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RESEARCH ARTICLE OPEN ACCESS

NEPHROPROTECTIVE EFFECTS OF *ELEUTHERINE AMERICANA MERR* AGAINST LEAD ACETATE-INDUCED CYTOTOXICITY IN MICE BALB/C

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ABSTRACT

Lead is a poisonous metal, hurtful to most human body organs if exposure surpasses a permissible level of 50 µg/m^3 and an action level of 30 µg/m^3. This occurs through the initiation of reactive oxygen species (ROS). Studies have indicated that Eleutherine americana Merr contains profound biological properties which protects against cancer cells, decreased in prothrombin level, and vessel vasoconstriction. It will be interesting to study the effect of the plant extract on kidneys exposed to lead acetate. This study aimed to evaluate the nephroprotective effects of Eleutherine americana Merr extract on lead acetate cytotoxicity in mice BALB/c. A total of 25 BALB/c mice were randomly divided into five groups. Group1 was given 0.5% Na-CMC orally while Group 2 was treated orally with 0.075 g/kg body weight lead acetate ($Pb(CH_3COO)_2$). Group 3 to 5 was given different dosages of Eleutherine americana Merr of 30, 60 and 120 mg/kg body weight accordingly and simultaneously with $Pb(CH_3COO)_2$. All treatment were for 35 days. Mice were sacrificed after 35 days, blood samples were collected for analysis of creatinine and urea while the kidneys were for histological studies. The levels of creatinine and urea was significantly higher in $Pb(CH_3COO)_2$ treated mice ($p<0.05$). Treatment with the plant extract significantly reduced the level of blood creatinine and blood urea at extract concentration of 30mg/kg body weight ($p<0.05$). Histology studies of the kidneys showed that $Pb(CH_3COO)_2$ caused glomeruli atrophy and tubular destructions. Treatment with the plant extract at dosages of 30 and 60 mg/kg body weight seemed to ameliorate the effect $Pb(CH_3COO)_2$ on the structure of the nephrons. Extract of Elutherine americana Merr was shown to have nephroprotective effect against the assault of $Pb(CH_3COO)_2$ in mice.

I. INTRODUCTION

Lead (Pb) is a conceivably poisonous component that, when consumed by the body, amasses in blood and bones, just as in organs like the liver, kidneys, cerebrum and skin. Its negative wellbeing impacts can be both intense and constant, on the grounds that the human body inadequately discharges Pb. Exposure to high levels of lead can result in adverse health outcomes in people, lead has been displayed to influence the capacity of reproductive, hepatic, endocrine, immune and gastrointestinal systems frameworks, some even leading to death [1-3] Exposure happens through ingestion of debased food,

drinking water, and residue, just as smoking and inward breath of dirtied air in regions with hefty traffic or industrial emissions [4, 5].

The kidney is one of the objective organs for lead harmfulness [5, 6] for being a significant course of discharge from the body and works with kidney harm by means of oxidative pressure and lipid peroxidation (LP). Intense lead harming (blood lead levels > 80–100 µg/dL) upsets both capacity both proximal tubular structure and function [6].

Lead might assume a significant part in producing oxidative stres [7] lead been known to be nephrotoxic at related at high-level [8] The mechanism of lead nephrotoxicity is oxidative stress and the imbalance between antioxidant capacity in the body and the formation of reactive oxygen species (ROS) in the kidney [9, 10].

A few investigations have detailed that lead has prompted oxidative pressure [7] Recent studies investigations show ROS or free extremists like superoxide particle (O2-), hydroxyl radical (OH-), and nitrogen oxide (NO) have a vital job in lead-actuated nephrotoxicity [11, 12].

Natural plants have consistently been utilized as the significant constituent of medication in the traditional system [13]. Natural products or medicinal plants having cancer prevention agent antioxidant properties for lessening free radical free revolutionary prompted tissue harm has been accounted for. Restorative plants have upper hands over the ordinarily utilized medications, which has a high price are pricey and known to have hurtful incidental effects for the treatment of different sicknesses [14].

The agent action antioxidantor hindrance of free radicals assumes an urgent part in against protection weighty metal incited nephrotoxicity. In this way, it has been guaranteed that defensive specialists against free radicals, like cancer prevention agents, may be useful therapeutics for weighty metal harmfulness in the kidneys [12]. In the current investigation, we zeroed in on the concentrate of plant Eleutherine Americana Merr pods as a natural antioxidant [15].

Indications of the content of Eleutherine Americana Merr extricates from hytochemical screening results containing: flavonoids, saponins, tannins, triterpenoids, alkaloids, anthraquinones, naphthoquinones, and steroids [16, 17]. Dayak onions have been displayed to have antioxidant [18, 19], antifungal [20] antimitotic [21] and antiacne [22].

II. METHODS

II.1 EXPERIMENTAL ANIMALS

This study was a true experimental study using randomization with a post-test-only control group design. The experiment was conducted at the Biomedical Laboratory of the Institute of Technology Health and Science, Wiyata Husada Samarinda. The experimental animals used were 25 male mice mus musculus BALB/C. The maintenance room was illuminated for 12 h (06.00–18.00). The experimental mice given food and ad libitum drinking water. The rats used were aged 3 months old, with an average initial body weight of 25–30 g. The experimental animals were divided into five groups, each group consisting of five mice.

II.2 RESEARCH DESIGN

The research was conducted in March 2021 at the Biomedical Laboratory, Fof the Institute of Technology Health and Science, Wiyata Husada Samarinda. The research design used a laboratory experiment with a completely randomized design, five treatments.

The treatment groups were detailed as follows:

Group 1 = Standard control, given 0.5% Na-CMC
Group 2 = Negative control, Exposed Pb Acetate 0.075 g/kg body weight
Group 3 = Exposed by Pb Acetate and had extract at a dose of 30 mg/kg body weight
Group 4 = Exposed by Pb Acetate and had extract at a dose of 60 mg/kg body weight
Group 5 = Exposed by Pb Acetate and had extract at a dose of 120 mg/kg body weight

II.3 RESEARCH PROCEDURE

II.3.1 Extract Preparation of Eleutherine Americana Mer (Dayak onion)

Dayak onion bulbs are peeled first, dried and then mashed. Dayak onion bulbs that have been dried are then ground and made into simplicia. Simplicia 500 grams was macerated with 96% ethanol for 48-72 hours and filtered using a Buechner funnel and concentrated using a rotaryevaporator.

II.3.2 Exposure to Lead Asetat and Intervention of Eleutherine Americana Merr Extract

Before interventions, the mice were put in adaptation in a standard cage for 7 days in a room with a temperature of 23 ± 2°C. The mice were individually caged. The lighting was set to 12 h of daylight-dark cycles. During the adaptation period, ad libitum (AL) food and drink were administered daily at 7 am and 5 p.m. After the adaptation, the mice were divided randomly into five groups, each consisting of 5 mice.

The normal control Group 1 was a standart control group with 0.5% Na-CMC administration. Days 1-3 Group 2 was given 0.5% Na-CMC, Group 3 - Group 5 was given Dayak onion extract at a dose of Group 3 (30mg/kgBW) Group 4 (60 mg/kgBW) Group 5 (120 mg/kgBW). Days 4-38 Group 2 was given 0.1 ml of lead acetate, Group 3 - Group 5 was given 0.1 ml of lead acetate + extract Group 3 (30mg/kgBW) Group 4 (60 mg/kgBW) Group 5 (120 mg/kgBW). Then on day 39 all mice were sacrificed for data examination.

II.3.3 Sampling

The collection of mouse blood serum begins with taking a certain volume of blood through the heart and then put it into a microtube. Then the blood was centrifuged at 4000 rpm for 20 minutes and the supernatant was taken at the top in the form of a slightly yellowish clear liquid. Blood serum was taken and stored at -20ºC until used for creatinine and blood urea analysis. In addition, after the abdomen was opened, the kidney was taken for Histological examination of tissue.

II.3.4 Ethical clearance

This research has been approved by the Veterinary Research Ethics Committee of the Faculty of Veterinary Medicine, Universitas Airlangga through approval letter No. 658/EC/KEPK/FKUA/2017.

III. RESULTS AND DISCUSSIONS

III.1 RESULTS

Before the interventions, the randomly grouped mice were first measured for the weights to ensure that they were in the same condition at the beginning of the study. The results of one-way ANOVA test showed no difference in the mean body weight among the groups before the treatment ($p > 0.05$).

III.1.1 Creatinine Levels

The result of experimental data on biochemical parameters in this study was the measurement of creatinine levels.Analysis of mice's creatinine levels showed that the highest was found in group G2 (2,41) followed by group G3 and group G5 (Figure 1). The one-way ANOVA test yielded $p < 0.05$ ($p = 0.024$), indicating a significant difference in the mean creatinine level between the five groups. The LSD (Least Significance Different) test also found a significant difference between the groups.

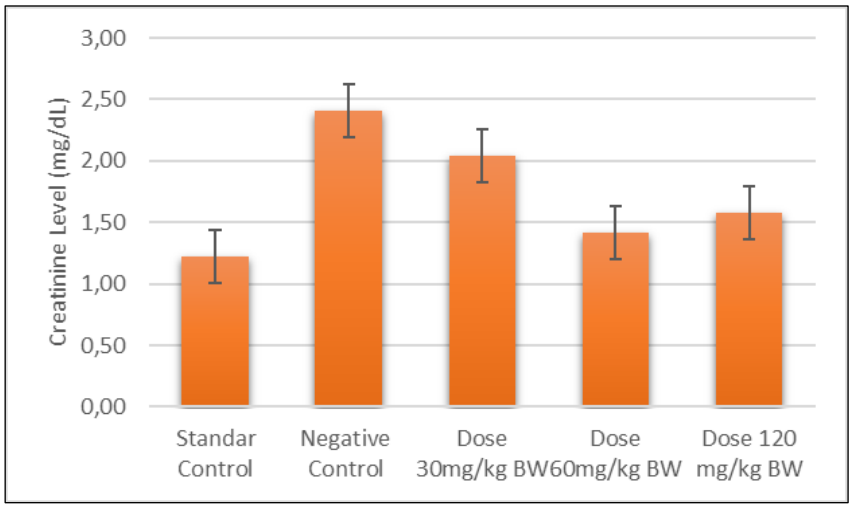

Figure 1: The Creatinine Levels.
Source: Authors, (2022).

Creatinine levels showed the results that a dose of 60 mg/kg showedowest creatinine level. Average result of each levelgroup is shown in Figure 1.

Normality test was performed by Kolmogorov-Smirnov test, Sig. > 0.05, creatinine data were normally distributed. Levene Sig test results. 0.91 ($p>0.05$), it means that the creatinine data is homogeneous.One Way Anova statistical analysis test shows the value of sig. 0.024($p<0.05$). There was a significant difference between the treatment groups for creatinine.LSD Post Hoc Test. The results showed that all doses had nephroprotective activity as seen from the significant difference with the negative control.The dose of 60 mg/kg did not show a significant difference with the dose of 120 mg/kg, meaning that the nephroprotective ability was the same as the treatment at the highest dose, but this dose showed a significant difference with the negative control.Meanwhile, the dose of 30 mg/kg showed a significant difference with the doses of 60 and 120 mg/kg, and also showed a significant difference with the negative control.

III.1.2 Blood Urea Levels

The result of experimental data on biochemical parameters in this study was the measurement of blood urea levels. Analysis of mice's blood urea levels showed that the highest was found in group G2 (73) followed by group G3 and group G5 (Figure 1). The one-way ANOVA test yielded $p < 0.05$ ($p = 0.000$), indicating a significant difference in the mean creatinine level between the five groups. The LSD (Least Significance Different) test also found a significant difference between the groups.

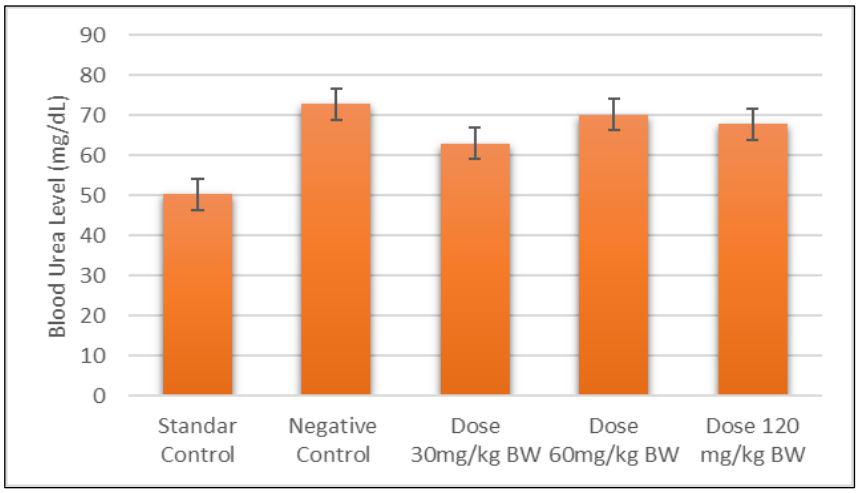

Figure 2: Blood Urea levels.
Source: Authors, (2022).

Blood Urea levels showed that the dose of 30 mg/kg had the lowest levels. The results of the average level of each group are shown in Figure 2.

Normality test was carried out by Kolmogorov-Smirnov and Sig. > 0.05. Urea levels were normally distributed.Levene, significant 0.058 (p> 0.05), homogeneously distributed data. One way ANOVA statistical analysis test showed that there was a significant difference between the treatment groups in urea. Post hoc using LSD analysis.

The results showed that the dose of 30mg/kg had nephroprotective activity as seen from the significant difference with the negative control. Doses of 60 and 120 mg/kg did not show a significant difference with negative control, which means that there is a decrease in nephroprotective activity.

III.1.3 Kidney Histopathology

Histopathological examination was carried out using a light microscope. Microscopic examination of normal kidneys showed intact tubules and glomeruli. In the lead acetate treatment group, kidney tissue showed the most severe glomerular atrophy and tubular destruction of all groups. Administration of Eleutherine Americana Merr extract at doses of 30 and 60 mg/kg BW but not at a dose of 120 mg/kg BW significantly changed histopathology to normal (Figure 3).

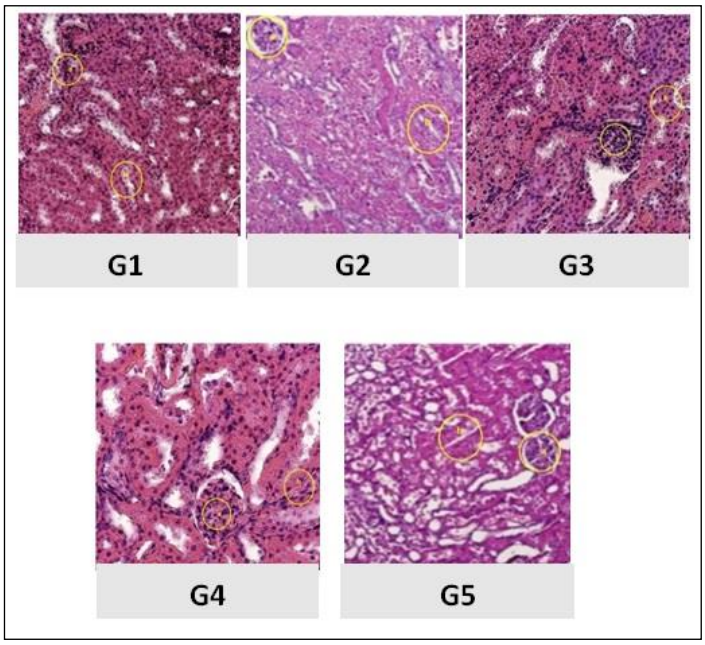

Figure 3: Histology of the kidney (40× objectives, scale bar 50 µm). G1: normal control, G2: negative control. G3, G4 and G5 were treated with 30, 60, and 120 mg/kg BW ethanolic extract of eleutherine americana merr, respectively. A: glomerular, B: tubules.
Source: Authors, (2022).

Nephrotoxicity occurs as a result of lead exposure because the kidneys are the main route of lead disposal. Lead is absorbed by the proximal renal tubular cells, where it binds to specific lead-binding proteins.Acute lead poisoning (blood lead levels > 80–100 g/dL) disrupts the structure and function of the proximal tubules, where protein pools that result in obstruction of delivery through the renal tubules also stimulate tubular necrosis.

The result shown in figure 5, the cast is in the negative control. In the treatment except normal controls, changes in the shape of the proximal tubule were found.

In the Pb Acetate negative control group, glomerular atrophy and tubular destruction were the most severe of all groups, and the administration of Eleutherine Amricana Merr could reduce the damage caused by Pb Acetate administration of 0.75 mg/kg bw. At the extract dose of 120 mg/kg bw there was still glomerular atrophy and tubular destruction, while at doses of 30 and 60 mg/kg bw the glomerulus and tubules were normal.

Glomerular damage (glomerular atrophy) causes disruption of the filtration process, thus causing kidney problems, namely reduced ability to filter blood. If the ability to filter blood is reduced, then blood cells and proteins can come out with the urine or even accumulate urea in the tubules because they can pass through the filtration process [23].

III.2 DISCUSSION

Elevated blood urea and creatinine levels were evident in group G2 which provided evidence that administration of 0.075g/kg BW of lead acetate could induce kidney injury. Administration of eleutherine americana merr extract at doses of 30 mg/kg and 60 mg/kg BW together with lead acetate significantly inhibited the increase in markers of kidney injury, namely blood urea and creatinine.

Creatinine is produced by the digestion of protein in the muscles, with most of the creatinine filtered through the blood by the kidneys and excreted in the urine. The glomerular filtration rate (GFR) is an important tool for measuring the renal excretion limit. In clinical practice, GFR is obtained from creatinine freedom in urine tests collected over 24 hours [23]. The level of creatinine clearance was significantly higher in the extract group with a dose of 60 (medium dose) this indicates the ability of eleutherine americana merr extract to remove creatinine from the blood into the urine, which in turn normalizes the creatinine content in the blood and extract at a dose of 120 (high dose) there was an increase in creatinine again this could be due to the active compound content in the eleutherine americana merr extract which had reached the highest level which in turn caused the active content in the extract to become toxic again.

In renal infection, serum urea accumulates and causes uremia because the rate of serum urea production exceeds the clearance rate [24, 25]. Significantly high blood urea in the G2 negative group indicates kidney injury. Administration of eleutherine americana merr extract prevented lead acetate-induced nephrotoxicity, significantly reduced urea accumulation in the 60 mg/kg bw extract group (medium dose) and the extract at 120 doses (high dose) increased blood urea return.

Lead acetate-induced nephrotoxicity is due to the formation of reactive oxygen species (ROS), especially the superoxide anion. Nephrotoxicity induced by ROS and NAPQI is largely offset by glutathione in the early stages of toxicity[26]

The content of creatinine and urea in the blood at a dose of 30 and a dose of 60 mg/kg bw decreased significantly compared to the control group, this provides evidence that eleutherine americana merr extract is able to minimize the toxic effects of lead acetate. The biochemical results were also confirmed by histologic findings, which showed preservation of the glomeruli and tubules.

Most lead acetate induces renal injury affecting the proximal tube, glomerulus, or more distal part of the nephron [27]. Gavage administration of lead acetate into the G2 negative group caused severe kidney damage, with tubular degeneration, wide lumina, damaged glomeruli, whereas pretreatment of eleutherine americana merr extract resulted in significant dose-

dependent nephroprotection against lead acetate-induced nephrotoxicity. Taken together, these results suggest that eleutherine americana merr extract may protect the kidneys from lead acetate-induced damage and may be a potential therapeutic candidate for lead acetate-induced nephrotoxicity.

Administration of lead acetate can cause substantial peroxidation of membrane lipids and depletion of antioxidants in renal tissue leading to severe kidney damage through the production of highly reactive free radicals [28, 29]. Decreased antioxidant status in renal tissue has been shown to partially explain the mechanism of lead acetate-induced nephrotoxicity as a result of free radical production [30]. In addition, previous studies have shown that eleutherine americana merr extract has antioxidant and anti-inflammatory effects and a protective effect against lead acetate-induced toxicity [31].

IV. CONCLUSIONS

The present study demonstrated the dose-dependent nephroprotective activity of the extract eleutherine americana merr in a mice strain BALB/C model of lead acetate-induced nephrotoxicity. Pretreatment with extract eleutherine americana merr dose-dependently prevented kidney injury as evidenced by serum and urine biochemical analysis and kidney histopathology. In conclusion, eleutherine americana merr is a potential nephroprotective agent against lead acetate-induced nephrotoxicity.

V. AUTHOR'S CONTRIBUTION

Conceptualization: Neti Eka Jayanti, Rozzana Mohd Said and Choo Chee Yan.
Methodology: Neti Eka Jayanti and Rozzana Mohd Said.
Investigation: Choo Chee Yan and Suhaidah Mohd Jofrry.
Discussion of results: Neti Eka Jayanti and Sa'adah Siregar.
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Writing – Review and Editing: Neti Eka Jayanti and Sa'adah Siregar.
Resources: Rozzana Mohd Said and Choo Chee Yan.
Supervision: Neti Eka Jayanti and Suhaidah Mohd Jofrry.
Approval of the final text: Neti Eka Jayanti and Suhaidah Mohd Jofrry.

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