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Histopathological evidences for effect beneficial of *Satureja hortensis* extract on hepatic lesion by cadmium–induced in Rat

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ABSTRACT

Background & Aim: Cadmium is an important industrial and environmental pollutant. Cadmium is one of the most toxic and carcinogenic heavy metals to organisms. This heavy metal mainly distributes to the liver and kidney in humans and animal and, causing acute hepatic injury.
Experimental: The ethanol extract of *Satureja hortensis* L. (Lamiaceae family), was evaluated for its activity against cadmium–induced in male Wister rats (150 – 180 g). The ethanol extract of *S. hortensis* (100 and 200 mg/kg/day for six weeks) was examined on serum bicochemical and hepatic histopathological characteristic of rats subcutaneously received with cadmium chloride ($CdCl_2$) at 3 mg/kg/day for six weeks.
Results: The biochemical results indicated that aspartate transminase (AST) and alanine transaminase (ALT) significantly increased in serum by cadmium–induced. The liver histopathological results revealed that the ethanol extract of *S. hortensis* treatment at 200 mg/kg/day significantly reduced toxicity by cadmium–induced. The ethanol extract of *S. hortensis* prevents the cadmium–induced lesions in hepatic function.
Recommended applications/industries: Known antioxidant, antimicrobial, antihepatotoxic, nephroprotective potentials of the extract of *S. hortensis* may be the mechanisms by which this plant protects animals against experimentally cadmium–induced.

1. Introduction

It has become evident that increasing industrial activities have modified the global cycle of heavy metals and metalloids, including the toxic non–essential elements like cadmium (Vinoth Kumar *et al*., 2010; Milton Prabu *et al*., 2012). Cadmium is a toxic heavy metal increasingly being recognized as a potential environmental pollutant. Cadmium accumulates in the biological system because of its long biological half–life (10 – 30 years) (Järup and Åkesson, 2009). Cadmium is used extensively in electroplating, although the nature of the operation does not generally lead to overexposures (Syers and Mackay, 1986). Increased concentrations of

cadmium in agricultural soils are known to come from human activities (Taylor, 1997), such as the application of phosphate fertilizer, sewage sludge, wastewater (Kara *et al*., 2004; Zhai *et al*., 2008), and pesticides, mining and smelting of metalliferous ores with high cadmium content (Tembo et al., 2006), and traffic (Nabulo *et al.*, 2006). Cadmium performs its effect on living organisms by accumulating in various tissues and affecting tissue antioxidant enzyme systems (Ozdemir and Dursun, 2009). Cadmium toxicity contributes to a large number of health conditions, including the major killer diseases such as heart disease, cancer and diabetes. Prolonged exposure to cadmium results in injury to the liver, lungs, kidney and testes (Zitkevicius *et al.*, 2011).

Cadmium and its compounds are highly toxic and exposure to this metal is known to cause cancer and targets the body's cardiovascular, renal, gastrointestinal, neurological, reproductive and respiratory systems (Goyer *et al.*, 2004). Several mitigative measures have been suggested to explain the damage induced by cadmium (Milton Prabu *et al.*, 2012). Parenteral administration of cadmium in rats causes a rapid accumulation of cadmium in the liver and at sufficient doses can give rise to severe hepatic injury in the form of hepatocellular necrosis (Andersen and Andersen, 1988).

Apoptosis seems to be a major mechanism for the removal of damaged hepatic cells, and constitutes the major type of cell death in nonparenchymal liver cells. Apoptosis of nonparenchymal cells is the basis of the pathogenesis of peliosis hepatis. The first peaks of necrosis and parenchymal cell apoptosis seem to evolve as a result of direct cadmium effects whereas the latter ones result from ischemia. The toxic effect of cadmium is due to its inhibition of liver metabolic enzyme systems containing sulfhydryl groups and uncoupling of oxidative phosphorylation in the mitochondria, which this results in increased lipid peroxidation, DNA damage, depletion of sulfhydryls, altered calcium homeostasis, hepatic congestion, ischemia and hypoxia (Bharavi *et al*., 2010; Habeebu *et al*., 1998).

The other possible mechanism of cadmium toxicity is the displacement of essential metals especially zincs requiring enzymes that are inactivated through direct displacement from their binding site by cadmium. Zinc, which is protective against cadmium, is becoming increasingly deficient in the soil and consequently in foods. Cadmium displaces zinc in many metallo–enzymes and many of the symptoms of cadmium toxicity can be traced to a cadmium–induced Zn deficiency (Gupta *et al*., 1991).

Summer savory or common savory (*Satureja hortensis* L.), belonging a mint family (Lamiaceae), is a widely distributed, annual plant, cultivated in many parts of the world. Infusion and decoction of aerial parts of savory are used to produce a tonic, carminative, antitoxic activity, digestive and expectorant and for the treatment of colds in Iranian traditional medicine (Zargari, 1990; Dadfar *et al*., 2012a). Phenolic compounds are widely known for their beneficial effects, such as preventing hormone–related cancers, potent antioxidant, and antibacterial properties (Burt, 2004). The essential oil and extract of *S. hortensis* with a higher percentage of phenols such as thymol and carvacrol have highest antioxidant activity (Ghasemi Pirbalouti, 2010). The antioxidant activity of the essential oils could be attributed to their hydrogen donating ability. The essential oil and extracts isolated from *S. hortensis* have been shown to have biological and pharmacological activities such as antibacterial (Mihajilov–Krstev *et al*., 2009), antifungal (Razzaghi–Abyaneh *et al*., 2008), antioxidant (Dorman and Hiltunen, 2004), insecticide (Pavela *et al.*, 2008), antispasmodic, anti–diarrheal, anti–nociceptive and anti–inflammatory (Hajhashemi *et al*., 2000; Hajhashemi *et al*., 2002), and treatment of rhinosinusitis diseases (Uslu *et al.*, 2003). The possible hepato–protective activity of *S. hortensis* extract against cadmium–induced hepatotoxicity has not been reported so far. Therefore, in current study, we aimed to evaluate the hepatoprotective effect of *S. hortensis* extract by using the cadmium–induced in rats.

2. Materials and Methods

2.1. Plant material

The aerial parts of *S. hortensis* were collected at a farm in Isfahan, Southwest Iran. The sample of the plant was identified by regional floras and authors with floristic and taxonomic references (Mozaffarian, 2008), and voucher specimen was deposited at the Herbarium of I.A.U of Shahrekord, Iran (IAUSHK–53).

2.2. Extract preparation

The aerial parts of *S. hortensis* were dried to constant weight in desiccant at room temperature (30°C). 200 g a

sample was extracted with 2000 mL ethanol (97%) at 25°C for 72 h. Ethanol was removed under reduced pressure in a rotary evaporator at 40°C. The concentrated extract was filtered using Whatman No. 1 filter paper and then lyophilized gave a green residue with yield 7%. The ethanolic extract of *S. hortensis* was reconstituted to a final concentration of 5% (w/v). The extract samples were stored in universal bottles and refrigerated at 4°C prior to use.

2.3. Experimental animals

Male Wister rats (150 – 180 g) of two months were used. The animals were housed in standard environmental conditions of temperature (22 ± 3ºC), humidity (60 ± 5%) and a 12–h light/dark cycle. During experimental time Wistar rats were given standard pellet diet (Pastor Institute, Iran) and water *ad libitum*. The rats were used for the experiment after one week of acclimatization period. All the procedures were approved by the Medical Ethics Committee of Shahrekord University of Medical Sciences. To determinate body weight, rats were placed into a container. Rat body weight and food intake and growth were monitored.

2.4. Experimental design

The rats were randomly divided into five groups of six rats in each group.

- Group I: Control rats subcutaneously received with 2 mL/kg/day normal saline.
- Group II: Rats subcutaneously received with ethanolic extract of *S. hortensis* at 200 mg/kg/day for six weeks.
- Group III: Rats subcutaneously received with cadmium chloride ($CdCl_2$) 3 mg/kg/day for six weeks.
- Group IV: Rats subcutaneously received with ethanolic extract of *S. hortensis* at 100 mg/kg/day followed by cadmium chloride 3 mg/kg/day for six weeks.
- Group V: Rats subcutaneously received with ethanolic extract of *S. hortensis* at 200 mg/kg/day followed by cadmium chloride 3 mg/kg/day for six weeks (Pari and Murugavel, 2005).

2.5. Activities of serum marker enzymes

After 42 days, blood samples collected from heart by cardiac puncture technique of unconscious rats with 100 mg/kg of ketamine hydrochloride and 16 mg/kg intraperitoneal xylazine 2%. Then blood was centrifuged (2000×g for 15 min) for the separation of serum. The activities of serum aspartate transaminase (AST) and alanine transaminase (ALT) were assayed spectophotometrically according to the standard procedures using commercially available diagnostic kits (Tehran Pars Azmoon, Iran).

2.6. Histopathological investigation

For evaluation of histopathological, liver tissue samples were removed, immersed in 10% formalin for 48 h, processed, and paraffin–embedded blocks were prepared. Sections of kidney (3 – 5 µm thick) were prepared and then stained with hematoxylin and eosin dye. Light microscopy was used to evaluate the histopathological of liver tissue.

2.7. Statistical analysis

The data were statistically analyzed using one–way ANOVA by the program SPSS (19.0). Means of characteristics were compared by Tukey test at $p < 0.05$ level.

3. Results and discussion

Statistically results indicated that cadmium–induced had a significant effect on AST ($p < 0.01$) with the highest AST (78.3 ± 8.1 IU/L) obtained from rats subcutaneously received with cadmium chloride ($CdCl_2$) at 3 mg/kg/day (Fig. 1). The lowest AST obtained from two treatments including control rats subcutaneously received with 2 ml/kg/day normal saline (56.1 ± 4.4 IU/L). Of course, Groups I, II and V i.e. controls and rats subcutaneously received with extract of *S. hortensis* at 200 mg/kg/day followed by cadmium chloride at 3 mg/kg/day had not significant differences (Fig. 1). Results demonstrated there was significant difference ($p < 0.05$) between different groups for ALT. The highest ALT obtained from Group III (36.4 ± 2.1 IU/L), and the lowest ALT obtained from Group I (26.2 ± 2.0 IU/L). In addition, no significant differences were observed among ALT obtained from Group I, Group II and Group V (Fig. 2).

At the 42^{ed} day the experiment, histological investigations were done for the treated and control samples. Comparison between controls, including rats subcutaneously received with normal saline and rats

subcutaneously received with ethanolic extract of *S. hortensis*, and some treated animals is shown in Fig. 3. Results of histopathological evaluation indicated that liver tissue samples of rats subcutaneously received with cadmium chloride ($CdCl_2$) 3 mg/kg/day (Group III) had cell swelling, and necrotic hepatocyte that replaced by inflammatory cells (Fig. 3a). According to results of microscopic examinations pathological of liver tissue in group II, rats received with ethanolic extract of *S. hortensis* at 200 mg/kg/day, no showed abnormal histological changes (Fig. 3b). Liver tissue samples of rats treated with ethanolic extract of *S. hortensis* at 100 mg/kg/day followed by cadmium chloride (Group IV) had mild inflammatory cells (Fig. 3c). The best results were obtained with rats subcutaneously received with ethanolic extract of *S. hortensis* at 200 mg/kg/day followed by cadmium chloride (Group IV), when compared to the other groups as well as to the control (Fig. 3d).

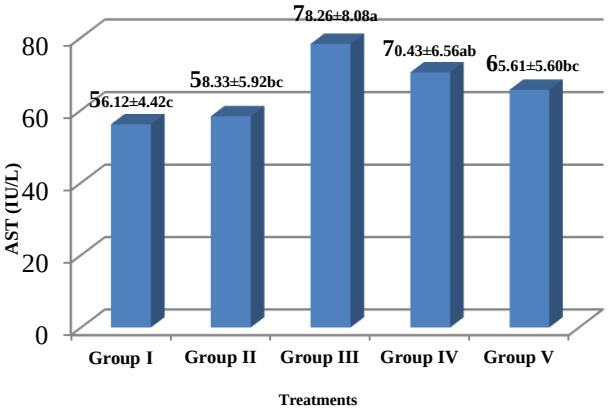

Fig. 1. Effect of ethanol extract of *S. hortensis* on AST in Cd–induced rats.
Values are mean ± SE from each group.
Significant different at $p < 0.05$ have been indicated with different letters.

Cadmium is a toxic metal that is widely used in different industries. It promotes an early oxidative stress and afterward contributes to the development of serious pathological conditions because of its long retention in some tissues (Bagchi *et al*., 1999; El–Demerdash *et al*., 2004). Cadmium induces a broad spectrum of toxicological effects and biochemical dysfunctions constituting a serious hazard to health. Cadmium interferes with antioxidant defense mechanisms together with the production of ROS, which may act as a signaling molecule in the induction of cell death (Waisberg *et al*., 2003; Renugadevi and Milton Prabu, 2009). In current study sub–chronic exposure with $CdCl_2$ caused liver damage, demonstrated by histopathological alterations. Histopathology evaluation revealed that $CdCl_2$ exposure caused a moderate hepatocyte degeneration (ballooning) and a discrete necrosis. Our results confirmed earlier reports that cadmium causes poisoning in various tissues of liver, kidneys, testes etc in humans and animals (Stohs *et al*., 2000).

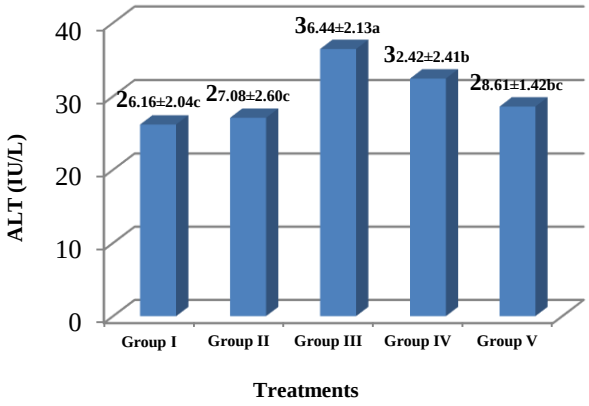

Fig. 2 Effect of ethanol extract of *S. hortensis* on ALT in Cd–induced rats.
Values are mean ± SE from each group.
Significant different at $p < 0.05$ have been indicated with different letters.

Results of this study indicated that aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities increased by $CdCl_2$ exposure. Our results are in agreement with results of study by Borges *et al*. (2008) that reported rats exposed to $CdCl_2$ presented increase in AST and ALT activities. In addition, Santos *et al*. (2005) concluded that cadmium exposed–mice presented an increase in plasma AST and ALT activities that could indicate a decrease in liver enzymes activity. El–Demerdash *et al*. (2004) have reported that cadmium caused alterations in transaminases of rats. Therefore, the increase on the plasma activities of AST and ALT could be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream, which could give an indication of the hepatotoxic effect of cadmium (Santos *et al*., 2005).

In current study confirmed that rats subcutaneously received with ethanolic extract of *S. hortensis* (at 200

mg/kg/day) significantly restored the liver function against the toxic effects of $CdCl_2$. Our earlier report (Dadfar *et al.*, 2012b) indicated that ethanolic extract and essential oil of *S. hortensis* had high antioxidant activity and having flavonoids, phenols and terpenoid (especially oxygenated monoterpenes).

Behravan *et al.* (2007) reported that both the ethanolic extract and the essential oil of *S. hortensis* were able to reverse the oxidative damage on rat lymphocytes induced by hydrogen peroxide. Results of previous studies demonstrated that phenolic compounds in medicinal plants may reduce toxic effects on induced by carbon tetrachloride on the liver and preventing the release of enzymes glutamic pyruvic acid, transaminase and alkalin phosphatase into blood (Marchishin, 1983).

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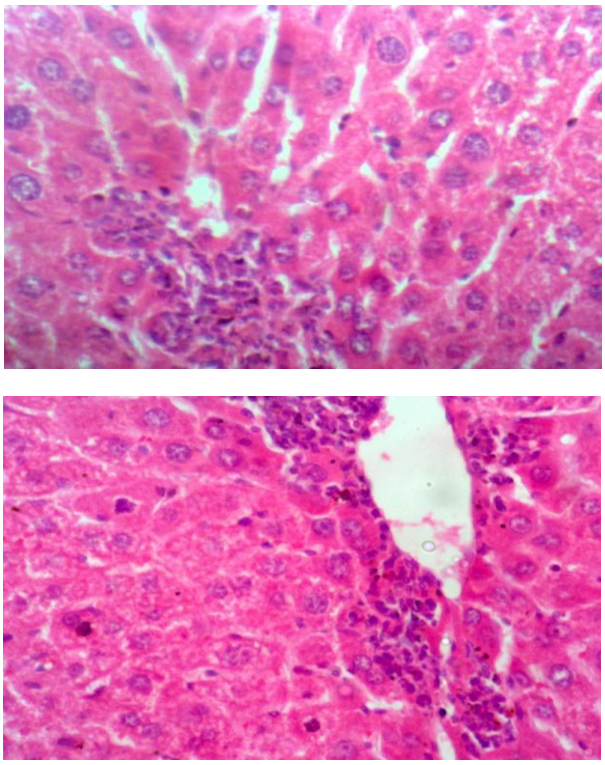
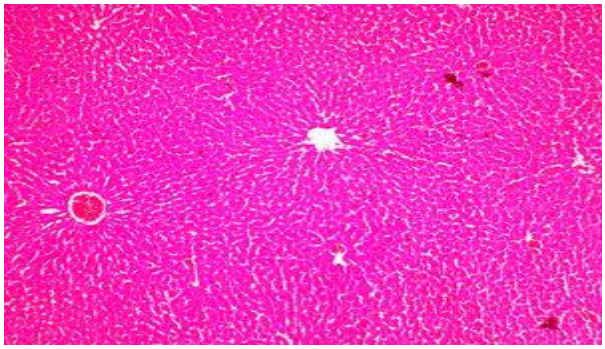
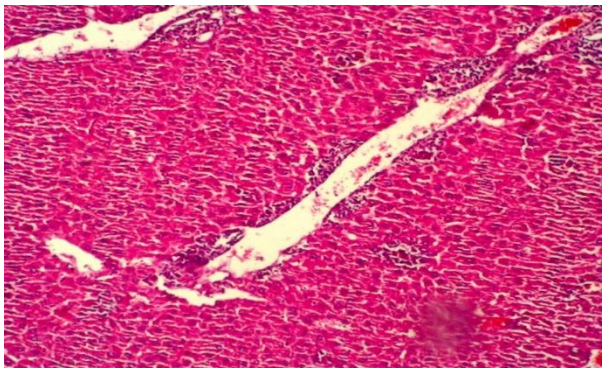


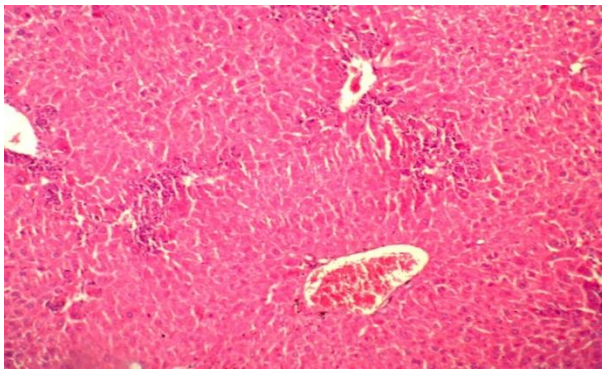
A. Rats subcutaneously received with $CdCl_2$ (Group III)



B. Rats subcutaneously received with ethanolic extract of *S. hortensis* at 200 mg/kg/day (Group II)



C. Rats treated with ethanolic extract of *S. hortensis* at 100 mg/kg/day followed by with $CdCl_2$ (Group IV)



D. Rats subcutaneously received with ethanolic extract of *S. hortensis* at 200 mg/kg/day followed by $CdCl_2$ (Group V).

Fig. 3. Histological evaluation of liver tissue samples of various treatments (magnification 400x).

4. Conclusions

The results suggest that the use of *Satureja hortensis* extract as an antioxidant seems to be useful in therapy of

cadmium poisoning, since it has the capability to alleviate many of the harmful effects of cadmium.

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