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Protective effects of *Etlingera elatior* extract on lead acetate-induced changes in oxidative biomarkers in bone marrow of rats

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ABSTRACT

Several environmental toxins with toxic effects to the bone marrow have been identified. Pathology associated with lead intoxication is due to the cellular damage mediated by free radicals. In the current study, we examined the effect of *Etlingera elatior* extract on lead-induced changes in the oxidative biomarkers and histology of bone marrow of rats. Sprague–Dawley rats were exposed to 500 ppm lead acetate in their drinking water for 14 days. *E. elatior* extract was treated orally (100 mg/kg body weight) in combination with, or after lead acetate treatment. The results showed that there was a significant increase in lipid hydroperoxide, protein carbonyl content and a significant decrease in total antioxidants, super oxide dismutase, glutathione peroxidase and glutathione – S-transferase in bone marrow after lead acetate exposure. Treatment with *E. elatior* decreased lipid hydroperoxides and protein carbonyl contents and significantly increased total antioxidants and antioxidant enzymes. Treatments with *E. elatior* extract also reduced, lead-induced histopathological damage in bone marrow. In conclusion, these data suggest that *E. elatior* has a powerful antioxidant effect, and it protects the lead acetate-induced bone marrow oxidative damage in rats.

1. Introduction

Human exposure to lead is still considered to be a serious health problem (Godwin, 2001; Goyer, 1990). Although blood lead levels continue to decline in our population, specific population groups are at disproportionately higher risk for lead exposure, especially the lower age groups such as infants and young children, and the working class with direct contact with lead in the industries. While the measures are in place to reduce lead exposure, there are two sources of exposure, i.e. lead based, paint and exposure to lead related industries, which are either inevitable or reducing at a rate so slow that is still a threat to public health (Martin et al., 2001). Lead absorption by ingestion depends on factors such as the particle size, physical form, gastrointestinal transit time and nutritional status of a person. Lead absorption increases, with increasing age, making children and infants more vulnerable to lead intoxication (Barry, 1981; Campbell et al., 2004; Pirkle et al., 1998). Besides acute toxicity, lead has an extremely long, half life in bone. Individuals with past exposure develop increased blood lead levels during periods of high bone turn over, or resorption, making chronic sub acute levels of lead exposure a serious health concern. Lead finds its way to the hard tissues like bone and teeth, where it accumulates, only to result in a sustained release and maintenance of an unacceptable blood lead level, many years after the exposure period (Escribano et al., 1997; Needleman et al., 1979; Pounds et al., 1991; Schwartz et al., 1986).

Being the ubiquitous environmental toxin, lead is known to induce a broad range of effects on many organ systems in humans (El-Nekeety et al., 2009; Hao et al., 2002; Royce et al., 1990). Even though there are many studies explaining the plausible mechanism of lead-induced physiological, biochemical and behavioral dysfunctions, the mechanism of some of the symptoms of the lead poisoning is not clearly explained yet (Gruber et al., 1999; Mateo et al., 2003; Wei et al., 2002). Many undesired effects of lead are thought to be via generation of reactive oxygen species (ROS) and by inducing oxidative stress. Several studies reported either elevated lipid peroxidation or decreased intrinsic antioxidant defense in different tissues after lead exposure, confirming the lead-induced oxidative deterioration of biological macromolecules as the possible contributor to the pathogenesis of lead poisoning (El-Nekeety et al., 2009; Hsu and Guo, 2002; Xu et al., 2005; Yiin and Lin, 1995). An

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oxidative stress study conducted by Monteiro et al. (1985) strongly suggested lead-induced oxidative damage when they discovered increased human erythrocyte super oxide dismutase and glutathione peroxidase activities, which showed the cellular responses to oxidative stress. Recent *in vivo* studies in lead exposed animals and workers showed the generation of reactive oxygen species, stimulation of lipid peroxidation and decreased antioxidant defense system stoutly supporting the function of oxidative stress in lead toxicity (Bolin et al., 2006; Patra et al., 2001; Patrick, 2006; Sandhir and Gill, 1995).

Etlingera elatior is an herbaceous perennial plant native to South East Asia. The plant is commonly known as the 'torch ginger' and is one of the 15 species of *Etlingera* spp. identified in Malaysia (Lim, 2001). It is used in Malaysian local dishes like *Penang laksa*, *nasi goreng* etc. (Khaw, 2001; Larsen et al., 1999; Noweg et al., 2003; Smith, 1979). The leaves and rhizomes of *E. elatior* are known to have high antioxidant properties (Habsah et al., 2005a; Ibrahim and Setyowati, 1999). Chan and Lim (2007) reported that, leaves of this plant have highest total phenolic content, ascorbic acid equivalent antioxidant capacity and tyrosine inhibiting activities. Four flavonoid compounds have been isolated from *E. elatior* by Williams and Harborne (1977) which contribute the high reactive species scavenging effect to this plant. In a recent study, Habsah et al. (2005a) have identified two newer compounds from extract of this plant which, contributed to the powerful antioxidant properties of this plant.

In the event that the generation of reactive oxygen species is mainly implicated in lead toxicity, a therapeutic strategy to increase the antioxidant defense system of the body may be of benefit for long term effective treatment of lead poisoning. The objective of the present study was to evaluate the protective effect of ethanol extract of *E. elatior* lead acetate-induced toxicity in the bone marrow of rats.

2. Materials and methods

2.1. Chemicals

Lipid hydroperoxide (LPO), protein carbonyl (PC), super oxide dismutase (SOD), glutathione peroxidase (GPx), glutathione S-transferase (GST) assay kits were purchased from Cayman Chemicals (Cayman Chemicals and Pierce Biotechnology, USA). Alkaline phosphatase (ALP) assay kit was obtained from Bioassay Systems (USA). Lead acetate was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Plant materials

E. elatior was collected from nursery in Sungai Buloh, Malaysia, authenticated by Department of Horticulture, University Putra Malaysia and the voucher kept in International Medical University (voucher specimen: IMU/PMR/2008/01).

2.3. Plant extraction

About 4 kg of inflorescence of *E. elatior* was collected, washed and cut into small pieces and again washed and soaked in running tap water. The fresh dried petals were powdered and extracted with ethanol. The mixture was filtered, evaporated in vacuum evaporator and lyophilized. The obtained extract was kept at −20 °C until it was used. The extract was standardized using HPLC examining the amount of gallic acid, which was 71 ± 2 μg/g.

2.4. Experimental animals

Three-month old male, Sprague–Dawley rats, with body weight ranging between 200 and 250 g were purchased from University Kebangsaan Malaysia (UKM), Kuala Lumpur, Malaysia. The rats were maintained in an air-conditioned room (24 ± 2 °C), at 12 h: 12 h light–dark cycle and fed with standard diet and water *ad libitum*. All experimental procedures were in accordance with the ethical guidelines for animal experimentation and the study protocol was approved by the Institute Research and Ethical Committee.

2.5. Experimental design

At the commencement of study, the animals were weighed and randomly assigned to different experimental groups. They were divided into five groups (eight rats per group); group I – control (without any treatment), group II – lead acetate alone for 14 days (500 ppm in drinking water), group III – *E. elatior* alone for 14 days (100 mg/kg body weight, orally), group IV – lead acetate with *E. elatior* for 14 days (100 mg/kg body weight), group V – lead acetate for 14 days and then *E. elatior* for next 14 days (100 mg/kg body weight). Lead acetate treatment was via drinking water and 24-h fluid intake of each rat was recorded. *E. elatior* extract was diluted with distilled water to the described concentration (100 mg/kg body weight) and the extract was, force fed via a feeding tube (0.5 ml/rat/day).

At the end of the experimental period, rats were weighed and anaesthetized with sodium pentobarbital, and blood samples were collected by cardiac puncture. Serum was separated and stored at −80 °C for biochemical analyses. The left and right femur bone of the animal was excised, the soft tissues on the bone were scrapped clean and the extreme ends of the femur were cut to reveal the marrow. A 3 mL syringe preloaded with the phosphate buffer solution was used to flush the bone marrow. The flushing and washing was repeated 3–4 times and this process ensured, complete removal of bone marrow contents from the femur bone. About 5 mL of marrow solution was collected from each rat. From the serum samples, lipid hydroperoxides (LPO), protein–carbonyl-content (PCC), super oxide dismutase (SOD), glutathione peroxidase (GPx), Glutathione S-transferase (GST) and total antioxidants (TA) were assayed using ELISA kits (Cayman Chemicals and Pierce Biotechnology, USA). The levels of antioxidant enzymes, lipid hydroperoxides, protein carbonyls and total antioxidants were expressed as units per milligrams of proteins of samples. Protein levels of the samples were estimated by protein assay kits obtained from Cayman Chemicals (Cayman Chemicals and Pierce Biotechnology, USA). The activities of alkaline phosphatase (ALP) in the bone marrow samples were measured using BioAssay System's Quantichrom ALP assay kit. Serum samples were assayed for blood lead levels via the graphite furnace atomic absorption spectrophotometry method.

For histopathological analyses, pelvic bone with proximal part of the femur was excised. Bone tissue was kept in 10% formalin until staining. Decalcification of the bone was done before processing into paraffin blocks. Five millimeter section were cut and stained with hematoxylin and eosin for histological examination.

2.6. Statistical analyses

All the data were expressed in means with standard deviation. Global comparison between all the groups was done using non-parametric Kruskal Wallis one way analysis of variance test. Pair wise comparison between the different groups was done using Mann–Whitney – U test and $p < 0.05$ was considered to show statistical significance. Comparison was done to explore the effect of lead toxicity, and nullifying effect of *E. elatior* on lead toxicity in terms of efficacy, and concurrent against post-exposure *E. elatior* treatment on lead toxicity. Graph Pad prism 5.0 software used for statistical analyses.

3. Results

3.1. Lipid hydroperoxides

Bone marrow lipid hydroperoxide levels increased significantly in the lead acetate alone treatment group ($p < 0.05$). A significant decrease serum LPO was observed in concurrent treatment with *E. elatior* group and also in post lead treatment group ($p < 0.05$). Treatment with *E. elatior* was able to reduce the serum LPO levels significantly ($p < 0.05$) to control levels (Table 1). No statistical significance difference was observed among concurrent *E. elatior* treatment group and post-lead-exposure *E. elatior* group.

3.2. Protein carbonyl content

Lead acetate alone treatment group showed a significant increase ($p < 0.05$) in bone marrow protein carbonyl content when compared to control rats. Concurrent *E. elatior* extract and lead acetate treatment group showed a significant decrease in PCC in bone marrow when compared to lead alone group. Post-lead-exposure, *E. elatior* group also showed a significant decrease ($p < 0.05$) in bone marrow PCC than lead alone group (Table 1). There was a more significant decrease in PCC level in *E. elatior* alone group ($p < 0.05$). PCC levels in these rats were significantly lower than control animals.