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Research Paper

Moringa Leaves Extract Relieves Oxidative Stress and Improves Kidneys Impairment due to Lead Toxicity in Rats

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ABSTRACT

Background: The kidneys are the main target of lead toxicity as lead is accumulated in and excreted through the kidneys. The main lead toxicity occurs through oxidative stress and lipid peroxidation, causing cellular damage in the kidneys. *Moringa* leaves contain high levels of flavonoids, with antioxidant effects that are useful in treating diseases accompanied by oxidative stress due to toxicity. Our goal was to analyze the attenuation effects of *Moringa* leaves extract on lead-induced nephrotoxicity in male Wistar rats.
Methods: This research was conducted based on an experimental design and post-tests. Forty-eight Wistar rats were randomly divided into four groups as follows: control group (K), which was given lead acetate at 750 mg/kg/day for 7 days, and three other groups of P1, P2 and P3, which were given lead acetate at the same dose for 7 days followed by administering with the ethanolic extract of *Moringa* leaves at 1,000, 1,500, and 2,000 mg/kg/day doses orally for 14 days, respectively.
Results: There were improvements in oxidative stress in the kidneys of the study rats, marked by an increase in the serum levels of GSH, GPx, CAT, and SOD, and a decrease in the kidney MDA levels. Kidney disorders can be improved by administering ethanol extract from *Moringa* leaves, which is characterized by a significant decrease in blood BUN and creatinine levels.
Conclusion: All doses of the ethanolic extract of *Moringa* leaves reduced the oxidative stress and improved the kidney function impaired due to acute exposure to lead in male Wistar rats.

Keywords: Lead acetate, *Moringa oleifera* extract, Oxidative stress, Renal function, Wistar rats

Introduction

Exposure to lead remains a worldwide health issue, both in developing and developed countries [1]. Lead exposure occurs primarily in the work environment, cosmetics, paints, solder, hair dyes, and shields for X-ray machines [2]. Lead is among the most hazardous heavy metals in nature, which causes biochemical, physiological, and behavioral disorders in humans [3, 4]. It is also one of the most significant environmental contaminants, which affects a variety of organ systems, from moderate impairment to complete malfunction when consumed in food and water or inhaled through the air in regions of high traffic and/or due to industrial emissions [2].

The kidneys assume a significant role in lead digestion and are the most delicate organs vulnerable to lead toxicity. Oxidative stress, inflammatory response, and histo-pathological changes are the consequences of nephrotoxicity by lead [5]. Oxidative stress has been proposed as a significant mechanism that might be associated with lead nephrotoxicity. In nephrons, oxidative stress occurs when there is an imbalance between reactive oxygen species (ROS) in the environment and the tissue antioxidants. It has been reported that the excessive production of ROS lowers antioxidant enzyme activities, such as catalase, glutathione peroxidase (GPx), and superoxide dismutase (SOD), and increases lipid peroxidation. Further, free radicals can profoundly affect cell membrane lipids, proteins, and DNA, and are a major cause of injury and rapid renal cell destruction [6-8].

Lead binds to the sulfhydryl group of reduced

glutathione (GSH) and inactivates it [5]. A decline in the activity of antioxidant enzymes and GSH results in damage to the cell membrane lipids by raising the malondialdehyde (MDA) levels. This compound is a product of lipid peroxidation and serves as a biomarker of renal cell damage. Previous studies have shown that lead acetate causes biochemical changes in the serum, such as increased blood urea nitrogen (BUN) and creatinine. The pathological profile of lead toxicity includes the formation of oxidative stress, necrosis, and apoptosis. These nephrotoxic events have been explained by changes in the molecular, cellular, and intracellular processes [3, 8].

Few anti-inflammatory and antioxidant drugs are available for the treatment of renal disease secondary to lead exposure; however, they have undesirable side effects. Recently, herbal extracts have been shown to protect against renal inflammation and oxidative damage due to lead, with flavonoids being the main therapeutic candidate. One of the plants that has been proven to overcome oxidative stress is *Moringa* leaves (*Moringa oleifera* Lam.), and flavonoids are abundantly found in *Moringa* leaves. It has been established that flavonoids share a common structure of benzo-pyrone rings and protect against tissue pathologic conditions linked to oxidative stress [9]. Limited studies have been conducted on whether *Moringa* leaves extract can relieve the nephrotoxicity due to acute exposure to lead.

Aim of the Study: Based on the above literature review, this study was planned to investigate whether *Moringa* leaves extract was effective in relieving the nephrotoxicity due to acute exposure to lead acetate in rats' kidneys.

Materials and Methods

Study Design: This research was conducted as an experimental study to examine the attenuating effects of the extract of *Moringa* leaves on lead-induced nephrotoxicity.

Animals: Forty-eight Wistar rats (*Rattus norvegicus*) were used based on the study's inclusion and exclusion criteria. Male rats aged 2.5 to 3 months old and weighed between 150 g and 200 g. The rats were physically healthy and had never been used in other studies. They were kept in appropriate cages in the laboratory and were acclimated for one week prior to the start of the experiments. Marking on the animals was made with 10% picric acid. Doses of the lead-acetate compound and the *Moringa* leaves extract were adopted from previous studies [10, 11].

***Moringa* Leaves Extraction:** The extraction of *Moringa* leaves in 50% ethanol was conducted using maceration methods. The extract solution was then freeze-dried in a lyophilizer, based on the successful methods described in previous studies [10].

Work Procedure: The 48 rats were divided randomly into four groups as follows: one control and three treatment groups (P1, P2, and P3). The control group was administered only 750 mg/kg of lead acetate for seven days. Groups P1, P2, and P3 were administered with 750 mg/kg of lead acetate for seven days. Then, they were given the *Moringa* leaves extract at 1,000, 1,500, or 2,000 mg/kg per day for a period of 14 days, respectively.

Blood and Kidneys Sampling: The rats' blood samples were collected from the orbital veins in 3-mL micropipette tubes. The blood samples were drawn into vacuum tubes containing EDTA and centrifuged at 3,000 rpm for 15 min. The serum samples were used to determine the blood BUN and creatinine levels.

On the 15th day of the experiments, when the animals had been treated with *Moringa* leaf extract for 14 days, both kidneys were dissected and stored in the fridge until later experiments. For collecting the kidneys, a trans-abdominal surgery was performed on each rat after they had been decapitated. The kidneys were immersed in a 10% phosphate-buffered saline (PBS) solution after being cleaned with distilled water to remove the animals' blood. The kidneys were then crushed, weighed, and 1 g sample from each specimen was placed in 10-ml PBS at pH 7.4. These samples were completely homogenized in a mortar and centrifuged at 3,000 rpm for 20 min. The supernatant from each sample was drawn into a test tube.

Estimation of GSH, GPx, SOD, CAT, and MDA levels: The GSH level was examined by the GSH assay protocol (BT Laboratories, Shanghai, China). The SOD, GPx, and MDA activities were measured using a spectrophotometric method following the instructions on the kits. The kits were obtained from Ransod, RandoxGPx, and Randox MDA Manual from Randox Laboratories Ltd, UK, respectively. The catalase (CAT) activity was measured spectrophotometrically as described in its kit provided by Sigma Aldrich (Steinheim, Germany).

Estimation of BUN and Creatinine Levels: The BUN levels were measured by the diacetyl monoxime (DAM) method, and the creatinine estimation was performed using the alkaline picrate method [12, 13]. The DAM method uses a Fearon reaction, in which urea condenses with diacetyl monoxime to form a diazine derivative under acidic conditions. The reaction forms a pink complex in the presence of ferrous ions and thiosemicarbazide, which is measured spectrophotometrically at 530 nm. Jaffe's creatinine method is based on alkaline picrate. At alkaline pH, the creatinine in the sample reacts with picrate and forms a creatinine-picrate complex. The rate of increase in the absorbance at 500 nm due to the formation of this complex is directly proportional to the concentration of creatinine present in the samples.

Data Analyses: The data normality was assessed using the Shapiro-Wilk test, and the variation was assessed using the Levene test [10, 11]. Since the data were homogeneous and normally distributed, the parametric repeated ANOVA method was applied.

Additionally, a post hoc test with the least significant difference was conducted to determine the significant differences among the treatment groups.

Results

The oxidative stress investigated in this study was observed non-enzymatically, based on the GSH levels, and the enzymatic pathways of three scavenger enzymes, i.e., SOD, CAT, GPx, and lipid peroxidase (MDA) in the rats' sera. The results of GSH levels are presented in Table 1. The lowest GSH levels were found in the control group (K), while the highest GSH levels were found in P3, among the treatment groups. The findings revealed that the serum levels of GSH differed significantly in group K versus other treatment groups. The results indicated that at doses of 1,000, 1,500, or 2,000 mg/kg/day for 14 days, the GSH levels significantly increased, indicating that the non-enzymatic pathways were able to relieve oxidative stress effectively.

The activities of anti-oxidant enzymes after the treatments are presented in Tables 2, 3, and 4. Antioxidant enzymes act as scavengers of oxidative agents in the body. Based on the data shown in Table 2, the GPx activity in all treatment groups was higher than that of the control. The data indicated that the higher dose of *Moringa* leaf extract led to the higher activity of GPx. The results of the statistical analyses demonstrated that all of the treatment groups had significantly different levels of GPx activity than did the control group. The analyses revealed that a daily oral intake of 1,000 mg/kg of *Moringa* extract for 14 days was the lowest recommended dose that increased the GPx activity significantly.

Table 3 reflects the data on CAT enzyme activity for all groups after the treatment. The statistical analyses of the data indicated that the CAT activity was significantly higher in all treatment groups than in the control group. However, the study findings revealed that the SOD activity level was lower in the control group than those in the treatment groups. These findings demonstrated that consumption of the *Moringa* extract boosted the SOD activity, thereby reducing the oxidative stress from the acute toxicity caused by lead exposure. The statistical analyses also revealed that the control group and each of the treatment groups had significantly different levels of SOD enzyme activity (Table 4).

Oxidative stress can promote lipid peroxidase, which is characterized by increased levels of MDA. Based on the data presented in Table 5, the MDA levels in the kidneys in the treatment groups that were given the *Moringa* leaves extract were lower than that of the control group. According to the data reflected in Tables 2 to 5, it was observed that the administration of *Moringa* leaves extract at a minimum dose of 1,000 mg/kg per day for 14 days could significantly relieve the oxidative stress and the peroxidase level in the kidneys of the rats exposed to lead acetate. Moreover, as presented in Table 6, the BUN levels in the control group were greater than those of the treatment groups.

Table 1. Kidney GSH levels after treatment with 50% ethanol extract of *Moringa* leaves and statistical test results with the control group (K) as the comparison.

No.	Group	Mean ± SD (mmol/L)	Minimum (mmol/L)	Maximum (mmol/L)	*P*-value
1	Control (K)	3,57 ± 0,49	2,84	4,24	0,000
2	Treatment 1 (P1)	5,03 ± 0,37	4,52	5,52	0,000
3	Treatment 2 (P2)	6,06 ± 0,34	5,60	6,78	0,000
4	Treatment 3 (P3)	7,06 ± 0,41	6,44	7,88	0,729

Table 2. Kidney glutathione peroxidase activity after treatment with 50% ethanol extract of *Moringa* leaves and statistical test results with the control group (K) as the comparison.

No.	Group	Mean ± SD (µmol/L)	Minimum (µmol/L)	Maximum (µmol/L)	*P*-value
1	Control (K)	4,79 ± 0,58	4,02	5,90	0,000
2	Treatment 1 (P1)	7,28 ± 0,33	6,84	7,85	0,000
3	Treatment 2 (P2)	8,85 ± 0,46	8,20	9,74	0,000
4	Treatment 3 (P3)	11,94 ± 0,73	10,98	12,95	0,000

Table 3. Kidney catalase activity after treatment with 50% ethanol extract of *Moringa* leaves and statistical test results with the control group (K) as the comparison.

No.	Group	Mean ± SD (µmol/L)	Minimum (µmol/L)	Maximum (µmol/L)	*P*-value
1	Control (K)	2,73 ± 0,34	2,20	3,15	0,000
2	Treatment 1 (P1)	4,23 ± 0,30	3,76	4,72	0,000
3	Treatment 2 (P2)	5,50 ± 0,47	4,85	6,25	0,000
4	Treatment 3 (P3)	7,16 ± 0,51	6,29	8,02	0,006

Table 4. Kidney superoxide dismutase activity after treatment with 50% ethanol extract of *Moringa* leaves and statistical test results with the control group (K) as the comparison.

No.	Group	Mean ± SD (mIU/L)	Minimum (mIU/L)	Maximum (mIU/L)	*P*-value
1	Control (K)	30,71 ± 4,24	24,42	36,50	-
2	Treatment 1 (P1)	43,20 ± 3,12	38,90	47,05	0,000
3	Treatment 2 (P2)	52,44 ± 3,37	48,16	58,29	0,000
4	Treatment 3 (P3)	60,75 ± 3,54	55,29	67,82	0,000

Table 5. Kidney MDA levels after treatment with 50% ethanol extract of *Moringa* leaves and statistical test results with the control group (K) as the comparison.

No.	Group	Mean ± SD (mmol/L)	Minimum (mmol/L)	Maximum (mmol/L)	*P*-value
1	Control (K)	3,07 ± 0,32	2,60	3,61	-
2	Treatment 1 (P1)	1,34 ± 0,10	1,19	1,49	0,000
3	Treatment 2 (P2)	1,05 ± 0,15	0,85	1,35	0,000
4	Treatment 3 (P3)	0,77 ± 0,10	0,58	0,92	0,000

Table 6. Blood BUN levels after treatment with 50% ethanol extract of *Moringa* leaves and statistical test results with the control group (K) as the comparison.

No.	Group	Mean ± SD (mg/dL)	Minimum (mg/dL)	Maximum (mg/dL)	*P*-value
1	Control (K)	53,84 ± 5,29	44,62	60,58	-
2	Treatment 1 (P1)	48,15 ± 1,81	44,95	51,16	0,000
3	Treatment 2 (P2)	34,88 ± 2,41	29,06	38,62	0,000
4	Treatment 3 (P3)	19,75 ± 3,06	16,20	26,49	0,000

Table 7. Blood creatinine levels after treatment with 50% ethanol extract of *Moringa* leaves and statistical test results with the control group (K) as a comparison.

No.	Group	Mean ± SD (mg/dL)	Minimum (mg/dL)	Maximum (mg/dL)	*P*-value
1	Control (K)	2,12 ± 0,31	1,68	2,61	-
2	Treatment 1 (P1)	1,77 ± 0,12	1,56	1,99	0,000
3	Treatment 2 (P2)	1,19 ± 0,27	0,89	1,95	0,000
4	Treatment 3 (P3)	0,70 ± 0,98	0,55	0,85	0,000

The statistical analyses indicated that the *Moringa* extract significantly lowered the blood BUN levels in the rats exposed to lead acetate. Lastly, as shown in Table 7, the treatment groups that received the Moringa leaves extract had lower blood creatinine levels than that observed in the control group. In this context, the P3 group had the lowest level of blood creatinine. The statistical analyses demonstrated that the controls and each of the treatment groups had significantly different blood creatinine levels. From the findings, it is inferred that the *Moringa* extract improved the impaired kidney function in the rats poisoned with lead acetate.

Discussion

The findings of this study suggested that the oral administration of *Moringa* extract at a minimum dose of 1,000 mg/kg per day for 14 days may significantly reduce the oxidative stress in the kidneys of the rats poisoned with lead acetate. The decline in the oxidative stress occurred in both enzymatic and non-enzymatic pathways.

Lead causes oxidative stress by interacting with sulfhydryl and other nucleophilic functional groups, and it disrupts some metabolic processes through enzymatic and non-enzymatic pathways [14]. By altering the activity and expression of antioxidant enzymes, lead lowers antioxidant defense in the enzymatic pathway. The covalent interactions of lead (Pb^{2+}) with the sulfhydryl groups of the defense systems of antioxidants have been linked to a number of toxic effects associated with lead in biological systems [15]. These interactions affect enzymes, such as GPx, glutathione-S transferase, SOD, and CAT, by uprooting the zinc ions that serve as important cofactors in the catalytic locations.

The levels of SOD, CAT, and GPx have also been shown to rise and fall in response to lead. Further, the effect of lead on boosting ROS generation can be considered a source of disequilibrium between the formation of free radicals in tissues and cellular structures, and the capacity of antioxidant enzymes to eliminate free radicals that harm cell membranes, DNA, and proteins. This finding is consistent with that reported in another study regarding *Moringa* leaves extract [16]. That study found that the extract significantly increased SOD and CAT in rats intoxicated with cobalt chloride ($CoCl_2$). This event can occur because *Moringa* leaves extract contains vitamins C, A, and E, flavonoids, phenolic acids, iridoids, and coumarins. These vitamins and compounds have antioxidant activities both directly by donating H^+ to free radicals or indirectly by stimulating the pathway of antioxidant response elements and nuclear factor-erythroid 2-related factor 2 (Nrf2), which promote the activity of antioxidant enzymes [17].

Vitamin C is an antioxidant and is important in maintaining the health of cells in the body [18]. As a free radical scavenger, ascorbic acid can directly react with superoxide anions, hydroxyl radicals, oxygen singlets, and lipid peroxide. Because of this property, the role of vitamin C is highly important in maintaining the integrity of cell membranes. Vitamin A serves as a strong antioxidant against ROS [18]. This vitamin stops the chain reactions by free radicals and binds O_2 as it has nine double bonds in its carbon chain [19]. As an antioxidant, vitamin E functions as a hydrogen ion donor and converts peroxyl moieties into tocopherol radicals, which are less reactive; as a result, they do not damage fatty acid chains [20]. Vitamin E also collaborates with oxygen to destroy free radicals [21].

Flavonoids are the main antioxidants in *Moringa* leaves, which contain ketone hydroxyl groups, acting as catalysts for lipid peroxidation [21]. They also have hydroxyl groups that stabilize ROS and donate hydrogen atoms to free radicals [21]. Although phenolic compounds are recognized as direct antioxidants, they also demonstrate indirect antioxidant activity by activating inborn protective enzymes and have favorable regulatory effects on some signaling

pathways. Since phenol groups are reactive and their aromatic rings contain hydroxyl substituents, phenolic acids act as antioxidants. Due to the reactivity of the phenol moiety, phenolic acids have a number of known mechanisms to support their antioxidant action [22]. However, the main method is thought to involve radical scavenging and donating hydrogen atoms. The substituents on the aromatic ring in phenolic acids affect the stability of the structure and consequently impact the ability to scavenge free radicals [23].

Iridoids are compounds that are widely found in plants. They have anti-inflammatory, antioxidant, neuroprotective, immunomodulatory, hepatoprotective, cardioprotective, anticancer, antibacterial, and antispasmodic effects [24]. According to previous studies [24, 25], Iridoids inhibit oxidative stress by increasing the activity of SOD, GSH, GPx, nitric oxide synthase, and nitric oxide production [24]. Coumarin belongs to the Benzopyrone family (i.e., benzo-alpha-pyrone), and has various benefits, such as anti-inflammatory, antioxidant, and neuroprotective functions. Coumarins activate the Keap1/Nrf2/ARE signaling pathway and promote the production of antioxidant enzymes [17].

Our study findings suggested that administering *Moringa* extract at least at 1,000 mg/kg/day for 14 days could significantly improve kidney function in Wistar rats poisoned with lead, as evidenced by the significant decline in the serum BUN and creatinine levels. Almost 90% of the lead that enters the body binds to albumin, a major erythrocyte protein [26]. By endocytosis and/or erythrophagocytosis, lead is deposited in various organs and tissues, including the kidneys, and causes oxidative damage to them by affecting the mitochondrial respiratory chain [26].

Another hypothesis exists that explains the dynamic of lead-induced kidney damage [26]. Hypothetically, Pb^{2+} competes with Ca^{2+} and dysregulates calcium homeostasis. As a result, the release of Ca^{2+} from mitochondria is stimulated, which initiates the opening of the mitochondrial transition pore, resulting in total mitochondrial damage and the formation of reactive species and oxidative stress, including altered lipid metabolism [26]. Other disruptive events include oxidative stress, ROS generation, and metabolism of modified lipids. Upon exposure to lead, the cells in the kidneys' proximal tubules suffer damage and apoptosis. Additionally, lead changes cellular structures and interferes with the stability of tight junctions [26]. The changes in the polarity and transport of epithelial cell vectors could result from atypical cell-cell junctional structures once the renal proximal tubules lumens are reduced and microvilli are lost [26].

Damages to the kidney proximal tubules cause impaired renal function, which can be evident as increased urea and creatinine in the blood and urine. Administration of the *Moringa* extract in the current study significantly reduced the rats' serum BUN and creatinine levels. These findings are consistent with those of Melebary and Elnaggar [27], who also reported similar results. Up to now, there has been no explanation of how *Moringa* leaves extract can reduce BUN and creatinine levels in the blood. However, the role of that extract is likely to be indirectly protecting against damage to cells in the kidneys' proximal tubules, thus improving the kidneys' function [28].

Finally, the high contents of anti-oxidants, including polyphenols, may explain the major improvement in kidney function in the rats acutely poisoned with lead acetate after the administration of the *Moringa* leaves extract. Numerous natural sources, including various types of fruits and vegetables, contain polyphenols, and their beneficial effects in attenuating heavy metal toxicity have been established in various disease models [4]. According to published reports, polyphenols remove toxic heavy metals, such as lead, from various organs, including the kidneys [4]. The potential mechanism of action of polyphenolic compounds is scavenging free radicals, such as ROS.

Limitations of the Study: This study had the limitation of lacking histopathological tests of the renal tissue samples. Otherwise, pathological examinations could provide first-hand evidence of the effect of *Moringa* extract on repairing the damaged renal cells. However, previous research has suggested that declines in the MDA, BUN, and creatinine levels in rats treated with *Moringa* extract provide sufficient evidence for structural repairs in renal cells. Elucidation of the mechanisms involved in the repair of renal cells warrants future histopathological investigations in animal and possibly human models.

Conclusions

This study concludes that the administration of 50% ethanol extract from *Moringa* leaves improves oxidative stress and reduces kidney function disorders induced by acute lead toxicity. In this study, the minimal effective dose for each rat that reduced oxidative stress and improved kidney impairment due to lead exposure was 1,000 mg/kg/day if administered orally for 14 days.

Conflict of Interests

The authors declared that there was no conflict of interests with any internal or external entities in conducting this study.

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Compliance with Ethical Guidelines

Ethical approval was received from the Ethics Commission of the Faculty of Medicine, Jenderal Soedirman University, on August 25, 2022 (Approval #: 017/KEPK/PE/VIII/2022).

Authors' Contributions

Agung Saprasetya Dwi Laksana was involved in all research processes, from designing, performing, and supervising the experiments; collected, analyzed, and interpreted data; and prepared, edited, and revised the manuscript draft. Lily Kusumasita Burkon and Madya Ardi Wicaksono conducted laboratory experiments, collected blood and kidney samples, examined samples in the laboratory, and analyzed and interpreted the data. Muhammad Zaenuri Syamsu Hidayat and Pugud Samodro proofread, edited, and revised the references. All authors discussed, reviewed, and approved the final version of the manuscript before submitted to this journal.

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