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Protective effects of ethanolic extract of rosemary against lead-induced hepato-renal damage in rabbits

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ABSTRACT

In traditional medicine, *Rosmarinus officinalis* L. leaf is used as a curative herbal therapy for the treatment of several diseases. The protective effects of rosemary in toxic effects of some environmental pollutants are known. However, there is paucity of information about its protective effects on lead acetate (LD) toxicity. To assess the protection of rosemary ethanolic extracts (REE) on LD-induced hepato- and nephro-toxicity, male albino rabbits were treated with REE (30 mg/kg) and/or LD (30 mg LD/kg) by gavage administration for 30 days. The total phenolic compound content in REE was estimated using Folin–Ciocalteu's assay and phyto-constituents were isolated and identified using gas chromatographic and mass spectrometry (GC–MS) analysis. The protective effect of REE in LD-induced liver and renal dysfunction and blood cells was evaluated by estimating blood biomarkers of liver and renal damage, histological, and biochemical examinations. Antioxidant enzyme activities, lipid peroxidation biomarker, protein and glycogen contents were estimated in both liver and kidney homogenates. The GC–MS analysis revealed that REE is rich in phenolic compounds including camphor, phytol, borneol, caryophyllene oxide, isopulegol, thymol, and verbenone. REE pre-treatment significantly ($P<0.05$) suppressed levels of LD induced hepatic and renal damage products as well as lipid peroxidation. In contrast, pre-treatment using REE significantly ($P<0.05$) decreased LD-induced depletion of antioxidant enzymes, protein, and glycogen content. Additionally, REE preserved blood cells and their structure and renal and hepatic architecture. In conclusion, these findings revealed that REE protects from toxic effects of LD possibly through its free radical-scavenging and antioxidant activities.

1. Introduction

A strong correlation between the exposures to various environmental pollutants and both renal and hepatic damage has been reported through several epidemiological studies (Gao et al., 2015). Lead (Pb), a potential pollutant, is known to induce hepatic- and nephro-toxicity in most animal species and humans (Soliman et al., 2015). In autopsy studies of Pb-exposed humans, among the soft tissues, liver is the largest depot (33%) of Pb followed by kidney cortex and medulla (Mudipalli, 2007).

The significance of Pb-toxicity lies in its wide distribution in ambient air, foods, drinking water, and as an antiknock agent in gasoline (Abdou and Hassan, 2014). Also, Pb is widely used in the industry because of its anti-corrosive properties and low melting point (Soliman et al., 2015). Food cans' soldering, lead-based paints, ceramic glazes, drinking water systems, and folk remedies are the main products containing Pb that pose hazard of Pb toxicity (Markowitz, 2000). Pb toxicity can lead to carcinogenicity (Landrigan et al., 2000), haematological abnormalities (Iavicoli et al., 2003), cardiac damage (Patra and Swarup, 2004), immunological alterations (Shah and Altindag, 2005), metabolic and reproductive disorders (Teijón et al., 2006) and nerve dysfunction (Ademuyiwa et al., 2007).

In earlier studies, several mitigation strategies have been adopted to alleviate Pb hazards. For instance, chelating agents such as calcium disodium ethyl diamine tetra acetic acid and magnesium dimercaptosuccinic acid have been reported to reduce Pb associated toxicological effects (Adikwu et al., 2013). Recent studies have shown that the plant extract based supplements are beneficial for prevention and attenuation of Pb toxicity. These supplements are cost effective, can be easily added to daily diet,

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and have very few side effects compared with the chemical therapy (Zhai et al., 2015).

Rosemary (*Rosmarinus officinalis*), a widespread household plant grown around the world, is used as flavouring agent for food, beverages, and cosmetics preparations (Ibarra et al., 2010). The extract of rosemary leaves contains a complex repertoire of biologically active phytochemicals including carnosol, carnosic acid, rosmanol, 7-methyl-epirosemanol, isorosmanol, rosmadial and caffeic acid (Ho et al., 1994). It possess pharmacological activities such as antiviral (Nolkemper et al., 2006), radioprotective (Del Bano et al., 2006), antimicrobial (Bernardes et al., 2010), anti-inflammatory (Minaiyan et al., 2011), neuroprotective (Azad et al., 2011), hypolipidaemic agent (Afonso et al., 2013), hepatoprotective (Ramadan et al., 2013) and nephroprotective (El Saied Azab, 2014).

The protective role of rosemary extracts in lead acetate (LD)-induced hepato-nephrotoxicity is not fully explored. Therefore, the present study was planned to evaluate hepato- or nephro-protective effects of rosemary in LD-induced toxicity by examining markers of liver and kidney function. Moreover, we also investigated the possible mechanisms of hepatic- and nephro-protective activity of rosemary.

2. Material and methods

2.1. Chemicals and other reagents

Lead acetate (99.6% purity) was purchased from El-Nasr Pharmaceutical Chemical Co. (Qaliubiya, Egypt). For experimental use, working stock solution of LD was prepared by diluting it in distilled water. Commercial colorimetric bioassay kits (BioMérieux, Marcy l'etoile, France) were purchased to estimate serum AST, ALT, ALP, urea, and creatinine, as well as of SOD, CAT activities and MDA (Biodiagnostics Co., Giza, Egypt). All other analytical grade reagents, chemicals, and stains were purchased from Sigma (Sigma, St. Louis, MO).

2.2. Plant material

The leaves of *Rosmarinus officinalis* purchased from a local herb market (Harraz Medicinal Herbs Market, Zagazig, Egypt) were verified and authenticated by Dr. Samir Salem Teleb, a Botanist of Taxonomy and Flora of Egypt, at the Department of plant, Faculty of Science, Zagazig University, Zagazig, Egypt. A voucher specimen (RM-2015) was deposited in the herbarium of same department. Plant leaves were dried at 40 °C in a vacuum oven and powdered fine in a mill and kept in a tightly closed container until extraction.

2.3. Preparation of rosemary ethanolic extracts (REE)

Following the method of Taha and Osman, (2015), the rosemary leaf powder (1700 g) was extracted three times in 80% ethanol (17 L) using a magnetic stirrer at room temperature and filtered through Whatman filter paper (No. 1). The residues were re-extracted by the same method. The extract was concentrated and dried using rotary evaporator (BÜCH-water bath-B-480, Switzerland) and lyophilised by Freeze-Dryer (Thermo-Electron Corporation – Heto power dry LL300 Freeze Dryer, Czech Republic). The dried extract was stored at −20 °C until further use.

2.4. Determination of total phenolic compounds

Total phenols concentration in the REE was determined by a UV spectrophotometer (Jenway-UV–vis Spectrophotometer), implying a colorimetric oxidation/reduction reaction, as previously outlined by Škerget et al. (2005). The diluted extract (10 mg in 10 mL solvent) 0.5 mL was added to 2.5 mL of Folin–Ciocalteu oxidising reagent (10 times diluted with distilled water) and 2 mL of Na_2CO_3 (75 g/L) and incubated at 50 °C for 5 min, then cooled. For control sample, 0.5 mL of distilled water was used. The absorbance was measured at 760 nm wavelength. Total phenolic content, expressed as gallic acid equivalent (GAE), was calculated using the following equation and the calibration curve:

$$y = 0.015x + 0.0533$$

$$R^2 = 0.9966$$

Where y is the absorbance and x is the concentration (mg GAE g^{-1} extract).

R^2 = Correlation coefficient

2.5. Gas Chromatography/mass spectrometry analysis (GC–MS) of REE

The REE samples were subjected to GC–MS analysis using a 1310 TRACE GC Ultra Gas Chromatographs (Thermo Fisher Scientific Inc., Waltham, MA, USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer) at the Regional Center for Mycology and Biotechnology, Al-Azhar University Campus, Nasr city, Cairo, Egypt. The GC–MS system was equipped with a DB5 MS column (30 m × 0.25 mm × 0.25 μm film thickness) (J&W Scientific, Folsom, CA, USA). Helium was used as a carrier gas at a flow rate of 1.5 mL/min at a split ratio of 1:10 and the following temperature program was used: 50 °C for 1 min; temperature incrementally increased by 10 °C/min to 150 °C and held for 2 min followed by increase of 5 °C/min to 250 °C and held for 1 min. The injector and detector were held at 250 °C and 300 °C, respectively. Diluted samples (1:10 hexane, v/v) of 0.2 μL of the mixtures were injected. Mass spectra were obtained by electron ionisation (EI) at 70 eV, using a spectral range of *m*/*z* 40–450. Most of the compounds isolated were identified using mass spectra library of authentic chemicals of Wiley spectral library collection and National Institute of Standards and Technology [NIST] library. The isolated components of the extract were identified by matching their mass spectra with the NIST published data (Adams, 2007).

2.6. Experimental design

Forty albino male rabbits (8 weeks of age and 900–1000 g of weight) were purchased from the Laboratory Animal Farm at Zagazig University and housed in stainless steel cages maintained in a pathogen-free environment at a controlled temperature (21–24 °C), relative humidity of 50–60% and a 12-h light-dark cycle. All rabbits were given standard pelleted diet (El-Nasr Co., Abou-Zaabal, Cairo, Egypt) and water *ad libitum*. Rabbits were acclimated for two weeks before their experimental use.

For experiments, rabbits were weighed and randomly divided into following four groups of 10 animals each and given a daily oral dose of different treatments by gavage method. Group I (control group) received distilled water for 30 days. Group II (REE group) treated with 30 mg REE/kg/day for 30 days. The dose of REE was chosen based on a pilot study of different doses ranging from 20 to 200 mg/kg of body weight (data not shown). The range of doses was taken from previous studies (Al-Hader et al., 1994; Bakırel et al., 2008). Group III (LD group) was administered 30 mg LD/kg/day for 30 days (Ahmed et al., 2012). Group IV (REE/LD) was treated with REE for 30 days followed by LD for 30 days at similar doses used in Groups II and III. The rabbits were carefully monitored throughout the experiment for signs of toxicity, morbidity, and mortality. Dose volumes were adjusted with weight of rabbit every