Rosemary extract ameliorates cadmium-induced histological changes and oxidative damage in the liver of albino rat

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Abstract The oxidative damage and histological changes of liver of rats exposed to cadmium and the ameliorative effect of rosemary extract against cadmium hepatotoxicity were studied. The results showed that exposure to Cd (30 mg/kg b.w., 5 consecutive days/week for 8 weeks) led to an increase in the MDA levels and a decrease in activity of SOD, CAT and the concentration of GSH versus controls. In contrast, administration of rosemary aqueous extract restored the changes in these parameters to nearly the normal levels. Treating animals with Cd led to different histopathological changes such as loss of the normal structure of hepatic cells, blood congestion, leukocytic infiltrations and fatty degeneration. Ultra structure results revealed abnormality in the nucleus, swelling of mitochondria, degeneration of endoplasmic reticulum, and increase of lysosomes and appearance of fat droplets. On the other hand, animals treated with Cd and rosemary showed an improvement in these changes and the tissue appears with normal structures. The results suggested that the ameliorative effect of rosemary extract may be due to its antioxidant properties in combating free radical-induced oxidative stress and tissue injury resulting from cadmium chloride.

Introduction In recent years the concentration of heavy metals increased in the environment (Valverde et al., 2000). Industries caused an increase in concentration of metals, which cause toxic effects in the environment, the harmful materials are found in every area of modern consumerism and inadvertently or inadvertently allow more and more exposure (Singh et al., 2010). Among the important heavy metals is cadmium, which is released into the environment by mining and smelting operations (primarily zinc, lead, copper, and cadmium (Zhang et al., 2012)), fuel combustion (Chen et al., 2014), incineration of municipal waste (Zhang et al., 2001), and sewage sludge (Edwards et al., 2013), and the application of phosphate fertilizer (Gill et al., 2013). Humans can get Cd from crops such as rice, potato, root and leaf of vegetables (Fan et al., 2009), and tobacco (Garcia-Esquinas et al., 2014), soil (Guo and Zhou, 2006), and fruits and oily seeds (Schwarz et al., 2014). It may be also found in animal milk (Gougoulas et al., 2014). Cadmium is a cytotoxic, carcinogenic, and mutagenic industrial product or by product (Du et al., 2014). It causes hepatotoxicity upon acute administration. Features of...
cadmium-induced acute hepatotoxicity encompass necrosis, apoptosis, peliosis and inflammatory infiltration (Kyriakou et al., 2013). Cadmium was also found to generate reactive oxygen species (ROS) that cause apoptosis (Wang et al., 2014).

Free radical cause damage which lead to peroxidation to biomembranes and DNA which lead to tissue damage, as a result caused many diseases. Antioxidants depart the effects of the free radical and may prevent the body from several diseases (Gupta and Sharma, 2006). Antioxidants known as radical scavengers, cause inhibition of lipid peroxidation and other free radicals mediate the process, and protect the human from several diseases resulting from the reaction of the radical. Many substances act as antioxidant such as flavonoids that are scavenging radicals (Czinner et al., 2001).

Natural herbs are widely consumed by humans on a daily basis, these natural products have many biologic and pharmacologic properties (Hosseinimehr, 2014). Rosemary (Rosmarinus officinalis) and its constituents especially caffeic acid derivatives such as rosmarinic acid have a therapeutic potential in prevention of bronchial asthma, spasmodic disorders, peptic ulcer, inflammatory diseases, hepatotoxicity, atherosclerosis, ischemic heart disease, cataract, cancer and poor sperm motility (Al-Sereiti et al., 1999). Results of many experiments showed that rosemary essential oil had antimicrobial, antioxidant, anti-carcinogenic, and cognition-improving effects (Faixo and Faix, 2008).

The antioxidant activity of rosemary extract can be attributed mainly to two components, carnosic acid and carnosol (Kadri et al., 2011; Machado et al., 2013). Extracts of rosemary can have both flavoring and antioxidative properties. In many cases both functions are used, but some extracts are to be used primarily for their antioxidant properties. In such cases the processing of the rosemary can be optimized to enhance the antioxidative function and to reduce that of the flavoring, antioxidants are required in foods to prevent oxidation of oils and production of off-flavors (Aguilar et al., 2008). The present work aims to explore the possible ameliorative effect of rosemary on cadmium chloride induced toxicity on liver of albino rats.

Materials and methods

Cadmium chloride (CdCl₂)

It is a chemical substance obtained from Raheja Centre, Mumbai, India. Cadmium chloride was dissolved in distilled water and was administrated orally to rats at a dose level 30 mg/kg b.w. for 5 consecutive days per week for 8 weeks according to Ohta et al. (2000).

Preparation of rosemary extract

Rosemary (R. officinalis) was collected from greenhouse in Faculty of Science, Menoufia University, Shebin El-Kom, Egypt. Rosemary extract was prepared according to Dorman et al. (2003). 50 g of the powdered herb was dissolved in 500 ml distilled water in a quick fit round bottom flask connected to a hydrodistillation apparatus. It was then left to slowly boil for 120 min. The water in flask was removed and replaced by another 300 ml of fresh distilled water and boiled for another 60 min, then filtered. The filtrate was subjected to lyophilization process by a freeze dryer under pressure 0.1–0.5 mbar and temperature −35 to −41 °C. The dry extract was stored at 4 °C until used.

Animals and treatments

Male albino rats (Rattus norvegicus) weighing 120 ± 5 g were kept in standard laboratory condition for at least one week before initiation of the experiments, being maintained on standard rodent diet, and were given free access to food and water. The animals were housed in especially designed plastic rodent cages in animal house in Faculty of Science, Menoufia University, Shebin El-Kom, Egypt. This study and all procedures were approved by the Animal Care and Bioethics of the Egyptian Committee, and the animal work was done at Faculty of Science, Menoufia University. The animals were divided into four groups:

**Group 1:** Animals served as the control group.

**Group 2:** Rats given rosemary orally at a dose of 220 mg/kg b.w. for 5 consecutive days per week for 8 weeks (Dorman et al., 2003).

**Group 3:** Animals were orally administrated with CdCl₂ at a dose level 30 mg/kg b.w. for 5 consecutive days per week for 8 weeks according to Ohta et al. (2000).

**Group 4:** Rats administrated orally CdCl₂ and rosemary extract for 5 days per week for 8 weeks.

Light and electron microscopic examination

The treated animals and their controls were anesthetized and dissected after 4 and 8 weeks of treatment. Livers were removed and fixed in 10% neutral formalin for 24 h, washed in running tap water for 24 h, and dehydrated in ascending grades of ethanol and two changes, cleared in two changes of xylene and embedded in paraplast and sections of 5 micrometer thickness were cut. Slides were stained with haematoxylin and eosin for histological examination. For ultrastructural examination very small pieces of liver were fixed in glutaraldehyde then rinsed in phosphate buffer, post fixed in buffered solution of 1% osmium tetroxide for 3 h at 4 °C, then processed with the standard steps: dehydration, infiltration, embedding and polymerization. The ultrathin sections were examined by using JEOL electron microscope (Karnovsky, 1965).

Biochemical assays

For biochemical study, livers were removed and homogenized in normal mammalian saline (0.9% NaCl) solution (1 mg tissue in 10 ml saline), using ultrasonic homogenizer. Tissue homogenate was kept in −20 °C deep freeze for one week to allow enzymes to liberate in the homogenate. Samples were centrifuged by cooling centrifuge and the supernatant was taken for biochemical analysis of enzymes. Glutathione (GSH) was estimated using the method of Buettler and Kelly (1963). Catalase (CAT) was determined according to the method of Goth (1991). Superoxide dismutase was determined according to Beauchamp and Fridovich (1971). Lipid peroxidation was measured according to Ruiz-Larrea et al. (1994).
**Statistical analysis**

The data were expressed as mean ± standard error. Data were analyzed by using Student’s *t*-test and homogeneity of variances (Levene test) using statistical program of social science (SPSS) software for windows. *P* < 0.05, *P* < 0.01 and *P* < 0.001 values were used.

**Results**

**Histological results**

*Light microscope observations*

Liver of control rat showed normal lobular architecture. The hepatic cells were found arranged in strands around the central vein and sinusoids appeared containing Kupffer cells (Fig. 1). Liver obtained from rats treated with rosemary extract exhibited the normal structure. Animals treated with CdCl₂ for 4 weeks revealed that the hepatic tissue was injured. The hepatic blood vessels were enlarged and congested (Fig. 2). Perivascular inflammatory infiltrates were observed (Fig. 3). Most of the hepatocytes showed cytoplasmic vacuolation with pyknotic nuclei (Fig. 4). After 8 weeks of treatment, these changes became intensive. The hepatic architecture was lost, masses of leukocytic infiltration were observed (Fig. 5) and cytoplasmic vacuolation was noticed in most cells. Fatty infiltrations of different sized fat droplets were observed (Fig. 6). After treatment with CdCl₂ and rosemary, an improvement was recorded in the hepatic tissue. In these specimens, the hepatocytes appeared normal with an increase of binucleated cells, few veins showed congestion and the sinusoids appeared with activated kupffer cells (Fig. 7).

*Ultrastructural observations*

Liver sections of control animals examined under electron microscope showed normal hepatocytes, each hepatocyte contained rounded nucleus containing one nucleolus. The nucleus was surrounded by nuclear envelop. The chromatin consists of dense clumping heterochromatin and lightly stained euchromatin. The cytoplasm appeared granular containing rounded and elongated mitochondria, rough endoplasmic reticulum, glycogen particles and Golgi apparatus (Fig. 8).

Many alternations were observed in hepatocytes of animals treated with CdCl₂. The nucleus was abnormal and showed outgrowth projections (Fig. 9). Rough endoplasmic reticulum was dilated and mitochondria appeared with disrupted cristae. Kupffer cell appeared in phagocytosis stage and cytoplasmic...
vacuoles were observed (Fig. 10). After 8 weeks more damage
was exhibited in the hepatocytes. Most of the nuclei were pyk-
notic and mitochondria became more degenerated (Fig. 11).
Fat vacuoles were abundant (Fig. 11). Hepatocytes of rats
given CdCl₂ and rosemary revealed that the nucleus appeared
normal with normal envelop, but with little heterochromatin

Figure 5  Liver section of a rat treated with Cd for 8 weeks
showing mass of inflammatory cells (H&E, scale bar = 0.068 µm).

Figure 6  Liver section of a rat treated with Cd for 8 weeks
showing fatty infiltrations (F) (H&E, scale bar = 0.068 µm).

Figure 7  Liver section of a rat treated with Cd and rosemary
showing restoration of structure of the liver tissue, Bi: binucleated
cell (H&E, scale bar = 0.068 µm).

Figure 8  TEM micrograph of a normal hepatocyte showing
nucleus (N), nucleolus (NU), nuclear envelop (NE), Golgi (G),
mitochondria (M) and rough endoplasmic reticulum (rER) (scale
bar = 2 µm).

Figure 9  TEM micrograph of liver of a rat treated with Cd for
4 weeks showing nucleus with abnormal envelop (arrow head),
and megamitochondria (M) (scale bar = 500 nm).

Figure 10  TEM micrograph of liver of a rat treated with Cd for
4 weeks showing Kupffer cell in phagocytosis stage (K), degener-
ated mitochondria (M) and fat droplets (arrow) (scale
bar = 2 µm).
and euchromatin (Fig. 12). Mitochondria and rough endoplasmic reticulum were normal. Binucleated hepatocytes were abundant (Fig. 13).

Biochemical results

Data in Figs. 14–16 show that there are no significant differences in levels of GSH, SOD and CAT in both control and rosemary extract groups. Animals treated with CdCl₂ revealed that levels of GSH, SOD and CAT decreased significantly ($P < 0.001$) after 4, 8 weeks. On the other hand, treating animals with CdCl₂ and rosemary extract led to an increase in these antioxidant enzymes. This increase was highly significant ($P < 0.001$) after 8 weeks of treatments. Lipid peroxidation marker (MDA) decreased significantly in rats given CdCl₂ (Fig. 17). Animals treated with CdCl₂ and rosemary showed a significant increase in MDA.

Discussion

The present results showed that CdCl₂ administration caused hepatotoxicity in rats. Histological and ultrastructural results revealed many alterations. In agreement with these results, Gaurav et al. (2010) observed cytoplasmic vacuolization, karyolysis, pyknosis and centrilobular necrosis in liver of rats after receiving single dose of CdCl₂. Marked changes in liver of male mice such as swelling and massive fatty degeneration in hepatocytes and large vacuoles in cytoplasm, pyknotic nuclei with comparatively poor staining affinity, due to damage of the hepatic cells were recorded after treatment with CdCl₂. Apoptosis was observed by Gathwan et al. (2012). Liver of cadmium treated female rat showed degeneration of liver, inflammatory leukocyte infiltration near the blood vessel and blood sinusoid dilatation (Mohammad et al., 2013). Dilated and congested central vein with massive hemorrhage extending to the nearby cells, mild periductal fibrosis around bile duct in the portal area, focal degenerative and necrotic changes along with inflammatory cell infiltration were noticed in male guinea pigs that received CdCl₂ (Azab et al., 2014). Fatty degeneration was observed in liver of CdCl₂-treated rats. Brody et al. (1961) attributed the fatty changes in the liver to excessive mobilization of free fatty acids from the fat depots induced by the lipolytic effects of the increased circulating catecholamines and the centrilobular necrosis to the catecholamine-induced decrease in hepatic flow.

Concerning the ultrastructure results, the current study revealed that cadmium chloride induced many alternations in liver tissue when examined by electron microscope, similar to results obtained by Kim and Yoon (2000), who reported that cadmium chloride induced nuclear membrane rupture and lysosomes were observed in cytoplasm, mitochondria destruction and glycogen appeared in liver sections of mouse. Cadmium chloride caused fragmentation in rough endoplasmic reticulum, and swollen mitochondria lost their cristae in liver of rats (Abdel-Moneim and Ghafeer, 2007). Damage of the nuclear membrane, regression of mitochondrial cisternae, deterioration of rough endoplasmic reticulum, loss of glycogen particles, and proliferation of smooth endoplasmic reticulum with condensation of the nuclear chromatins appeared in liver of rats treated with CdCl₂. The hepatocytes also appeared to contain many cytoplasmic fat droplets and many vacuoles (Mahran et al., 2011). According to Leo et al. (1982), the vacuolation of the liver cells can be attributed to swelling of the mitochondria and proliferation of the endoplasmic reticulum.
The obtained results showed an increase in lipid peroxidation marker MDA and decrease in the antioxidant enzymes, CAT and SOD. These results are similar to those of Ramesh and Satakopan (2010) who reported a decrease in CAT and SOD, and increase in MDA in liver of rats treated with CdCl₂. Bekheet et al. (2011) recorded a decrease in the activities of SOD and CAT, with a significant increase in the level of MDA in liver of rats exposed to CdCl₂. Acute exposure to CdCl₂ leads to an increase in lipid peroxidation (LPO), with a decrease in levels of CAT and SOD enzymes in liver of mice.

Figure 14  Effects of cadmium chloride and rosemary extract on liver (GSH) (mg/g tissue).

Figure 15  Effects of cadmium chloride and rosemary extract on liver (CAT) (kU/L).

Figure 16  Effects of cadmium chloride and rosemary extract on liver (SOD) (% inhibition).
Effect of rosemary extract on cadmium induced toxicity on liver

Abdel-Moneim caused a decrease (Abd was observed by treated on liver. This was manifested led to a significant decrease in liver GSH and this may be attributed to its

ring in liver by CdCl₂ non-lipid systems (Haraguchi et al., 1995). In this work rosemary is a good scavenger of peroxyl radicals and it is able of anti-proliferation and pro-apoptosis (Zhang et al., 2011).

acid is one of the rosemary components acting as a preventive agent for fibrosis progression in liver partly due to its activity of anti-proliferation and pro-apoptosis (Zhang et al., 2011). Rosemary is a good scavenger of peroxyl radicals and it is able to block the formation of the hydroxyl radical generated in non-lipid systems (Haraguchi et al., 1995). In this work rosemary extract was found to ameliorate the alternations occurring in liver by CdCl₂ and this may be attributed to its antioxidative action.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this work.

Effect of rosemary extract on cadmium induced toxicity on liver (LPO) (μmol MDA formed/h/g tissue).

References

Paul et al., 2013). The non-enzymatic antioxidant, GSH decreased in liver of rats treated with CdCl₂. Abdel-Moneim and Ghafeer (2007) reported a decline in GSH activity in liver of adult male rats treated with cadmium chloride. Administration of CdCl₂ led to a significant decrease in liver GSH (Ramesh and Satakopan, 2010). Non-enzymatic (GSH) antioxidants were significantly decreased in liver of CdCl₂ treated rats (Kumar et al., 2010). Depletion in reduced glutathione (GSH) in liver of rats injected with CdCl₂ was observed by Ibrahim (2013).

This work revealed that rosemary aqueous extract ameliorated the toxic effects of CdCl₂ on liver. This was manifested by the histological observation, decrease of MDA and increase in liver CAT, SOD, and GSH. These results are in agreement with Parmar et al. (2011), who reported that treatment with aqueous extract of rosemary leaves prevents oxidative stress caused by Dimethylbenz[α]anthracene (DMBA). Abd El-Ghany et al. (2012) found that after rosemary treatment almost healthy liver with no histopathological changes was observed in rats treated with CCl₄. Rosemary powder and extracts caused an increase in SOD and a decrease in MDA in patients with hepatic failure caused by CCl₄ (Abd El-Ghany et al., 2012). Virk et al. (2013) reported that rosemary aqueous extract given with CdCl₂ caused a decrease in MDA, increase in SOD and a decrease in GSH activity in liver of adult rats treated with CdCl₂ by Dimethylbenz[a]anthracene (DMBA).

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