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Research Article

Protection by Methanol and Flavonoid-rich Leaf Extracts of *Talinum triangulae* Against Lead-induced Toxicity in Rats

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Abstract

Background and Objective: Lead is an environmental pollutant. The painful invasive chelation therapy makes it exigent to continue the protracted treatment against lead toxicity. This study is aimed at evaluating the protective effect of the methanol and flavonoid-rich leaf extracts of *T. triangulae* on lead-induced toxicity. **Materials and Methods:** Thirtysix male albino rats were used and were distributed into five groups; A, B, C D and E. Groups D and E were further subdivided into three; D_1, D_2, D_3, E1, E_2 and E_3. Both groups and subgroups contained four rats each. All groups except A were intraperitoneally injected a single dose of 50 mg kg^{-1} b.wt., of lead chloride and commenced treatment within 30 min of lead injection. Group A and B were given distilled water and 50 mg kg^{-1} b.wt., of lead chloride only, respectively. Group C was treated only with 40 mg kg^{-1} vitamin C while groups D_1, D_2, D_3 and E_1, E_2, E_3 were treated with 50, 100 and 200 mg kg^{-1} b.wt., of methanol and flavonoid-rich leaf extract of *T. triangulae*, respectively for 14 days once daily. Blood were collected for determination of antioxidant indices in plasma using standard method and data were analyzed using SPSS version 20. **Results:** The result showed that methanol and flavonoid-rich extracts significantly ($p<0.05$) decrease the Malondialdehyde (MDA) level that was triggered by the increase oxidative stress when treated with lead. The levels of glutathione and activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) were increased significantly when treated with the extracts. **Conclusion:** The outcome of this study showed that the extracts have a protective effect on lead-induced toxicity.

Key words: Lead toxicity, biochemical system, oxidative stress, flavonoid, antioxidants, *Talinum triangulae*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Lead is a naturally occurring toxic metal found in the Earth's crust. It has many uses, including in the manufacture of lead-acid batteries for motor vehicles and energy storage in pigments and paints, solder, ammunition, ceramic glazes, jewellery, toys and in cosmetics and traditional medicines[1]. During the 1970's and 80's, concern over lead related health problems led to the implementation of policies, such as; the introduction of unleaded gasoline and a ban on using lead-based paint; however, lead continues to be used in some aviation fuels (Avgas) for piston-engine aircraft[2]. The processing, use and disposal of lead and its products can result in environmental contamination and human exposure. As lead is an element once released into the environment it persists as a potential source of exposure.

The adverse impacts of lead range from slight alterations of biochemical and physiological systems to serious damage in some vital organs leading to death of the organism[3]. Its wide spread has resulted to environmental contamination, human exposure and significant public health problems in many parts of the world[1]. Lead contamination globally has been reported to be due to increase in its circulation in soil, water and air because of human activities related to industries, food and smoking, drinking water and other domestic sources[4]. According to Institute for Health Metrics and Evaluation (IHME)[5], it was estimated that lead exposure accounted for 63.2% of the global burden of idiopathic developmental intellectual disability, 10.3% of the global burden of hypertensive heart disease, 5.6% of the global burden of the ischemic heart disease and 6.2% of the global burden of stroke. Also, it was estimated that lead exposure accounted for 1.06 million deaths and 24.4 million years of healthy life lost worldwide due to long-term effects on health[5]. The highest burden was in low and middle-income countries[5]. The levels of lead in the blood that were initially thought to be safe has now been proven to compromise health and cause injury to multiple organs even when overt symptoms are not present.

Lead toxicity is known to occur through lead-induced oxidative stress[6]. Flora *et al.*[7] reported that the machinery of lead-induced oxidative stress was the depletion of antioxidative reserves and the augmented generation of Reactive Oxygen Species (ROS). Toxicity also arises from lead replacing divalent cations, which are necessary for cellular activity[3,8].

Management of lead-mediated chronic health hazards principally depends on the chelation therapy where, disodium calcium edentate, Ethylenediaminetetraacetic Acid (EDTA), British Anti Lewisite (BAL) and Dimercaptosulfonic Acid (DMSA) are extensively used[3]. Prolonged use of the above agents is questionable due to its painful intramuscular route of treatment with moderate to severe side effects[3,9] among the patients. Hence, the mitigation of lead toxicity with the use of a safe, cheap, non-invasive strategy is becoming a new challenge via nutraceuticals or drug from the herbal source. Among these, the vegetable, *Talinum triangulae* is common, easily accessible and is reasonably inexpensive throughout Nigeria especially in Eastern region. Therefore, the present study was conducted to assess the defensive effects of methanol and flavonoid-rich leaf extracts of *Talinum triangulae* in the blood of lead-exposed rats (Plate 1).

Plate 1: Leaves of *Talinum triangulae* (water leaf)
Source: Emmanuel *et al.*[10]

MATERIALS AND METHODS

Duration of research: The research was conducted in the Department of Biochemistry, Ebonyi State University, Abakaliki, Nigeria, for four months from August-November, 2019.

Plant collection and extraction: The leaves of *Talinum triangulae* (water leaf) were obtained in August, 2019 from a farmland in Abakaliki in Ebonyi state, Nigeria. The leaves were authenticated by a taxonomist in the Department of Biological Sciences of Ebonyi State University, Abakaliki. The leaves were afterward cleaned, air-dried and blended. The blended leaves were extracted with 90% methanol for 72 h. The methanol extract was concentrated using a rotary evaporator, freeze dried and preserved for further use. The protocol of Joseph *et al.*[11] was adopted.

Preparation of flavonoid-rich extract: The preparation of flavonoid-rich extract was done according to the method described by Chu *et al.*[12]. About 12 g of methanol extract was dissolved in 60 mL of 10% H_2SO_4 in a small flask and was hydrolyzed by heating on a water bath for 30 min at 100°C. The mixture was placed on ice for 15 min, so as to allow flavonoids aglycones precipitate and the cooled solution was filtered. Then, the filtrate was liquefied in 50 mL of warm 95% ethanol (50°C) and the resulting solution was again filtered into 100 mL volumetric flask which was made up to the mark with 95% ethanol. The filtrate collected was concentrated to dryness using rotary evaporator.

Preparation of methanol and flavonoid-rich extracts: Almost 10 and 6.60 g of methanol and flavonoid-rich extracts were dissolved in 38 and 25 mL of distilled water respectively to get stock solution of 0.26 g mL^{-1}.

Preparation of lead chloride solution: Five grams of lead chloride was dissolved in 20 mL of distilled water to get stock solution of 0.25 g mL^{-1}.

Preparation of ascorbic acid solution: About 6.60 g of ascorbic acid was dissolved in 25 mL of distilled water to get stock solution of 0.26 g mL^{-1}.

Experimental design: The protocol of Obafemi *et al.*[13] was adopted. A total of 36 male albino rats weighing between 120-250 g were acclimatized to laboratory conditions for a period of 1 week and they had access to feed and water *ad libitum*. Animal studies were adhered to the Principles of Laboratory Animal Care (NIH publication # 85-23)[14]. The male albino rats were divided according to body weight into five groups namely; A, B, C D and E. The rats in groups D and E were further subdivided into three; D_1, D_2, D_3, E1, E_2 and E_3. Both groups and subgroups contained four rats each. All groups except A were intraperitoneally injected a single dose of 50 mg kg^{-1} b.wt., of lead chloride after overnight fasting. Treatment commenced within 30 min after injection of the lead. Group A was given distilled water only and served as control while B was injected 50 mg kg^{-1} b.wt., of lead chloride only. Group C was treated only with 40 mg kg^{-1} vitamin C while groups D_1, D_2, D_3 and E_1, E_2, E_3 were treated with 50, 100 and 200 mg kg^{-1} b.wt., of methanol leaf extract and flavonoid-rich leaf extract respectively for 14 days once daily via oral intubation.

Collection and preparation of blood for oxidative stress markers: After 14 days of treatment, the male albino rats were euthanized and blood was collected through ocular puncture using capillary tube into lithium heparin container. Plasma was, thereafter, obtained by centrifuging the blood sample for 10 min at 3,000 rpm. Malondialdehyde (MDA) and glutathione (GSH) levels, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) activities were estimated in the plasma. The protocol of Uraku *et al.*[14] was adopted.

Determination of MDA levels in the plasma: The MDA was measured to justify the lipid peroxidation status in the plasma. MDA-reactive products were measured using thiobarbituric acid. The MDA was determined by spectrophotometric method as described by Wallin *et al.*[15]. Exactly 0.1 mL of plasma, 0.9 mL of distilled H_2O, 0.5 mL of TCA reagent and 0.5 mL of TBA reagent were added to a test tube. The test tube was incubated at 95°C for 30 min in a water bath at (37°C). After which, the test tube was allowed to cool in water and exactly 0.1 mL of Sodium Dodecyl Sulphate (SDS) was added to the test tube. The absorbance of the sample was read against the blank test reagent at 532 and 600 nm. Calculation was done according to Wallin *et al.*[15]:

$$\text{TBARS (\%)} = \frac{\text{532-A60C}}{0.52\text{-}8 \times 0.1\ 1} \times 100$$

Determination of GSH levels in the plasma: Glutathione was determined according to the method of Jollow *et al.*[16]. A 20% hemolysate of sera was prepared in distilled water and 0.3 mL of this was mixed with 0.7 mL of 0.2 M sodium phosphate buffer (pH 8) and 2 mL of 0.6 mM DTNB (prepared in 0.2 M buffer, pH 8). After 10 min, the yellow color obtained was measured at 412 nm against a blank which contained 0.1 mL of 5% TCA in place of the supernatant. A standard graph was prepared using different concentrations (10-50 n mol^{-1}) of GSH in 0.3 mL of 5% TCA. The GSH content was calculated with the help of this standard graph and expressed as moles mg^{-1} protein. The GSH level was expressed as µmol mL^{-1} of blood.

Determination of SOD activities in the plasma: The method described by Misra and Fridovich[17] was used to determine the activity of Superoxide dismutase (SOD). The plasma, 0.02 mL was mixed with 2.95 mL 0.05 M sodium carbonate buffer (pH 10.2) and 0.03 mL of epinephrine in 0.005 M HCl was used

to start the reaction. Similarly, exactly 2.95 mL buffer, 0.03 mL of epinephrine and 0.02 mL of distilled water were added to the blank. The SOD activity was measured by considering changes in absorbance at 480 nm for 5 min, $\sum = 4020\ M^{-1}\ cm^{-1}$:

$$\text{SOD activity (U mg}^{-1}) = \frac{\Delta A\ \text{min}^{-1} \times V_T}{\sum \times V_S}$$

where, ΔA is the change in absorbance, V_T is the total volume, V_s is the sample volume and $\sum$ is the molar extinction.

Determination of CAT activities in the plasma: Catalase (CAT) activities were assayed according to the method described by Aebi[18]. Exactly 2.5 mL of phosphate buffer, 2 mL of hydrogen peroxide (H_2O_2) as well as 0.5 mL of the plasma were added to a test tube. Exactly 2 mL of dichromate acetic acid reagent was added to 1 mL portion of the reaction mixture. The CAT activity was measured in absorbance at 240 nm against the blank at a minute interval by a formula[18] given below:

$$\text{Catalase concentration (U L}^{-1}) = \frac{\Delta A\ \text{min}^{-1} \times V_T}{\sum \times V_S}$$

where, ΔA is the change in absorbance, V_T is the total volume, V_s is the sample volume and $\sum$ is the molar extinction.

Determination of GPX activities in the plasma: This was determined by using the method of Rotruck *et al.*[19]. A mixture of 0.1 M phosphate buffer (pH 7.4), 10 mM sodium azide, 4 mM GSH, 2.5 mM H_2O_2 and 0.5 mL of plasma was incubated at 37°C for 3 min followed by the addition of TCA (10%) and centrifugation of mixture at 3,000 rpm for 5 min. Two milliliters of K_2HPO_4 and 1 mL of 0.04% DNTB were added to 1 mL of the supernatant obtained and the absorbance was read at 412 nm against a blank.

Statistical analysis: The experimental data were expressed in terms of Mean±SD, N = 5 of different groups. The differences between the mean values were evaluated by ANOVA and the group means were compared by Duncan's Multiple Range Test (DMRT). Values were considered statistically significant at $p<0.05$.

RESULTS

Effect of *T. triangulae* extracts on MDA level: The MDA levels of treated and untreated rats are shown in Fig. 1. It demonstrated a significant high levels of MDA at $p<0.05$ in

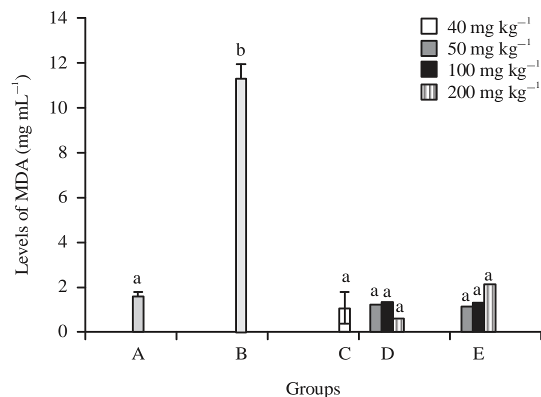

Fig. 1: Effect of methanol and flavonoid-rich leaf extracts of *T. triangulae* on MDA levels in lead treated rats
Bars with different alphabets showed significant changes at $p<0.05$ and vice versa, A: Control (distilled water only), B: Lead-induced rats, C: Lead chloride+vitamin C, D: Lead chloride+methanol leaf extract, E: Lead chloride+flavonoid-rich leaf extract

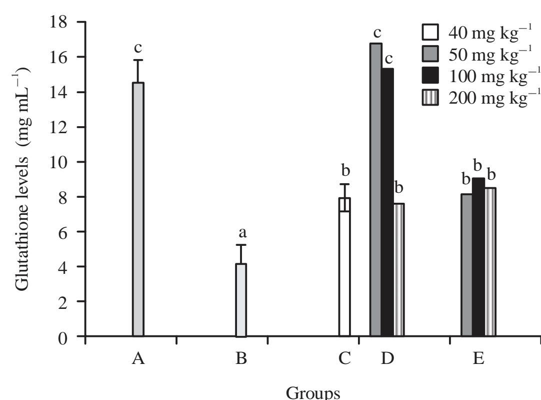

Fig. 2: Effect of methanol and flavonoid-rich leaf extracts *of T. triangulae* on glutathione levels of rats treated
Bars with different alphabets showed significant changes at $p<0.05$ and vice versa, A: Control (distilled water only), B: Lead-induced rats, C: Lead chloride+vitamin C, D: Lead chloride+methanol leaf extract, E: Lead chloride+flavonoid-rich leaf extract

lead administered untreated rats. In the treated groups, the methanol and flavonoid-rich leaf extracts *of T. triangulae* as well as vitamin C showed no significant decrease at $p>0.05$. However, only methanol leaf extracts *of T. triangulae* demonstrated little changes in non dose dependent style.

Effect of *T. triangulae* extracts on GSH level: The GSH levels of treated and untreated rats are shown in Fig. 2. A significant reduction levels of glutathione was shown in lead administered untreated rats at $p<0.05$. in the treated groups, the methanol and flavonoid-rich leaf extracts of *T. triangulae* together with vitamin C demonstrated significant increase at $p<0.05$ and the effect was in non dose dependent manner.

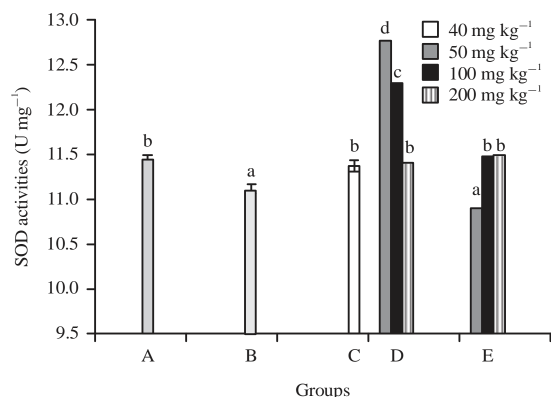

Fig. 3: Effect of methanol and flavonoid-rich leaf extracts of *T. triangulae* on SOD activities of rats treated
Bars with different alphabets showed significant changes at p<0.05 and vice versa, A: Control (distilled water only), B: Lead-induced rats, C: Lead chloride+vitamin C, D: Lead chloride+methanol leaf extract, E: Lead chloride+flavonoid-rich leaf extract

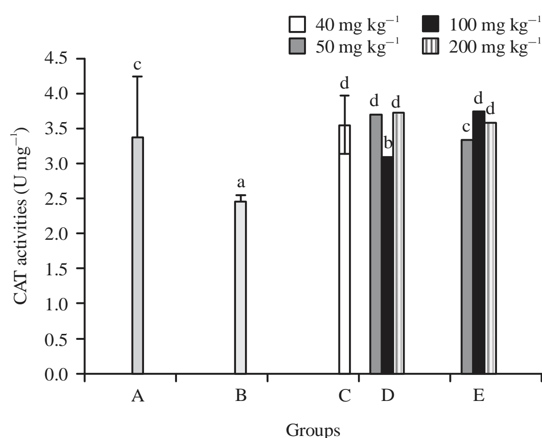

Fig. 4: Effect of methanol and flavonoid-rich leaf extracts of *T. triangulae* on catalase activities of rats treated
Bars with different alphabets showed significant changes at p<0.05 and vice versa, A: Control (distilled water only), B: Lead-induced rats, C: Lead chloride+vitamin C, D: Lead chloride+methanol leaf extract, E: Lead chloride+flavonoid-rich leaf extract

Effect of *T. triangulae* extracts on SOD, CAT and GPX activities: Figure 3 showed a significant decrease in the activities of SOD in lead administered untreated rats and in rats treated with only 50 mg kg^{-1} of flavonoid-rich leaf extracts *of T. triangulae*. The administration with methanol and flavonoid-rich leaf extracts *of T. triangulae* as well as vitamin C demonstrated significant increase at p<0.05 but the increase was higher in 50 and 100 mg kg^{-1} of methanol rich leaf extract treated rats.

Figure 4 showed a significant decrease in the activities of CAT in lead administered untreated rats. The administration with methanol and flavonoid-rich leaf extracts of *T. triangulae*

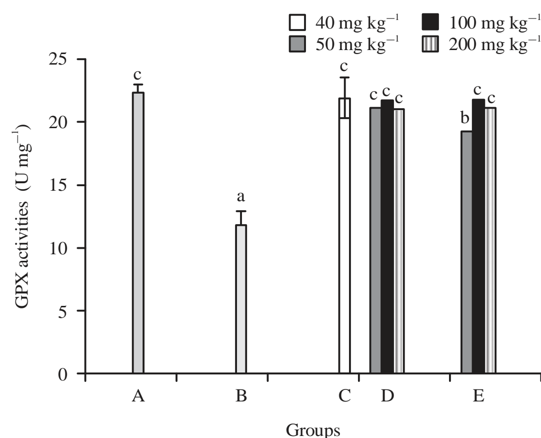

Fig. 5: Effect of methanol and flavonoid-rich leaf extracts of *T. triangulae* on glutathione peroxidase activities of rats treated
Bars with different alphabets showed significant changes at p<0.05 and vice versa, A: Control (distilled water only), B: Lead-induced rats, C: Lead chloride+vitamin C, D: Lead chloride+methanol leaf extract, E: Lead chloride+flavonoid-rich leaf extract

showed significant increase at p<0.05 which compared favorably vitamin C treated rats. However, the increase was higher in 100 and 200 mg kg^{-1} of flavonoid-rich leaf extracts *of T. triangulae* and in 50 and 200 mg kg^{-1} of methanol leaf extract treated rats.

Figure 5 showed a significant decrease in the activities of GPX in lead administered untreated rats and in rats treated. The administration with methanol and flavonoid-rich leaf extracts of *T. triangulae* showed significant increase at p<0.05 which compared favorably with normal and vitamin C treated rats.

DISCUSSION

Virtually, lead is a ubiquitous environmental and industrial pollutant that has been perceived in all phase of environment and biological system. The persistence nature of lead has quite often been allied with considerable health risks in human and animal tissues[13,20]. Ascorbic acid is probably the most widely considered vitamin when it comes to the prevention of lead-induced oxidative stress. Its property of scavenging free radicals along with metal chelation makes it a potential detoxifying agent for lead[21]. However, the therapeutic and prophylactic use of plant extracts to ameliorate lead-induced organ damage has been severally explored[22].

From the result of the experiment in Fig. 1, the MDA level increased significantly (p<0.05) in-group B which indicated that there was a boost in oxidative stress. Upon treatment

with vitamin C and the plant extracts, the level was reduced. Group C when compared to other treated groups compared favorably each other as well as group A and showed non-significant reduction in the MDA levels at $p>0.05$. The result of this study is in conformity with the report of Obafemi *et al.*[13], who reported that the methanol and flavonoid rich extracts of *Synsepalum dulcificum* significantly ($p<0.05$) lowed MDA levels. Also, the result is accordance with the work of Mohammad *et al.*[23], who reported that concentration of MDA in plasma of painters was higher than controls; 7.48 ± 1.3 vs. 3.08 ± 0.56 nmol mL^{-1}. MDA level is an important pointer of LPO in biological systems. Lead is known to produce oxidative damage in the blood by enhancing peroxidation of lipid membranes[24] and LPO is deleterious process solely carried out by free radicals[25].

In Fig. 2, the result showed that gluthathione level in group B was reduced significantly ($p<0.05$) compared to other groups, but treatment with vitamin C and plant extracts increased the level. However, the increment was higher in animals treated with 50 and 100 mg kg^{-1} b.wt., of methanol leaf extract which compared favorably with group A while only 200 mg kg^{-1} of methanol leaf extract, all doses of flavonoid rich extracts and vitamin C showed similar value. This is in accordance with report of Mohammed *et al.*[23], who observed a significant depletion of GSH in painters compared to controls. A significant change was also observed in the oxidised form of GSH, i.e., GSSG. Also, the present study is corroborated by the observations of Gargouri *et al.*[26] and Abdel-Moneim *et al.*[27]. GSH plays a pivotal role in the protection of cells against oxidative stress. It can act as a non-enzymatic antioxidant by direct interaction of the SH group with ROS or it can be involved in the enzymatic detoxification reactions for ROS as a cofactor[28]. Many studies have shown a decrease in GSH levels during lead toxicity[29,30].

From the result in Fig. 3, the activity of SOD was lower in group B is when compared to other groups but administration of vitamin C and plant extracts revised the effect. Though, the increase in SOD activity was higher in groups treated with 50 and 100 mg kg^{-1} of methanol leaf extract but 200 mg kg^{-1} of methanol leaf extract, all doses of flavonoid-rich extract as well as vitamin C and group A did not show any significant increase. The research of Obafemi *et al.*[13] agreed with the result of this present study who reported that Sodium Dismutase (SOD) activity was significantly lower at $p<0.05$ in the lead-induced untreated group compared to both the control and ascorbic acid-treated groups. Similarly, Jakie *et al.*[31] reported that superoxide dismutase in serum showed a significant decrease at $p<0.05$ in lead-treated group and a significant increase in *E. elatior* treated groups compared to the control group at $p<0.05$.

Findings from Fig. 4 and 5 showed that catalase and GPx activities was reduced in the group B when compared to other groups, but upon treatment with vitamin C and methanol and flavonoid rich leaf extracts increased the CAT and GPx activities. The increase compared favourably with all the treated groups together with group A. The work of Kandimalla *et al.*[32] and Berrahal *et al.*[33] corroborated with the outcome of this study in CAT and GPX activities. Antioxidants have been employed in pharmaceuticals as they play wonderful roles in pathological conditions as well as in food industries as food additives. The consumption of plant based antioxidants is considered useful because of its usefulness in circumventing scores of ailments associated with oxidative stress. Consequently, the rebellion application of natural antioxidants in food industries, medical and pharmaceutical field indicates its potential as promising alternative to synthetic once.

CONCLUSION

Based on the present study, it can be concluded that the methanol and flavonoid rich leaf extracts of *Talinium traiangulare* have protective effect against lead-induced toxicity in rats. And that the methanol leaf extract of *Talinium traiangulare* was more effective than flavonoid-rich leaf extract. So, further investigation is necessary to identify and isolate the actual phytochemicals responsible for its protective efficacy and this will help to elucidate the actual mechanism of action.

SIGNIFICANCE STATEMENT

This study discovered the use of *T. triangulae* leaf as a significant natural antioxidant which can be beneficial as a possible remedy for diseases associated with oxidative stress. This study will help the researcher to uncover the critical areas of the use of noninvasive treatment strategy against toxic metal pollution via nutraceuticals or drug from vegetable source that many researchers were not able to explore. Thus, a new theory on preventive role of leaf extracts of *T. triangulae* against lead may be arrived at.

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