interstitial inflammation and tubular atrophy (Fig. 5).

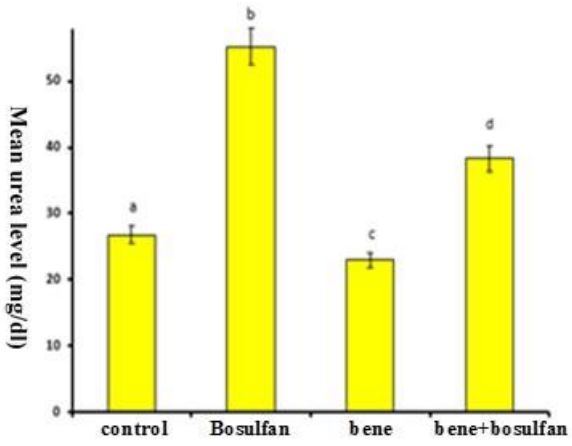

Figure 1. Mean urea level in the serum of mice in the studied groups based on mg/dl

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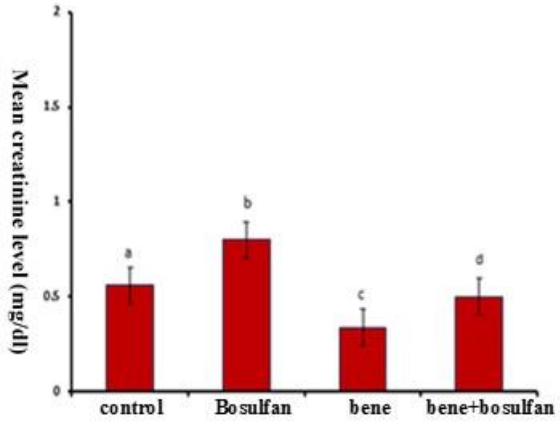

Figure 2. Mean creatinine level in the serum of mice in the studied groups based on mg/dl

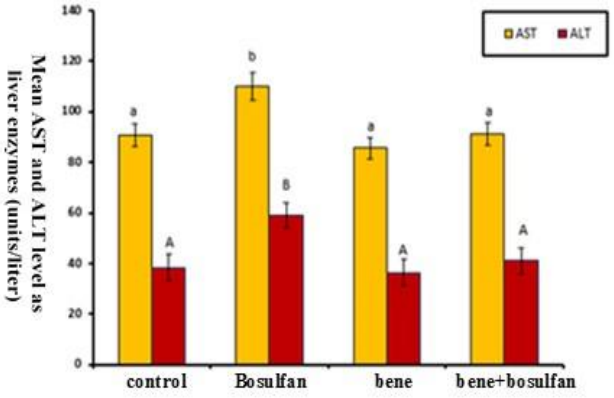

Figure 3. Mean ALT and AST enzyme levels in the serum samples of mice in the studied groups in units per liter.

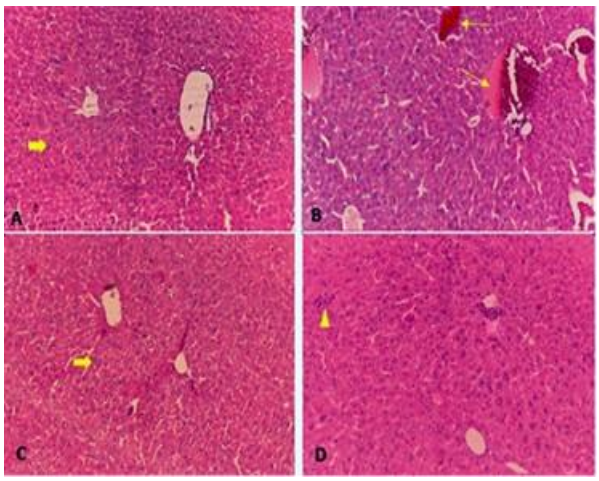

Figure 4. Liver image of mice in different groups taken with a 20% lens. group. A: control group, b: busulfan group, c: corm group, d: bene+bosulfan group. Staining: Hematoxylin and Eosin. Thick arrow: Hepatocyte cell, Thin arrow: Congestion, Arrow tip: spot necrosis

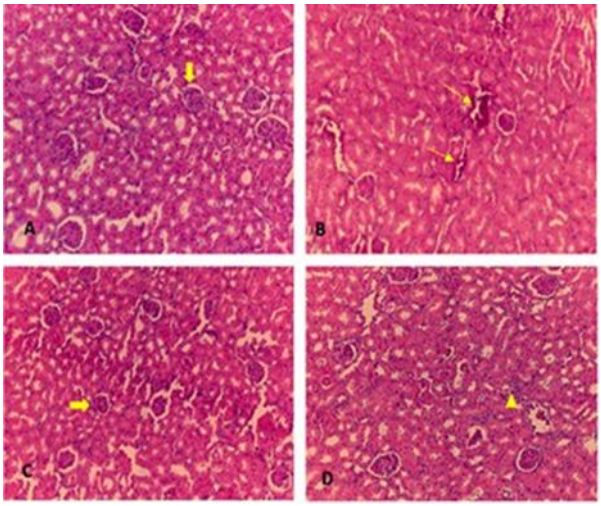

Figure 5. Image of all mice kidneys in different groups taken with 20× lens. group. A: control group, b: busulfan group, c: bene group, d: bene+bosulfan group. Staining: Hematoxylin and Eosin. Thick arrow: Kidney glomeruli, Thin arrow: Congestion, Arrow tip: Mild interstitial nephritis

Discussion

Results of this study showed that administration of 10% bene improved liver and kidney histopathology and decreased serum urea and creatinine level in comparison to busulfan treated group. In addition, bene treatment reduced the levels of liver enzymes alanine aminotransferase and aspartate aminotransferase. Omidi et al. investigated the effects of bene powder on the lipid profile and phosphatidin phosphorylase levels in the 15- and 60-day treatment periods. The results showed that 15-day treatment with bene increased all lipoprotein fractions and decreased triglyceride. Although liver phosphatidin phosphorylase levels decreased by 11%, it was not statistically significant. In the treatment group that had been treated with bene for 60 days, there was no difference in lipid profile with the control group but with a significant decrease in triglycerides. Liver phosphatidin phosphorylase levels in this group decreased by 16% (25). In the study of Tolooei et al., bene as an antioxidant reduced MDA and increased the level of antioxidant superoxide dismutase and catalase and improved liver damage. In addition, the levels of glutamate pyruvate transaminase (GPT), glutamic oxalacetate transaminase (GOT) and alkaline phosphatase (ALP) in the bene-treated group decreased compared to the carbon tetrachloride group (19). Another study showed that bene administration reduced ABCG4 gene expression in rat liver (31). In the present study, bene was effective on liver functional parameters including alanine transaminase and aspartate transferase

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enzymes and improved busulfan-induced liver symptoms. The results of the study by Heidarian et al showed that administration of bene increased the antioxidant enzymes catalase, superoxide dismutase, vitamin c and also improved the histopathology of the kidney. While urea, uric acid, creatinine and malondialdehyde levels decreased (18). In line with our study, administration of 10% bene diet reduced urea and creatinine levels and improved renal symptoms induced by busulfan. In addition, liver and kidney histopathology improved. Bene consumption decreased significantly in the bene group compared to the control group, which may indicate a positive effect of bene in healthy individuals. It seems that the possible mechanism of the effect of bene administration on liver and kidney structure and function is that bene with strong antioxidant properties and compounds such as gallic acid, quercetin and luteolin reduce oxidative stress. The first major defense line of the body is the superoxide dismutase and catalase enzymes, and the next is the glutathione peroxidase enzyme, which reduces free radicals in the body. If the food does not contain the necessary amount of antioxidants, enzymatic defense against oxidants may be harmed. The results showed that administration of 10% bene for 35 days improved liver and kidney histopathology and liver and kidney functional parameters in busulfan-induced rat liver-kidney injury model. Considering that bene is native to our country, as a cheap and affordable compound that has potent antioxidant properties, it is suggested that in the next study, histopathological examination of liver and kidney tissues as well as oxidative stress levels will be measured and in addition the levels of urinary enzymes alkaline phosphatase and lactate dehydrogenase as well as liver enzymes alkaline phosphatase and gamma glutamyl transferase should also examined.

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