

Protective Effects of Hydro Alcoholic Extract of Sebestan (*Cordia myxa*) Fruit Against Cadmium-Induced Liver Toxicity in Adult Male Rats

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Abstract

Cadmium is a heavy metal with toxic effects on various organs, including liver, kidneys and testicles. In the present study, the protective effects of hydro alcoholic extract of sebestan (*Cordia myxa*) fruit on hepatic functional tests as well as its histological changes following cadmium chloride toxicity were investigated in adult male rats. 48 adult male Wistar rats were divided into 6 groups of 8, as follows: The Control group, the sham group I received 2 mg / kg cadmium chloride, the sham group II received 125 mg / kg hydro alcoholic extract of sebestan fruit, the experimental group I, II and III received 125, 250 and 500 mg / kg extract for 21 days respectively followed by 14 days of 2mg / kg cadmium chloride administration. At the end of the 35-day period, blood samples were obtained and used for measuring serum concentrations of ALT, AST and ALP. The results were statistically analyzed using SPSS software, and ANOVA and Tukey tests; and $P \leq 0.05$ was considered statistically significant. Mean serum levels of ALP, ALT and AST showed a significant increase in the sham I compared to the control and the sham II. Conversely, the mean concentrations of ALP, ALT and AST in the experimental group receiving Cordia extract (500 mg / kg) plus cadmium chloride showed a significant decrease relative to the group receiving cadmium chloride alone. It is concluded that Cordia fruit extract has a significant protective effect against liver toxicity induced by cadmium chloride.

Keywords: *Cordia myxa*, Cadmium chloride, ALP, ALT, AST, Adult male rat

Introduction

Cadmium (Cd) is a relatively scarce heavy metal with atomic number 48 (Hosseinzadeh et al., 2005; Zhou et al., 2004). It is a soft, malleable and flexible white-bluish metal easily cut with a knife (Hosseinzadeh et al., 2005; Kopp et al., 1982, Yamano et al., 1998). Studies have shown that cadmium disrupts cellular activity through peroxidation of unsaturated fatty acids culminating in

disruption of protein synthesis as well as lipid, carbohydrates and amino acids metabolism (Duble et al.1985; Germano et al.,1998; Gaurav et al.,2010; Weast et al.,1988).

The mechanism of toxicity induced by cadmium is not very clear (Hoste et al.,2009). However, It has been shown that factors such as increasing the production of reactive oxygen species (ROS) and consequently, the oxidative stress induced by ROS production neutralizes antioxidant enzymes in the tissues . Following exposure to cadmium, mitochondrial function in damaged cells is disrupted, and the production of ROS is increased. The unbalanced ROS and internal antioxidant system leads to oxidative stress and cellular damage (Shiota et al.,2006).

The development of organ toxicity, such as liver damage, by cadmium has been well documented (Robert and Donald,1982); even the involvement of chronic Cd toxicity in carcinoma has been suspected (Rashed et al.,2015). The adverse hepatic changes include: increased levels of AST and ALT enzymes, as well as microscopic changes in the liver tissues such as focal necrosis, inflammatory cell accumulation, apoptosis, and sinusoidal dilatation (Rashed et al.,2015). In addition, the toxic effects of cadmium on liver are both time- and dose-dependent.

Next to the skin, liver is the largest organ of the body located in the abdominal cavity below the diaphragm. It receives and processes the absorbed food from the gastrointestinal tract, and stores them for use in other parts of the body. Another word, It is located between the bloodstream and the digestive tract (Limdi and Hyde,2003).

About 80% of the liver cells are made of hepatocytes, which store fat and sugar; in addition, they are responsible for the production of albumin, prothrombin and fibrinogen (Limdi and Hyde,2003)

The clinical index for liver injury is the hepatic functional tests such as measuring the serum levels of ALT, AST and ALP enzymes (Limdi and Hyde,2003). Although most of these tests are not specific for liver, if their levels are too abnormal, their origin could be related to the hepatic dysfunction. The most common enzymes considered as indicators of liver damage are Transaminases, including aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) (Qunitans et al.,2013).

Sebestan tree or *Cordia* species belong to the plant family Boraginaceae. Of the five species in the world, one species i.e. *Cordia myxa* (lasura and Assyrian) is found in southern parts of Iran; under several local names such as sebestan, sarbestan, Luz, etc (Limdi and Hyde,2003; Qunitans et al.,2013). The initial screening of hydro alcoholic extract of sebestan fruit by

phytochemical method has shown that it contains flavonoids, sterols, phenolic acids, saponins, tripenoids, alkaloids, coumarin and tannin (Abdallah et al.,2011).

In Malaysia and Ivory Coast, this herb is used to treat the wounds. In Tanzania, its pulps are used to treat rheumatoid arthritis and to remove roundworms. In some African areas, fresh leaves of this herb is orally used in the treatment topically for the Tsetse mosquito bites (Abdallah et al.,2011).

Since no study has thus far been carried out on the protective effects of sebestan extract on liver against hepatic toxicity caused by Cadmium, in this study, we try to investigate the possible effects of hydro alcoholic extract of Cordia fruit on the serum levels of ALP, ALT and AST following induction of cadmium chloride toxicity in male rats.

Materials and Methods

In this experimental study, 48 adult male Wistar rats weighing approximately 200-225 g and about 2.5-3 months old were used. They were obtained from the animal breeding center of Kazerun Azad University, and kept in 40×25 ×15 cm polycarbonate cages with lattice roofs made of steel. Six cages were used each containing 8 male rats. The floor of cages was covered with saw dust, which was replaced daily. Cages were washed twice a week with water and disinfectants.

Throughout the experiment, the mice were exposed to 12 hours of darkness and 12 hours of light under 23 ° C. They used urban tap water freshly provided daily. They were fed dry food (Pars Animal Feed Company). Animals had unlimited access to water and food throughout the experiment, and ethics of working with animals were observed.

Preparation of the hydro alcoholic extract

Fresh sebestan fruits were collected in gardens of Kazerun city, Fars province. Following seed removal, they were blended well in a mixer. 500 g of the resulting mixture was put in a percolation device, and 70% ethanol was added until the solution level was several centimeters above the fruit mixture. It was kept at room temperature for 72 hours, after which the valve of the percolator was loosen to collect The extract drop by drop. At the same time, 70% hydro alcoholic solution was added from the top until the extracted drops lost the color of the fruit. The extract was Concentrated by rotary at 40-50° C, and was dried completely in a desiccator for 24 hours.

Animal grouping and treatment

The rats were randomly divided into the following groups: (1) the control group left untreated (received regular water and food); (2) the sham group I or negative control intraperitoneally received 2 mg / kg Cadmium chloride (CdCl_2) for 35 days; (3) the sham group II or positive control orally received 125 mg / kg body weight sebestan extract for 35 days; (4) the Experimental group I orally received 125 mg / kg body weight Cordia extract for 21 days followed by 14 days of intraperitoneal administration of 2 mg / kg body weight CdCl_2 ; (5) the Experimental group II received 250 mg / kg body weight Cordia extract for 21 days followed by 14 days of intraperitoneal administration of 2 mg / kg body weight CdCl_2 ; and (6) the Experimental group III received 500 mg / kg body weight Cordia extract for 21 days followed by 14 days of intraperitoneal administration of 2 mg / kg body weight CdCl_2 .

At the end of the trial, all rats were subjected to ether anesthetic, and two stages of blood sampling was carried out from the left ventricle: (a) From the control, sham I, sham II and experimental group I at the end of the 21st day, and (b) from the experimental groups II and III at the end of the 42th day. Blood samples were placed inside the carefully labeled test tubes, and centrifuged at 5000 rpm for 15 minutes. The resulting serum samples were frozen at -20°C to be used in enzyme measurements.

Hepatic enzyme levels were measured using Pars exclusive kits (pars Azmun company) as well as autoanalyzer (Immunoassay Analyzers -IA) using the kinetic method recommended by the Society for Clinical and Laboratory Chemistry (IFCC).

statistical analysis

The results were analyzed using SPSS software, and ANOVA and Tukey tests. $P \leq 0.05$ was considered statistically significant between different groups.

Results

Statistical comparison of ALT serum concentration in different groups is shown in Table 1. Based on these results, there was no significant difference between the mean concentration of alanine transferase enzyme (ALT) in control group (394.16 ± 15.54) and sham II (376.5 ± 17.22). However, its level in the sham group I (805.00 ± 45.45) was significantly higher than the control group (394.16 ± 15.54) ($P < 0.05$). Similarly, serum level of ALT in the experimental group I

(692.00 ± 29.39) was significantly higher than control (394.14 ± 15.54) and sham II (376.5 ± 17.22) ($P < 0.05$). Also, its serum levels in experimental group II (582.85 ± 38.20) and experimental group III (424.5 ± 40.38) were significantly higher than the control group (394.16 ± 15.54) and sham II (376.5 ± 17.22) ($P < 0.05$). Conversely, the level of ALT enzyme in the experimental group III (422.56 ± 40.38) was significantly lower than the experimental group I (692 ± 29.39) and experimental group II (582.85 ± 38.20).

Statistical comparison of serum levels of AST enzyme is presented in Table 2. According to these results, there was no significant difference between mean serum concentration of alanine aminotransferase enzyme (AST) in the control group (93.50 ± 7.34) and the sham II (91.5 ± 5.59). In contrast, the mean concentration of AST in the sham group I (254.31 ± 8.52) was significantly higher than the control group (93.50 ± 7.34) ($P < 0.05$). Also, its serum level in experimental group I (207 ± 15.09) was significantly higher than the control (93.50 ± 7.34) and sham II (91.5 ± 5.59) ($P < 0.05$). The levels of AST enzyme in the experimental group II (151.00 ± 11.16) and experimental group III (119.71 ± 19.40) were significantly higher than the control group (93.5 ± 7.34) and sham II (91.5 ± 5.59) ($P < 0.05$). Conversely, its serum levels in the experimental group 3 (19.11 ± 11.49) compared to the experimental group II (151 ± 11.16) and experimental group I (207 ± 15.09) showed a significant decrease at the level of $P < 0.05$.

Table 3 shows the mean serum levels of ALP enzyme. As seen, there is no significant difference between mean serum concentrations of ALP in the control group (682.32 ± 21.97) and sham II (675.37 ± 18.69). The serum level of this enzyme in the sham group I (726 ± 3.74) relative to the control group (682.32 ± 21.97) was significantly higher at the level of $P < 0.05$, whereas its level in the experimental group I (671.42 ± 34.21) showed a significant decrease relative to the control group (682.32 ± 21.97) and the sham II (675.37 ± 18.69) ($P < 0.05$). Similarly, serum level of ALP in the experimental group II (675 ± 22.23) compared to the control group (682.32 ± 21.97) showed a significant decrease. Also, the level of this enzyme in the experimental group II (675 ± 22.23) relative to the sham group II (675.37 ± 18.69) showed an increase, but this elevation was not significant at the level of $P < 0.05$. The level of ALP in the experimental group III (653.27 ± 23.63) was significantly lower than the control group (682.32 ± 21.97) and sham group II (675.37 ± 18.69) ($P < 0.05$). The level of ALP in the experimental group III (653.27 ± 23.63) was significantly lower than the experimental group II (675 ± 22.23) and the experimental group I

(671.42 ± 34.21). Serum level of ALP in the experimental group II (675 ± 22.23) showed a significant increase relative to the experimental group I (671.42 ± 34.21) (P <0.05)

Table 1. Comparison of mean serum ALT concentration in experimental groups receiving different doses of sebestan extract with control and sham groups.

($\bar{x} \pm SEM$) ALT enzyme (U/L)	Groups
(394.16 ± 15.54)	control group
(805.00 ± 45.45)	sham group I
(376.5 ± 17.22)	sham group II
(692.00 ± 29.39)**	experimental group I
(582.85 ± 38.20)**	experimental group II
(422.56 ± 40.38)**	experimental group III

Values are based on the mean ± standard error of the mean ($\bar{x} \pm SEM$).

* indicates a significant difference with the control group at the level of P <0.05.

** indicates a significant difference between experimental groups at P <0.05.

Table 2. Comparison of mean serum AST concentrations in experimental groups receiving different doses of sebestan extract with the control group.

($\bar{x} \pm SEM$) AST enzyme (U/L)	Groups
(93.5 ± 7.34)	control group
(254.31 ± 8.52)*	sham group I
(91.5 ± 5.59)	sham group II
(207 ± 15.09)*	experimental group I
(151.00 ± 11.16)*	experimental group II
(119.71 ± 19.40)*	experimental group III

Values are based on the mean ± standard error of the mean ($\bar{x} \pm SEM$).

* indicates a significant difference with the control group at the level of P <0.05.

** indicates a significant difference between experimental groups at P <0.05.

Table 3. Comparison of mean serum ALP levels in experimental groups receiving different doses of Cordia extract with the control group.

$(\bar{x} \pm SEM)$ ALP enzyme (U/L)	Groups
(682.32 \pm 21.97)	control group
(726.00 \pm 3.74)	sham group I
(675.37 \pm 18.69)	sham group II
(671.42 \pm 34.21)	experimental group I
(675 \pm 22.23)	experimental group II
(653.27 \pm 23.63)	experimental group III

Values are based on the mean \pm standard error of the mean ($\bar{x} \pm SEM$).

* indicates a significant difference with the control group at the level of $P < 0.05$.

** indicates a significant difference between experimental groups at $P < 0.05$.

Discussion

According to our results, activity of hepatic enzymes (ALT, AST and ALP) in the cadmium chloride receptor group (Sham I) increased significantly at the level of $P < 0.05$ relative to the control and sham II groups (tables 1, 2 and 3), indicating hepatic damages. It has been shown that The serum level of Alanine aminotransferase, a cytoplasmic soluble enzyme naturally found in the liver, increases rapidly during hepatic damage, liver cirrhosis, liver tumors, obstructive jaundice or hepatic toxicity induced by drugs or foreign substances (Dorian et al.,1992). Although it cannot be said that this enzyme is exclusively located in the liver, the highest concentration of ALT is found in this organ (Qunitans et al.,2013).

In addition, Aspartate aminotransferase with two cytosolic and mitochondrial isoenzymes is present in the liver, muscle, heart, kidneys and the brain (Shaban et al.,2003). If these organs are damaged, serum level of AST will increase (Shaban et al.,2003).

Similarly, alkaline phosphatase is found in various organs such as the liver, bone and to a lesser extent in the kidneys, intestines and placenta. ALP is a transpeptidase that increases in bone diseases such as rickets, bone metastases as well as hepatic disorders (Limdi and Hyde,2003).

Moreover, alkaline phosphatase is a membrane protein that can play a role in the membrane permeability and transport of substances in and out of the cells (26). This enzyme is also present in the biliary duct epithelium and in the canalicular membrane of the hepatocytes. The release of ALP enzyme in blood circulation is much lower than AST and ALT. Likewise, in this

study, the level of ALP in the group exposed to CdCl_2 increased significantly, which indicates its adverse effect on bile duct epithelium.

Induction of toxicity by cadmium in different organs, including the liver is well documented, and even its carcinogenic effects has been indicated during chronic Cd toxicity.

Tandon et al. (1992) Reported that intraperitoneal injection of 1 mg cadmium per kg body weight for 7 days resulted in an increase in AST and ALT enzymes in rat (Tandon et al.,1992). Robert et al. showed that injection of 3.9 milligrams of cadmium per kg body weight every 10 hours cause liver necrosis and increased activity of liver enzymes (Robert and Donald,1982). Also, studies have shown that intraperitoneal injection of CdCl_2 increases the peroxidation of unsaturated fatty acids, free radical accumulation as well as decrease in the Concentrations of copper, zinc, iron, selenium, glutathione and Superoxide dismutase, catalase and glutathione peroxidase enzymes in the liver and kidney of rat (Csalino et al.,2002). In the same way, it has been shown that oxidative stress and the production of free radicals such as ROS produced in the presence of cadmium are responsible for the induction of toxic effects of this heavy metal in various organs and cells, and can act as signals for induction of cell death (Satatug and Haswell – Elkins,2000). The results of this study showed that intraperitoneal injection of CdCl_2 elevates the hepatic enzymes such as ALT, AST and ALP, which agrees with other studies. Since these enzymes are intracellular and, in cases where cell damage occurs, they enter the bloodstream, it is concluded that CdCl_2 has damaged liver cells. It is noteworthy that enzymes AST and ALT are found in the mitochondria (Zitkevicius et al.,2011). One of the most important destructive effects of free radicals is the onset of lipid peroxidation, which leads to cell membrane damage and changes in enzyme activity, culminating in cell damage and liver necrosis. The latter leads to increase in the serum levels of enzymes that are released from liver into the bloodstream. The overall increase in liver enzymes is recognized as a marker for detecting liver damage caused by drugs, alcohol and viruses.

According to our results, activity of hepatic enzymes (ALT, AST and ALP) in the experimental group receiving 500 mg / Kg hydro Alcoholic extract plus Cadmium chloride compared to the sham I declined significantly and was close to the level of control group (tables 1, 2 and 3) indicating the positive effects of the *Cordia* extract. Studies have shown that antioxidants and phenolic compounds present in medicinal herbs can prevent toxic effects of drugs on the liver and reduce the release of liver enzymes into the bloodstream. Citrus antioxidants inhibit the

oxidative stress caused by cadmium in the liver (Robert et al.,1982). In the present study, experimental groups I, II and III, treated with 125, 250 and 500 mg / kg hydro Alcoholic Extract of *Cordia* respectively, showed a decline in the levels of ALT, AST and ALP enzymes relative to the increase in the dose of received extract. Also, they showed a significant decrease compared to the group exposed to Cd (sham I). The decline of these enzymes to their normal levels relative to the doses of sebestan extract is a clear sign of protective effects of this plant. This effect is likely to be the results of antioxidant properties of phenolic compounds present in *Cordia*. These compounds can protect the liver cells against oxidative stress by inhibiting free radicals, decreasing lipid peroxidation, increasing the activity of antioxidant enzymes, and securing membrane stability, which prevent the release of liver enzymes into the bloodstream. At the same time, phenolic compounds can chelate the metals due to their antioxidant properties. Therefore, it is likely that the protective effects of sebestan extract results from its ability to chelate cadmium (through the formation of a Phenol-cadmium complex). In this way, they can possibly withstand the destructive effects of the produced free radicals.

Conclusion

The results of liver enzyme tests in rats showed that hydro alcoholic extract of sebestan fruit is effective in protecting liver against CdCl₂ toxicity and can reduce toxic effects of cadmium. This protective property might depend on the antioxidant and phenolic compounds present in *Cordia*, although further studies are needed to clarify this finding.

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