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Protective Effect of Aqueous Leaves Antidote of *Piper crocatum* Against Lead Induced Oxidative Stress in Mice Liver

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Present study investigated whether the *Piper crocatum* leaves antidote has given the protective effect against Pb(II) induced oxidative stress in mice liver. Oral administration of lead significantly increased serum hepatic markers (SGPT and SGOT), oxidative stress markers (malondialdehyde) and creatinine levels. Pre oral administration with *Piper crocatum* leaves antidote in lead intoxicated rats were significantly recovered the parameters. The histopathology analysis of liver section of experimental rats revealed that *Piper crocatum* leaves antidote exhibit the protective effect against lead(II) induced hepatic damage in mice.

Keywords: *Piper crocatum*, Oxidative stress, Serum hepatic markers, Lead.

INTRODUCTION

Lead is known as a persistent and ubiquitous environmental pollutant that has no beneficial biological role in organisms [1]. The toxicity of lead mainly attributed by induced oxidative stress by elevation of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radicals [2]. The liver is considered as one of the target organs that could affect by lead toxicity owing its site of storage after exposure. Absorbed lead is stored in soft tissues *via* the portal vein, mainly in the liver. So the liver is considered as the first organ to examine the changes in morphological or tissue damage due to lipid peroxidation using histopathological analysis [3]. Considering that lead toxicity is one of the most serious problems in worldwide, the specific and safe treatment is necessary. The common treatment for lead toxicity is using the chelators such as $CaNa_2EDTA$ and DMSA, but moreover, these chelators are potentially toxic to human and often fail to remove the lead content in body tissues [4]. Therefore it is necessary to increase the study related to therapeutic potential of medicinal plant to reduce the toxicity of heavy metals induced tissue injury [5]. Betel plant (*Piper* sp) known has many medical active compounds as if flavonoid, alkaloid, tannin and essential oil. Generally it is used to overcome body odor and bad breath, nose bleed, itching and ulceration. *Piper crocatum* as one of betel plant is known as medicinal plant that traditionally used by Indonesians. *Piper crocatum* is also known has anti proliferative effect [6], antimicrobial activities [7], has high activity of antioxidant [8]. High activity of antioxidant may play an important role in protecting cells and organs from oxidative stress. This study was carried out to investigate the potential of *Piper crocatum* leaves antidote against Pb toxicity induced oxidative stress and changed of biochemical parameters and histopathology analysis in liver in intoxicated mice.

EXPERIMENTAL

The experimental plant *Piper crocatum* was collected from local market in Padang, West Sumatera, Indonesia.

Preparation of antidote of *Piper crocatum*: The leaves are washed with clean water then wind dried at room temperature for a week. After dried, the leaves crushed with a blender into a powder form. 2 g of *Piper crocatum* powder mashed with distilled water while crushed, then transferred into a beaker glass and then add distilled water to 120 mL. The solution then heated to boil and filtered and then stored in a bottle.

Male white mice weighing approximately 140-160 g were obtained from Andalas University, West Sumatera Indonesia for experimental purpose. The animal ethic committee of Andalas University has approved the protocol of the experimental. The mice were provided with adequate diet and drinking water *ad libitum* during the experimental

Lead nitrate was used of analytical grade obtained from Merck (Darmstad, Germany).

Experimental design: Adult white mice were randomly divided into 3 groups, each groups contained 3 animals. The first group served as control and was given only distilled water and normal diet. The second group was given 5 mL × bw/200 g bw of mice antidote *Piper crocatum* for one week, continued with intraperitoneally injection of 1 mL × bw/200 g bw Pb 1000 mg/L. The third group was only given 1 mL × bw/200 g bw Pb 1000 mg/L intraperitoneally. After 5 h, the animals were killed using chloroform anesthesia and the blood drawn for biochemical analysis and oxidative stress. The mice liver then excised and fixed in Bouin's solution for histological studies.

Biochemical analysis and lipid peroxidation: Lipid peroxidation was estimated by malondialdehyde (MDA) content in serum. Briefly, 3 test tube each containing distilled water, standard solution of malondialdehyde and serum were prepared. In each test tube 2.5 mL of trichloroacetic acid (5 %) was added, homogenized and then centrifuged for 10 min (2000 rpm). The filtrate from each tube was pipetted and added into each test tube. Into each test tube 1,5 mL sodium thiobarbituric acid was added, homogenized using vortex and then heated in water bath. Read the absorbance at 530 nm. SGPT, SGOT, creatinine and ureum content were measured using two reagents method (Substrate Start).

Histopathology analysis: The liver were removed, washed with sterile saline and fixed in Bouin's solution. At room temperature for 17 h, after fixing process, the tissue then washed under running water and dehydrated. Sections of the tissue (5-6 μm) were prepared by using rotary microtome and stained with hematoxylin and eosin dye and examined under light microscope.

Statistical analysis: The data was analyzed using the Statistical Package for Social Science program (SPSS ver. 16). The analysis was done using analysis of variance (ANOVA) followed by Tukey test and Bonferroni test.

RESULTS AND DISCUSSION

Biochemical analysis and lipid peroxidation: Blood from control group, Pb treatment and antidote pre treatment were drawn up and the serum were analyzed biochemically (Table-1).

From Table-1 it could be determined that there is an increase in most of the parameters in the groups of mice treated with lead. There are significant decrease of the value of the parameters of the group III compared with group II. Lead exposure will increase some blood biochemical parameters such as ureum, uric acid, creatinine and also bilirubine levels, caused an elevation in enzymatic activity of both SGPT and SGOT and interfere lipid profile. This might be suggested that the lead exposure induced adverse effect on liver and kidney function. The increase of liver enzyme such as SGPT and SGOT indicated inflammation or damage to liver cells. The damage liver cells will leak higher amounts of enzyme into the blood stream which could lead elevated liver enzyme in blood [9]. Lead exposure is also known lead to kidney damage in both animals and humans. The kidney serves as a main organ of Pb excretion and accumulation and also a sensitive organ target for Pb exposure [10]. The rats exposed to Pb 0.4 mg/100 mL for 10 days significantly elevated the levels of urea and creatinine compared with the control so it could be concluded that Pb is nephrotoxic [11]. Based on the result, pre treatment with *Piper crocatum* antidote were able to reduce the levels of malondialdehyde, urea, creatinine, SGOT and SGPT significantly compared with the group that were exposed to Pb only. This protective effect is probably due to the antioxidant activity contained in *Piper crocatum*. Betel plant generally has a high content of antioxidant and has a several bioactive compounds such as hydroxycavicol, eugenol, chavibetol and allylpyrocatechol. The reported antioxidant could reduce lipid peroxidation and oxidative stress in mice characterized by decreasing level of malondialdehyde [12]. The result showed that there is an increased in SGPT. The elevated levels of SGPT and SGOT are indicators of the damage of plasma membrane permeability, cellular damage and toxicity metabolic disorder due to Pb toxicity. The increased activity of SGPT also act as a marker of hepatocellular damage. SGOT is present mainly in muscles and the increase level of SGOT related to the leakage of enzyme from muscles because of muscular activity induced by intoxication [3].

Histopathology analysis: The protective effect of *Piper crocatum* antidote and Pb(II) administration intraperitoneally are described in changes of liver histopathology. The effects of cell structure damage in mice that exposed with Pb and with antidote pre treatment was shown in Fig. 1.

Fig. 1 shows that pre treatment administration orally of *Piper crocatum* antidote for 7 days is able to protect the liver

TABLE-1
CONTENT OF MALONDIALDEHYDE, UREA, CREATININE, SGPT AND SGOT OF THE EXPERIMENTAL MIC

Parameters	Group I Distilled water	Group II 5 mL × bw/200 g bw antidote for seven days, continued with 1 mL × bw/200 g bw Pb 1000 mg/L in the last days	Group III 1 mL × bw/200 g bw Pb(II) 1000 mg/L
Malondialdehyde (mg/dL)	3.61	4.08*	6.88**
Ureum (mg/dL)	18.36	23.41*	24.42**
Creatinine (mg/dL)	0.21	0.42*	0.57**
SGOT (U/L)	11.98	33.04*	77.15**
SGPT (U/L)	25.88	23.72*	59.43**

*P < 0.05 compare to control group (group I); **P < 0.05 compare to antidote treated group (group II)

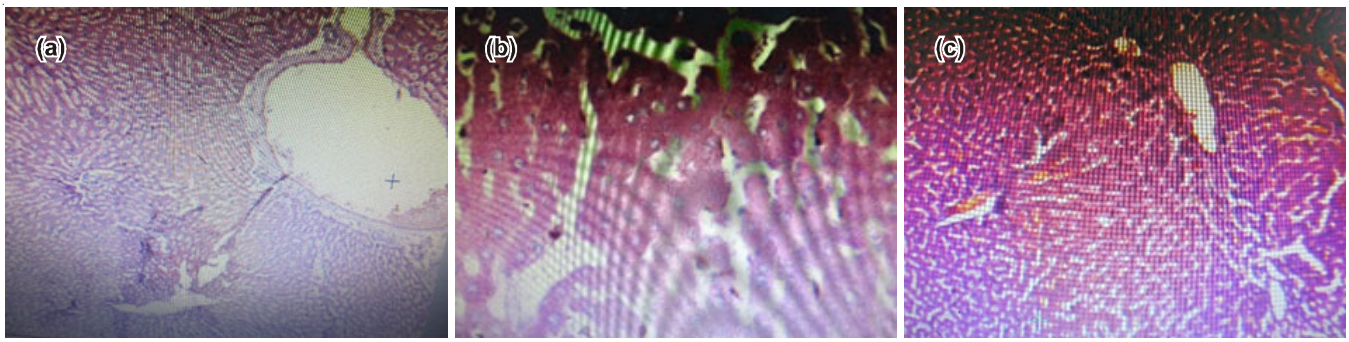

Fig. 1. A photomicrograph of mice liver group I (control); (a) showing normal and hepatic architecture; (b) photomicrograph of mice liver group III (Pb(II) 1000 mg/L treatment), veins dilatation occurs and there are distribution of lymphocytes. There are occurs cloudy swelling and accumulation of fat; (c) photomicrograph of mice liver group II (antidote pre treatment), liver lobules and central venous looks normal. Magnification 100x

from damage so the *Piper crocatum* antidote predicted to have preventive action against Pb exposure. A study reported that the mice which exposed to Pb(II) 500 mg/kg bw experienced a severe structural failure of the liver. The hepatocytes appeared irregularly arranged with disorganization of hepatic architecture. The central vein appeared dilated and congested with massive hemorrhage extending to nearby cells. In previous study, co-administration of sesame oil significantly improved the structural changes of liver and could significantly lower the activities of serum alanine aminotransferase, aspartate aminotransferase, alkaline phospatase and γ-glutamyltransferase, indicated that sesame oil showed effective hepatoprotective action against lead acetate toxicity [13]. Protective effect against Pb exposure also shown by the aqueous extract *Ipomoea aquatic* by inhibiting oxidative stress and apoptosis. Treatment using aqueous extract *Ipomoea aquatic* could significantly restore the parameters of biochemical serum almost to normal and reduce the effects of damage to organs [5]. In addition to having phenolic and flavonoid content, *Piper crocatum* leaves are known to have high antioxidant content so that the protection mechanism of *Piper crocatum* leaves against toxicity of Pb(II) induced oxidative stress occurs through reactive oxygen species scavenging [14].

Conclusion

The exposure of Pb(II) in experimental mice leads to changes in the levels of biochemical serum parameters and leads to oxidative stress. Administration of herbal antidote *Piper crocatum* leaves could restore the levels of serum biochemical parameters into normal. The exposure to Pb(II) also leads to abnormal changes on liver histology in the form of central vein dilation distribution of lymphocytes. Pre treatment with *Piper crocatum* antidote could reduce the damage of the liver so it is expected that antidote *Piper crocatum* has preventive effect against Pb(II) toxicity.

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