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Protective Effects of Ethanol Extract of Mangosteen (*Garcinia mangostana L*) Pericarp Against Lead Acetate-induced Nephrotoxicity in Mice

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Abstract: Lead is one of the most toxic metals, producing nephrotoxicity in animals and humans. Oxidative stress reported to play an important role in lead acetate induced kidney injury. This study was carried to investigate the role of ethanol extract of mangosteen pericarp in protecting against lead acetate-induced nephrotoxicity in male mice. The sample used 50 male mice were divided into 5 groups: negative control (Mice were given daily with aquadest) ; positive control (Mice were given daily with lead acetate 20 mg/kg BW orally once in a day for 21 days) and the treatment group (Mice were given the mangosteen pericarp extracts 200 mg; 400 mg; 800 mg/kg BW orally once in a day for 25 days and on 4th day, were given lead acetate 20 mg/kg BW one hour after the mangosteen pericarp extracts administration for 21 days). On day 25 measured levels of Creatinine, Blood Urea Nitrogen (BUN), Malondialdehyde (MDA), Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx). The Creatinine, BUN, MDA, SOD and GPx data were analyzed with one-way ANOVA, followed by LSD test. The results showed that, Oral administration of lead acetate 20 mg/kg BW for 21 days resulted in a significant increase in Creatinine, BUN and MDA level. Moreover, significant decrease in SOD and GPx. Treatment with the mangosteen pericarp extracts 800 mg/kg BW but not 200 mg/kg BW and 400 mg/kg BW significantly ($P< 0.05$) decreased the elevated Creatinine, BUN and MDA levels as compared to positive control group. Treatment with the mangosteen pericarp extracts 800 mg/kg BW also significant increase in SOD and GPx as compared to positive control group. From the results of this study concluded that the mangosteen pericarp extracts could be a potent natural herbal product provide a promising nephroprotective effect against lead acetate induced nephrotoxicity in mice.

Key words: Mangosteen pericarp extracts · Lead acetate · Creatinine · BUN · MDA · SOD · Gpx

INTRODUCTION

The environmental contamination by heavy metals has increased drastically along with the rapid development of modern industry. Among these metals is lead, of which its levels have increased substantially during the last few years. Lead-exposure occurs through the respiratory and gastrointestinal systems and lead which is ingested and absorbed is stored mainly in liver, kidney and bone.

Elevated lead levels in the body have been associated with nephrotoxic, hepatotoxic, neurotoxic and cardiovascular disease [1, 2].

In living systems, kidney is considered to be highly sensitive to toxic agents. Ponce-Canchihuamán *et al*. [3] administered 25 mg/0.5 mL of lead acetate intraperitoneally to rats weekly. It was found that activities of SOD, CAT and GSH in rat kidney were significantly decreased while level of MDA was significantly increased with respect to the control. The mechanism behind lead nephrotoxicity is the oxidative stress and it develops when there is an imbalance between the generation of reactive oxygen species (ROS) and the scavenging capacity of antioxidants in the kidney [4, 5]. A previous study confirmed the possible involvement of reactive oxygen species (ROS) or free radicals such as superoxide ion (O_2^-), nitrogen oxide (NO) and hydroxyl radical (OH^-) in lead-induced toxicity [6, 7]. MDA, one of the well known secondary products of lipid peroxidation after exposure to reactive oxygen species and free radicals, may be used as an indicator of cell membrane injury. The increase in MDA levels in kidney suggests enhanced lipid peroxidation

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leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals [8, 9]. The most widely used assay for lipid peroxidation is the MDA formation. The level of MDA, the end product of lipid peroxidation is measured by the thiobarbituric acid reactive substance (TBARS) method. The concentration of MDA is the direct evidence of toxic processes caused by free radicals [10, 11].

Antioxidant activity or inhibition of generation of free radicals plays a crucial role in providing protection against such kidney damage. Vitamins are ideal antioxidants to increase tissue protection from oxidative stress due to their easy, effective and safe dietary administration in a large range of concentrations [12]. Antioxidants such as vitamin E and vitamin C were found to improve kidney conditions significantly when treated in animals with lead acetate induced damage. The antioxidant activity of vitamin E is targeted primarily towards the lipid component of cells. Antioxidants such as vitamin E and vitamin C have been shown to inhibit free radical formation and are effective in minimizing lipid peroxidation in several different biological systems. Vitamin E and vitamin C is a natural antioxidant and prevents the increased production of free radicals induced by oxidative damage to lipids and lipoproteins in various cellular compartments and tissues [13].

Recently, there has been an increased interest in the therapeutic potential of plant products or medicinal plants having antioxidant properties in reducing free radical-induced tissue injury. Medicinal plants are commonly used for the treatment of various ailments, as they are considered to have advantages over the conventionally used drugs that are much expensive and known to have harmful side effects [10]. Many authors tried various ameliorating agents like *Curcuma longa*; Ginger; green tea; *Pongamia pinnata* against lead toxicity [5, 14-16].

Mangosteen (*Garcinia mangostana L.*) is one of the most famous fruits in Indonesia. Previous studies have shown that the mangosteen extracts from various parts contain varieties of secondary metabolites such as prenylated and oxygenated xanthones. Xanthones such as α-, β- and γ-*mangostins, garcinone E,* 8-*deoxygartanin* and *gartanin* could be isolated from pericarp, whole fruit, bark and leaves of mangosteen [17]. Several studies have shown that obtained xanthones from mangosteen have remarkable biological activities such as antioxidant, antitumoral, anti-inflammatory, antiallergy, antibacterial, antifungal and antiviral activities [18-20]. The present study is intended to investigate nephroprotective activity of ethanolic extract of the mangosteen peel against lead acetate induced kidney damage in mice.

MATERIALS AND METHODS

Chemicals: Lead acetate was purchased from Sigma-Aldrich chemic (USA). All other chemicals and solvents used in this study were of highest purity and analytical grade and purchased from Sigma-Aldrich chemic (USA). Reagent kits for assay of Creatinine and BUN were obtained from Kristalindo (Indonesia). Reagent kits for determination of MDA, GPx and SOD were purchased from Kristalindo (Indonesia).

Experimental Animal: Male Swiss albino mice (*Mus musculus*) weighing approximately 25–30 g (2.5-3 months) were obtained from Veterinary Farma Surabaya Indonesia for experimental purpose. They were housed in plastic cages in an air-conditioned room with temperature maintained at 25°C±3°C, relative humidity of 50% ± 5% and 12 h alternating light and dark cycles. The mice were provided *ad libitum* with tap water and fed with standard commercial mice chow. The Animal Ethics Committee of Airlangga University, Surabaya Indonesia has approved experimental protocol.

Preparation of Ethanol Extract of Mangosteen Pericarps: Plant material and extract preparation Mangosteen pericarps were collected from Surabaya, Indonesia. Fruits were cleaned with running tap water and fresh pericarps were chopped into pieces. They were dried under shade at ambient temperature for 5 days and the air-dried pericarps were then ground to powder for extraction. The powdered pericarp (1 kg) was macerated with ethanol (5 L) for a week at 37°C. The supernatant was then collected and filtered through Whatman No. 1 filter paper in a Buchner funnel under vacuum. The filtrate was concentrated by evaporation with a vacuum rotary evaporator at 45°C. The extract was dried at reduced pressure, stored at 0-4°C and used for the experimentation.

Experimental Design: The fivety male mice (*Mus musculus*) were divided randomly into five groups as the following: negative control group (Mice were given daily with aquadest); positive control group (Mice were given daily with aquadest and lead 20 mg/kg BW orally once in a day for 21 days) and the treatment group (Mice were given the mangosteen pericarp extracts ethanol 200 mg; 400 mg; 800 mg/kg BW orally once in a day for 25 days and lead acetate 20 mg/kg BW were given on 4th day, one hour after the mangosteen pericarp extracts administration for 21 days). On day 25, blood samples were taken by

cardiac puncture into chilled tubes and centrifuged at 3000 rpm for 20 minutes; then sera were stored at -85°C until assay.

Biochemical Assays: Serum biochemical markers activities of BUN, Creatinin and MDA whereas enzymatic antioxidants (SOD and GPx) were assessed in kidney.

Estimation of blood urea nitrogen was carried out by the diacetyl monoxime method. Protein-free filtrate was prepared by adding serum and equal amount of 10% TCA, then mixture was centrifuged at 2000 r.p.m. and supernatant was taken. To 0.5 ml of protein free filtrate, were added 3.5 ml of distilled water, 0.8 ml diacetylmonoxime (2%) and 3.2 ml sulphuric acid-phosphoric acid reagent (Reagent was prepared by mixing 150 ml 85% phosphoric acid with 140 ml water and 50 ml of concentrated sulphuric acid). The reaction mixture was placed in a boiling water bath for 30 min and then cooled to room temperature. The absorbance was read at 480 nm.

Creatinine was estimated by the alkaline picrate method. Protein-free filtrate was prepared. To 1.0 ml serum were added, 1.0 ml sodium tungstate (5%), 1.0 ml sulfuric acid (0.6 N) and 1.0 ml distilled water. After mixing thoroughly, the mixture was centrifuged at 800 X g for 5 min. The supernatant was added to a mixture containing 1.0 ml picric acid (1.05%) and 1.0 ml sodium hydroxide (0.75 N). The absorbance at 520 nm was read exactly after 20 min.

The level of serum MDA was determined spectrophotometrically with a thiobarbituric acid (TBA) solution. In brief to 150 ìl serum sample added the followings: 1ml trichloroacetic acid (TCA) 17.5%, 1ml of 0.66% TBA, mixed well by vortex,incubate it in boiling water for 15 minutes, & then allowed to cool. Then add 1ml of 70% TCA and let the mixture to stand at room temperature for 20 minutes, centrifuged at 2000 rpm for 15 minutes and take out the supernatant for scanning spectrophotometrically.

Portions of kidney was immediately washed in ice cold physiological saline and homogenized in 50 mM potassium phosphate (pH 7.4) to render 10% homogenate. The homogenate was centrifuged at 4000 rpm for 15 min at 4°C. The supernatant was used for SOD and GPx analysis.

Histopathological Study: After 10 days, all animals from every group were sacrificed and separated the kidneys by dissection procedure. Pieces of kidneys obtained from each group were immediately fixed in 10% formalin solution. The fixed formalin fixed kidneys were embedded in paraffin and serial section were made and stained with haemotoxylin and eosin. The stained sections were examined under light microscope.

Statistical Analysis: Data were presented as means ± standard errors. One-way ANOVA was carried out and the statistical comparisons among the groups were performed with LSD test using a statistical package program (SPSS version 17.0).

RESULTS

Effects of Mangosteen Pericarp Extract on Lead Acetate Induced Changes in The Serum Creatinine and BUN: An increase in the serum creatinine and BUN indicates kidney damage. Analysis of these creatinine and BUN has been done to evaluate the nephroprotective effect of ethanol extract of mangosteen pericarp in lead acetate treated mice. Positive control (Lead acetate treated mice) showed a significant (p <0.05) increase in serum creatinine and BUN comparing with the negative control. In contrast, the groups pretreated with ethanol extract of mangosteen pericarp (800 mg/kg BW) showed significantly (P <0.05) decreased serum creatinine and BUN level in a dose dependent manner with respect to the positive control to towards normalization and close to the negative control group (Table. 1).

Effects of Mangosteen Pericarp Extract on Lead Acetate Induced Changes in SOD and Gpx: Lead acetate enhances the intracellular formation of reactive oxygen species causing hepatic damage. In the present study we analyze the hepatic levels of several antioxidants (SOD and GPx) and MDA. Positive control (Lead acetate treated mice) showed significant (P< 0.05) decrease in the level of SOD and GPx compared with negative control group, meanwhile a significant (P< 0.05) increase in MDA level was detected. Groups pretreated with ethanol extract of mangosteen pericarp (800 mg/kg BW) showed a significant (P <0.05) increase in the level of SOD and GPx with significant (P< 0.05) decrease in MDA level compared with lead acetate treated mice towards the normal level and close to the negative control (Table. 2).

Effects of Mangosteen Pericarp Extract on Lead Acetate Induce Kidney Injury: Histopathological study was performed using light microscopy. Microscopic examination of normal kidney showing tubular brush borders and intact glomeruli without any structural alterations in renal tissues. In lead acetate treated, renal

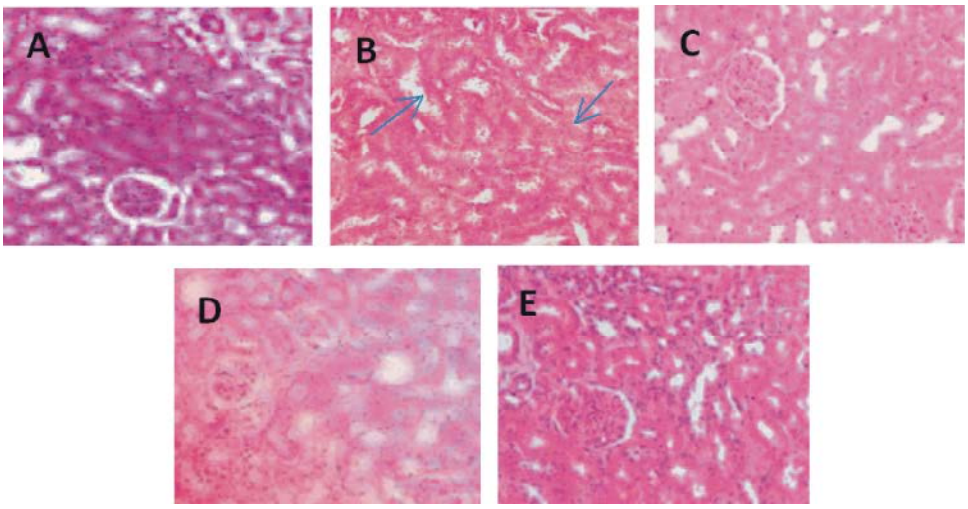

Fig. 1: Histological study of kidney tissue in control and experimental groups of rats. Normal morphological view of renal sections in control group (A). Histopathological view of renal sections in lead acetate treated group showed the degeneration, desquamation, necrosis in tubules and glomerulus (Indicated by arrows) as compared to control group (B). Mice treated with mangosteen pericarp extract 200 mg/kg BW and 400 mg/kg BW showed karyopicnosis and slight tubular degenerative and necrotic changes (C and D). Mice treated with mangosteen pericarp extract 800 mg/kg showed regeneration in tubular epithelial cells (E).

Table 1: Effects of mangosteen pericarp extract on lead acetate induced changes in the serum creatinine, BUN and MDA

	Means±SD		
Groups	BUN (mmol/L)	Creatinine (mmol/L)	MDA (nmol/L)
Negative Control	7.24±1.27^{a}	31.53±4.75^{a}	4.97±0.66^{a}
Positive Control	24.13±3.49^{b}	64.24±8.63^{b}	9.04±0.97^{b}
Mangosteen 200 mg/kg BW	26.20±5.65^{b}	66.30±6.21^{b}	8.74±1.10^{bc}
Mangosteen 400 mg/kg BW	18.83±4.87^{c}	58.20±7.15^{c}	7.94±0.92^{cd}
Mangosteen 800 mg/kg BW	13.62±3.61^{d}	41.80±5.15^{d}	6.02±0.82^{cd}

Superscript within each column indicate significant difference between the means ($p < 0.05$)

Table 2: Effects of mangosteen pericarp extract on lead acetate induced changes in SOD and GPx

	Means±SD	
Groups	SOD (U/mg)	GPx (U/mg)
Negative Control	34.32±1.07^{a}	44.36±5.71^{a}
Positive Control	17.82±1.81^{b}	29.85±3.62^{b}
Mangosteen 200 mg/kg BW	16.98±1.69^{b}	31.21±5.63^{bc}
Mangosteen 400 mg/kg BW	20.21±2.72^{c}	34.62±5.96^{cd}
Mangosteen 800 mg/kg BW	27.12±2.13^{d}	39.67±3.45^{d}

Superscript within each column indicate significant difference between the means ($p < 0.05$)

tissues showed swelling and massive and diffuse cell necrosis in proximal tubules of kidneys indicates cell injuries. Pretreatment with ethanol extract of mangosteen pericarp extract were significantly prevented histopathological changes towards normal. Treatment with ethanol extract of mangosteen pericarp extract highly ameliorated the toxicity manifestations in the kidney (Figure 1).

DISCUSSION

Lead is one of the most toxic metals, producing severe organ damage in animals and humans. Studies have shown that the kidney is one of the primary targets in lead associated toxicity [1, 4]. Lead produces oxidative damage in the kidney by enhancing lipid peroxidation and cause kidney dysfunction and increase free radical damage [11, 21]. Antioxidant enzyme levels are applied as markers of oxidative stress. Based on the present study lead induced toxicity might result in decreased tissue activities of enzymatic antioxidants SOD and GPx. The decrease of SOD and GPx activities might predispose the examined tissue of mice to oxidative stress, because these enzymes catalyze the decomposition of ROS [6, 22]. The levels of these antioxidants might provide a clear indication on the extent of cytotoxic damage that occurs in kidney tissue. Therefore, some authors have postulated that antioxidants should be one of the important components of an effective treatment of lead poisoning [1, 4].

Since plant based natural products are the current field of interest for the researchers due to their cheap and high therapeutic potential without much of the side

effects associated with synthetic drugs, the present study evaluated the nephroprotective property of Mangosteen.

Mangosteen is one of the famous fruits and its pericarp extracts have been widely used in traditional medicine. Most of the scientific researches have been focused on phytochemical studies in order to find novel constituents. Pharmacological activities and mechanisms of actions are scarcely available. From phytochemical studies, mangosteen pericarp consists of more than 90% xanthones as major polyphenolic compounds, especially α-*mangostin* (80–90%) and β-mangostin (5–10%), named *panaxanthone* [17]. These xanthones are of great interest because of biological and pharmacological properties, such as antioxidant, antitumor, anti-inflammatory, antibacterial, antifungal and antiviral properties [18, 19].

The present study is intended to investigate nephroprotective activity of ethanolic extract of the mangosteen pericarp against lead acetate induced kidney damage in mice. In lead induced nephrotoxicity, a significant elevation in the levels of creatinine and BUN were observed which serves as an indicator of impaired renal functions. The serum creatinine and BUN are recommended for the assessment of kidney injury in preclinical studies as it is considered a more specific and sensitive indicator of kidney damage. Low levels of serum creatinine and BUN are normally found in the blood, but when the kidney is damaged or diseased, which makes creatinine and BUN levels go up. Most increases in serum creatinine and BUN levels are caused by kidney damage [23]. The current work revealed an increase in the level of creatinine and BUN in lead acetate treated mice in comparison to the negative control and this may be due to the degeneration of kidney by necrosis. Similar observation was reported by Pratap [5] have reported that lead acetate treatment induced significant elevation of serum creatinine and BUN activities. Our results indicated that ethanol extract of mangosteen pericarp has a nephroprotective activity against lead acetate-induced nephrotoxicity, where the pretreated groups with ethanol extract of mangosteen pericarp 800 mg/kg b.w, showed an improvement in the creatinine and BUN levels. This might be through its direct action on free radicals of lead acetate to protects the kidney cellular damage by maintaining its membrane integrity.

Lead toxicity leads to generation of free radical damage by two separate pathways including hydroperoxides, singlet oxygen and hydrogen peroxides, evaluated by MDA levels as the final products of lipid peroxidation and the direct depletion of antioxidant reserves [22]. The present investigation resulted significantly increase of MDA levels in the kidney of lead acetate treated mice in comparison to the negative control. This means that it increased the oxidative stress in the lead acetate treated mice.Therefore, the significantly lower levels of MDA in the tissues of treated groups as compared with the lead acetate group indicate attenuation of lipid peroxidation. It is known that lead acetate-induced oxidative stress tissue damage could be caused by two mechanisms: increased generation of ROS and by causing direct depletion of antioxidant reserves [4, 9]. Intense lipid peroxidation caused by lead exposure may affect the mitochondrial and cytoplasmic membranes causing more severe oxidative damage in the tissues and consequently releasing lipid hydroperoxides into circulation which reflects the induction of oxidative stress [13, 16]. The ethanol extract of mangosteen pericarp, which behaves as a powerful antioxidant and free radical scavenger, can decrease MDA level perturbed by lead acetate in mice liver, as observed in this study. Treatment of mice with ethanol extract of mangosteen pericarp at a dose of 800 mg/kg body weight prevented the levels of lipid peroxidation (MDA) to rise when the mice were challenged with lead acetate. This means that ethanol extract of mangosteen pericarp minimized the toxic effect of lead acetate via its antioxidant activity. The antioxidant protective mechanism decreases the oxidative stress and scavenges the free radical which responsible for the kidney damage and thus inhibit the lipid peroxidation (MDA). The findings of this study suggest that ethanol extract of mangosteen pericarp could attenuate oxidative stress by decreasing the lipid peroxidation (MDA level) in lead-treated kidney. A similar result had been showed that vitamin C and Vitamin E enhanced the antioxidant status and inhibited lipid peroxidation (MDA) in rats with lead acetate induced kidney injury. These findings indicate that the antioxidant activity of Vitamin C and vitamin E are targeted primarily towards the lipid component of cells. Antioxidants such as Vitamin C and viyamin E have been shown to inhibit free radical formation and are effective in minimizing lipid peroxidation in several different biological systems [13].

SOD and GPx are important antioxidant enzymes. They constitute a mutually supportive defense mechanism against ROS. SOD decomposes superoxide radicals (O_2-) to produce H_2O_2. GPx is a selenoenzyme which plays a major role in the reduction of H_2O_2 and hydroperoxide to produce nontoxic products. Therefore, the activities of these enzymes have been used to assess oxidative stress in cells. Many studies have shown that

lead has high affinity for SH groups in several enzymes such as SOD and GPx, thus it can alter antioxidant activities by inhibiting functional SH groups in these enzymes [1]. In the present study, the activity of SOD and GPx in mice kidney was dramatically decreased by lead acetate treatment. This decreased SOD and GPx activity with lead acetate treatment is in agreement with previous studies [11]. This suggested that lead acetate exposure induced oxidative stress by inhibiting the activity of this antioxidant enzyme. Interestingly, the administration of ethanol extract of mangosteen pericarp increased the activities of SOD and GPx in the kidney of lead-treated mice, which might be due to the ability of extract of mangosteen pericarp to reduce the accumulation of free radicals. Ethanol extract of mangosteen pericarp acts as a scavenger for the oxygen-derived free radicals, thus protecting from cellular damage.

Histopathological results demonstrating structural changes in renal tissue of aminoglycoside metal toxic such as lead acetate were reported by some researchers. Histopathological view of renal sections in lead acetate treated group showed the degeneration, desquamation and necrosis in tubules and swelling in glomerulus, as compared to control negative group. Glomerular and tubular epithelial changes were considerably mild in the groups treated with ethanolic extract of the mangosteen pericarp 400 mg/kg showed karyopicnosis and mild tubular epithelial changes while in case of animal treated with ethanolic extract of the mangosteen pericarp 800 mg/kg showed regeneration in tubular epithelial cells. We think that, morphological changes in kidneys were because of lead acetate, but these changes tended to be considerably mild in lead acetate plus ethanolic extract of the mangosteen pericarp treatment. In

summary, our data indicate that lead acetate-induced nephrotoxicity might be related to oxidative damage. Co-administration of ethanolic extract of the mangosteen pericarp lessened the effects of lead acetate-induced nephrotoxicity possibly by inhibiting free radical mediated process. Further investigation of these promising protective effects of ethanolic extract of the mangosteen pericarp against lead acetate-induced renal injury may have a considerable impact on developing clinically feasible strategies to treat patients with renal failure.

It could be concluded that, ethanol extract of mangosteen pericarp may exert its protective actions against lead-induced kidney injury in rats possibly through its antioxidant mechanisms. Ethanol extract of mangosteen pericarp can be a future natural product for counteract the lead acetate intoxication. This Results showed that ethanol extract of mangosteen pericarp has a potential nephroprotective effect in a dose dependant manner that minimize or diminish compounds the nephrotoxic effect induced by lead acetate intoxication.

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