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PROTECTIVE EFFECT OF HYDROALCOHOLIC EXTRACT OF EPHEDRA MAJOR ON AN EXPERIMENTAL MODEL OF BILE DUCT LIGATION IN RATS

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INTRODUCTION
The liver is the largest organ of the body that regulates several important functions such as metabolism. The liver also is an important organ for detoxification of hepatotoxins which can cause hepatic injury during metabolic reaction (Giannelli et al., 2003).

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Cirrhosis of the liver due to the high prevalence of debilitating and also sometimes fatal complications is the main problems of the health care system. Cirrhosis is the final and irreversible scarring of liver fibrosis that responds to a variety of long-term waste-inflammatory and damages (Braunwald et al., 2008).

The pathogenesis of the liver damage involves all cell types via death and regeneration processes and progress to chronic hepatitis, fibrosis and cirrhosis (Giannelli et al., 2003). Biliary cirrhosis is one type of cirrhosis, in which one form of scar tissue around the bile ducts is created (Braunwald et al., 2008).

An experimental model in rats which characterized by rapidly progressing biliary fibrosis is Bile Duct Ligation (BDL). The initial stages are represented by acute cholestasis, in which oxidative stress and inflammation play essential roles (Lotkova et al., 2011).

Medicinal plants, due to their low side effects, have been used as alternative to chemical drugs. Plants of the Ephedra genus, including Ephedra major and others have traditionally been used for a variety of medicinal purposes, including treatment of hay fever, asthma, and the common cold. Members of this genus contain various medicinally active alkaloids (but notably ephedrine) and they are widely used in preparations for the treatment of asthma (Abourashed et al., 2003). Ephedra major with alkaloidy compounds have effective role in treatment of many diseases. The alkaloids ephedrine and pseudoephedrine are the active constituents of the Ephedra genus. Derivatives of ephedrine are used to treat low blood pressure, but alternatives with reduced cardiovascular risk have replaced it for treating asthma. The whole plant can be used at much lower concentrations than the isolated constituents—unlike using the isolated ephedrine, using the whole plant rarely gives rise to side-effects (Kapner, 1997).

Aim: The aim of this study was to investigate the effect of hydroalcoholic extract of Ephedra major on laboratory (serum bilirubin) and pathological parameters on a model of induced cholestasis in rats.

**MATERIALS AND METHODS**

**Plant Material**

The leaves of Ephedra major were collected during summer 2012 from the surrounding areas of Karaj city, Alborz province, Iran. The leaves were identified by faculty member of Islamic Azad University of Karaj.

The freshly collected leaves were cleaned from dirt and they were dried under shade and then coarsely powdered manually. The powder was macerated by using 70% ethanol for a period of 3 days and then the percolated solution was filtered, concentrated and dried. The material was stored at 4°C until used (Shah et al., 2009).

**Animals**

In this study, forty male Sprague-Dawley rats (prepared by the Research Center for proliferation and maintenance of laboratory animals, Ahvaz JundiShapur University of Medical Sciences) weighing 200 to 250 g were used. Animals kept in separate cages under standard laboratory (temperature of 25±2 ºC and 12: 12 h light- dark cycle) with free access to food and water. Protocol implementation was approved by local research committee Ahvaz JundiShapur University of Medical Sciences and Islamic Azad University of Karaj.
Chemical
Ketamine (10%) and xylazine (2%) were purchased from Alfasan Co. (Holland).

Experimental Design
The animals randomly divided into five groups (sham, cirrhotic, cirrhotic treated with two doses of extract and extract) (n=8).

Biliary cirrhosis in animals (extrahepatic cholestasis) induced by chronic (28 days) double BDL. Each rat was anesthetized using ketamin (50 mg/kg) and xylazine hydrochloride (10 mg/kg) (Mitchell et al., 1998) and after a midline abdominal incision closed to the sternum, the common bile duct was identified; a double ligature was made with 3/0 silk, after that a cut was made between both ligatures (Liu et al., 2003; Rivera-Huizar et al., 2006). Sham group underwent laparotomy and the common bile duct was only dissected from the surrounding tissue. Then the animals were allowed to recover in separate cage with free access to food and water. In the cirrhotic treated with extract groups, extract was administrated daily (100 and 200 mg/kg/day, gavage) (Shah et al., 2009) for a period of 15 days, and other groups received a similar volume of normal saline (in sham and cirrhotic groups) or extract (200 mg/kg/day, gavage) (Fadillioglu et al., 2004; Ebrahimkhani et al., 2008).

Bilirubin Analysis: Blood samples collected from the rat's hearts for measure serum bilirubin in plasma as a marker to show the occurrence cholestasis (Fernández et al., 2006). Total and conjugated bilirubins tested with kit (pars azmoon, Iran) and were measured by colorimetric method using an autoanalyzer (BT3000).

Preparation of Histological Samples
In all groups four weeks (28 days) from the beginning of the experiment or since the BDL, animals were anesthetized with ketamin (50 mg/kg) and xylazine hydrochloride (10 mg/kg) (Mitchell et al., 1998). The liver was isolated and washed with normal saline, immersed in 10% formalin solution then subjected to histopathological examination.

Samples were embedded in paraffin. The sections were made at 5 micrometers with a microtome and stained by haematoxiline-eosine (H&E) method (Olteanu et al., 2012). Then the slides were examined under a microscope Olympus BH2-RFCA.

STATISTICAL ANALYSIS
Results were analyzed by using student t-test and one way ANOVA. P<0.05 was considered as significant level.

RESULTS
The bilirubin levels (conjugated and total) between all groups (Figure 1) shows that not only the amount of conjugated and total bilirubin in cirrhotic and cirrhotic treated with extract groups was significantly higher than the sham group (P<0.001), but also significant difference between the bilirubin levels in cirrhotic and treated with extract (100 and 200 mg/kg/day, 15 days) groups was observed (P<0.01). There was no significant difference between the bilirubin levels in non cirrhotic groups (sham and extract).

The liver morphological changes induced by BDL were shown at Figure 3 and 4. The livers of the rats from sham group and extract group which received extract (200 mg/kg/day) had no histological changes (Figure 2A, B).
Figure 1: Comparison of bilirubin levels in different groups, 4 weeks after surgery in rat (n=8). Values are expressed as Mean± SEM. *significantly different compared to sham group at p< 0.001, a significantly different compared to sham group at p< 0.01. One-way ANOVA was used, followed by LSD test.

Figure 2: Hematoxylin and eosin histological stained of the Liver tissue (*600) Sham (A) and Extract (200 mg/kg/day) (B) groups showing normal and intact basic liver structures.

Figure 3: Hematoxylin and eosin histological stained of the Liver tissue. C: Cirrhotic group showing liver parenchimal necrosis (white arrow) and Collagen aggregation (dark arrow) (*300). D: Cirrhotic group showing fatty change (white arrow) (*600).

Severe histopathological changes were observed in livers of all the rats from cirrhotic group. The predominant lesions were collagen aggregation, extensive degeneration and necrosis cells. The histological appearance of the liver showed cytoplasmic vacuolation that was indicated fatty change (Figure 3C, D). Administration of Extract (100 and 200 mg/kg/day) ameliorated the changes induced by BDL. The lesions in the liver of cirrhotic rats that received extract were conspicuously less than those in the cirrhotic rats (Figure 4E, F).
DISCUSSION

In the present study, the effect of hydroalcoholic extract of *Ephedra major* was determined laboratory (serum bilirubin) and pathological parameters on a model of induced cholestasis in rats. *Ephedra major* has a reduction effect on bilirubin and histopathological changes which administrated four weeks to control hepatic complications caused by cirrhosis.

![Figure 4: Hematoxylin and eosin histological stained of the Liver tissue. E: Cirrhotic group received extract (100 mg/kg/day) showing liver parenchimal necrosis (white arrow) and Collagen aggregation (dark arrow) (*300). D: Cirrhotic group received extract (200 mg/kg/day) showing liver parenchimal necrosis (white arrow) and Collagen aggregation (dark arrow) (*300)](image)

A well-known model to induced cirrhosis in animals is BDL (Liu *et al.*, 2003).

BDL induced cirrhosis in groups, which has reflected by a significant increase in serum bilirubin levels. After induced of BDL, pathological parameters in the cirrhotic group showed significantly increased in comparison with sham group. A serum bilirubins level has changed by *Ephedra major* extract treatment. Results showed that not significant differences in bilirubins level between groups which received two doses of extract (100 and 200 mg/kg/day).

Previous study had shown that the liver damage markers were elevated bye BDL induced cirrhosis. Chronic BDL significantly increases most of plasma and hepatic cytokine levels (Fernández *et al.*, 2012). Several studies have shown that treatment with free radicals remarkable decreases liver damage following the closure of the bile duct in rats (Dianat *et al.*, 2012). Also other results shown that oxidative stress associated with lipid peroxidation is involved in the development of liver damage in cholestatic rats by BDL (Coban *et al.*, 2008). This study has shown that Pathological parameters were reduced in cirrhotic groups treated with two doses of extract compared to cirrhotic group.

Previous study has shown that administration of *Ephedra* extract to rats were reduced the amount of cardiac injury markers, i.e., serum LDH, SGOT and CPK levels (Shah *et al.*, 2009). Therefore, the findings indicate that *Ephedra* extract through interfering with the free species decreased oxidative stress in cholestatic rats. It seems that protective effects of *Ephedra* extract are mediated via, antioxidant, free radicals scavenging, and inhibition of microsomal enzymes activities and has a hepatoprotective effect.
CONCLUSION
Significant decrease in bilirubin and pathological parameters in cirrhotic group treated by Ephedra major extract suggests the protective effect of Short-term consumption of low dose Ephedra major in cirrhotic patients.

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