Histopathological evidences for effect beneficial of walnut extract on hepatic lesion by cadmium-induced in Rat

Elham Moghtadai Khorasgani, 1,2 Abdollah Ghasemi Pirbalouti and Shahriar Adibi

1Department of Pathology, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, P O Box: 166, Shahrekord, Iran
2Department of Medicinal plants, Shahrekord Branch, Islamic Azad University, P O Box: 166, Shahrekord, Iran

Abstract

Cadmium (Cd) is an important industrial and environmental pollutant. Cd 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. The ethanol extract of walnut leaf extract, a native plant from Iran, was evaluated for its activity against Cd-induced. The ethanol extract of walnut leaf (100 and 200 mg/kg/day for 6 weeks) was examined on serum biochemical and hepatic histopathological characteristic of males rats, which are Cd-induced (3 mg/kg/day for 6 weeks). The biochemical results indicated that aspartate transaminase (AST) and alanine transaminase (ALT) significantly increased in serum by cadmium-induced in all treatments. The liver histopathological results revealed that the ethanol extract of walnut leaf treatment at 200 mg/kg/day significantly reduced toxicity by cadmium-induced. The ethanol extract of walnut leaf prevents the cadmium-induced lesions in hepatic function. Known antioxidant, antimicrobial, antipathotoxic, nephroprotective potentials of the extract of walnut leaf may be the mechanisms by which this plant protects animals against experimentally cadmium-induced.

Key words: Cadmium, Walnut, Aspartate transaminase (AST), Alanine transaminase (ALT)

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). Cd 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) (Jarup, 2002). Cd is used extensively in electroplating, although the nature of the operation does not generally lead to overexposures (Syers and Mackay, 1986).

It is also reported that increased concentration of cadmium in agricultural soils is known to come from the application of phosphate fertilizers, sewage sludge, waste water and pesticid. Cd performs its effect on living organisms by accumulating in various tissues and affecting tissue antioxidant enzyme systems (Ozdemir and Dursun, 2009). Cd toxicity contributes to a large number of health conditions, including the major killer diseases such as heart disease, cancer and diabetes. Prolonged exposure to Cd results in injury to the liver, lungs, kidney and testes (Zitkevicius et al., 2011). Cd 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, 1991). Several mitigative measures have been suggested to explain the damage induced by Cd (Milton Prabu et al., 2012).

Parenteral administration of Cd 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. This results in increased lipid peroxidation, DNA damage, depletion of sulfhydryls, altered calcium homeostasis, hepatic congestion, ischemia and hypoxia (Bharavi et al., 2010 and Habeebu et al., 1998).
The other possible mechanism of cadmium toxicity is the displacement of essential metals especially zincons requiring enzymes that are inactivated through direct displacement from their binding site by Cd (Gupta et al., 1991). Zinc, which is protective against cadmium, is becoming increasingly deficient in the soil and consequently in foods. Cd displaces zinc in many metallo-enzymes and many of the symptoms of cadmium toxicity can be traced to a Cd-induced Zn deficiency. Walnut Juglans regia is a plant with the scientific name and a base that grows well in temperate regions of the earth. In Iran, northern forest regions such as Astara, Talysch, Bandar Gaz roudbar and there can be planted in gardens in other parts of the country Walnut leaf extract and bacterial germicidal kill the pain. Tonic and blood purifier walnut leaves and bark, leaves and bark, especially for the treatment of diseases of the skin are helpful. It is also used to treat and control diarrhea and the excessive loss of blood during menstruation. It has been found effective against ringworm, tapeworm and other parasites. Historically, it has been used to fight poison, venomous bites, bites by rabid dogs, gangrene, and boils. There is some evidence that it has anti-tumor properties. study investigated the preventive effect of intraperitoneal injection of 200 mg/kg walnut leaf hydroalcoholic extract on alloxan-induced diabetic rats 4 weeks before and 4 weeks after diabetes induction, and concluded that the use of walnut leaf hydroalcoholic extract was effective in preventing diabetes (Asgari et al., 2008).

Walnut leaves are widely used in traditional medicine to treat Chronic diseases are used. The property Anticancer, antioxidant that proved. Walnuts contain two main groups of phenolic compounds. The phenolic compounds, especially flavonoids Protective effect against liver damage caused by toxins (Amad et al 2002)

Materials And Methods

2.1. Plant material:
The aerial parts of Walnut leaves 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 ground the aerial parts of Walnut leaves 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 Walnut 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±3°C), 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 Walnut leaves at 200 mg/kg/day for six weeks.
• Group III: Rats subcutaneously received with cadmium chloride (CdCl₂) 3 mg/kg/day for six weeks.
• Group IV: Rats subcutaneously received with ethanolic extract of Walnut leaves fat 100 mg/kg/day followed by cadmium chloride 3 mg/kg/day for six weeks.
• Group V: Rats subcutaneously received with ethanolic extract of Walnut leaves at 200 mg/kg/day followed by cadmium chloride 3 mg/kg/day for six weeks (Eidi et al., 2011).

2.5. Bilirubin content
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
(2000xg for 15 min) for the separation of serum. In finally, ALT and AST were estimated spectrophotometrically using commercial diagnostic kits (Tehran Pars Azmoon, Iran).

2.6. Evaluation of histopathology:
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 was 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.

Result:

3.1. Bilirubin content:
An analysis of variance indicated that Cd-induced had a significant effect on AST ($p < 0.01$) with the highest AST (78.3 IU/L) obtained from rats subcutaneously received with cadmium chloride ($CdCl_2$) at 3 mg/kg/day (Fig. 1). The lowest AST (78.3 IU/L) obtained from two treatments including control rats subcutaneously received with 2 ml/kg/day normal saline (56.1 IU/L) and rats subcutaneously received with extract of Walnut leaves at 200 mg/kg/day followed by cadmium chloride at 3 mg/kg/day (56.6 IU/L). No significant differences were observed among AST obtained from Group I and Group V (Fig. 1).

Statistical analysis indicated that there was significant difference between five groups for ALT. The highest ALT obtained from Group III (36.4 IU/L) and the lowest ALT obtained from Group I. Of course, no significant differences were observed among ALT obtained from Group I, II and V (Fig. 2).

3.2. Histopathological observations:
At the 42nd day the experiment, histological evaluation was 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 Walnut leaves, 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 Walnut leaves at 200 mg/kg/day, no showed abnormal histological changes (Fig. 3b). Liver tissue samples of rats treated with ethanolic extract of Walnut leaves 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 Walnut leaves 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).

Discussion:
Cadmium is a toxic metal that is widely used in different industries. It promotes an early oxidative stress and contribute to the development of serious pathological conditions because of its long retention in some tissues (Bagchi et al., 2000; EI-Demerdash et al., 2004). Cd 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). In present 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).

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 and 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 Walnut (at 200 mg/kg/day) significantly restored the liver function against the toxic effects of CdCl_2. Our earlier report (Eidi et al., 2011) indicated that ethanolic extract and essential oil of Walnut had high antioxidant activity and having
flavonoids, phenols and terpenoid (especially oxygenated monoterpenes). Several studies indicate that flavonoids can inhibit free radical formation and the propagation of free-radical reactions by chelating of transition metal-ions reported that both the ethanolic extract and the essential oil of Walnut 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 alkaline phosphatase into blood (Marchishin, 1983).

In conclusion, the results suggest that the use of Walnut 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.

![Fig. 1: Effect of ethanol extract of Walnut on AST in Cd--induced rats.](image1)
Values are mean ± SE from each group.
Significant different at $p < 0.05$ have been indicated with different letters.

![Fig. 2: Effect of ethanol extract of Walnut on ALT in Cd--induced rats.](image2)
Values are mean ± SE from each group.
Significant different at $p < 0.05$ have been indicated with different letters.

A. Rats subcutaneously received with CdCl$_2$ (Group III)

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

C. Rats treated with ethanolic extract of Walnut at 100 mg/kg/day followed by with CdCl$_2$ (Group IV)
D. Rats subcutaneously received with ethanolic extract of Walnut at 200 mg/kg/day followed by CdCl₂ (Group V).

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

References


