



## AMELIORATIVE EFFECTS OF TANNIN FRACTIONATE OF *CYPERUS ESCULENTUS* TUBER ON LEAD-INDUCED HEPATOTOXICITY IN WISTAR RATS

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### Cite this article:

Hassan, L. A., Adamu, S. P., Lawal, R. T., Ojo, F. O., Abdul, D. I., Hasiya, S. B. (2025), Ameliorative Effects of Tannin Fractionate of *Cyperus esculentus* Tuber on Lead-Induced Hepatotoxicity in Wistar Rats. African Journal of Biology and Medical Research 8(2), 25-35. DOI: 10.52589/AJBMR-Q6JRFXA

### Manuscript History

Received: 14 Mar 2025

Accepted: 9 Apr 2025

Published: 1 May 2025

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**ABSTRACT:** *Aims: Hepatoprotective activity of the tannin fraction of Cyperus esculentus was studied against lead-induced hepatotoxicity in Wistar rats. Materials and Methods: Twenty adult male Wistar rats were divided into four (4) groups (A-D) (n=5). Group A was administered normal saline only. Groups B, C, and D were fed 30mg/kg body weight of lead. Additionally, groups C and D were administered with 50mg/kg body weight and 100mg/kg body weight of tannin fraction of Cyperus esculentus, respectively, orally for 28 days. The following day after the last administration, the animals were sacrificed under 50 mg/kg body weight of thiopental anaesthesia, and blood samples were obtained through the intra-cardiac puncture to assay the levels of liver enzymes [Aspartate transaminase (AST) and Alanine transaminase (ALT)] and oxidative stress markers [malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT)]. The liver of the rats was harvested, weighed, and fixed in 10% formol saline and sectioned for histological studies using hematoxylin and eosin. Results: Results showed that treatment with lead was associated with decreased levels of SOD, CAT, GSH, and increased MDA activities. Also observed is a significant increase in liver enzymes (AST and ALT) in the Lead-treated group. The histological result revealed alterations of hepatic structure, including hepatocytic vacuolations and sinusoidal congestion following lead treatment. Treatment with tannin fraction of Cyperus esculentus prevented and reversed lead-induced hepatic injury. Conclusion: Tannin fraction of Cyperus esculentus enhanced innate antioxidant activity and ameliorated the Lead-induced liver injury and can be used to protect against hepatotoxicity.*

**KEYWORDS:** *Cyperus esculentus*, Tannins, Lead, Liver, hepatotoxicity.



## INTRODUCTION

The liver plays an important role in the biotransformation of drugs and toxins. For this reason, drugs and chemicals-induced hepatotoxicity, which we are exposed to daily, is a major concern in our society (Singh *et al.*, 2011). More than 900 drugs and chemicals have been found to cause liver injury (Friedman *et al.*, 2003). Lead is a very common environmental toxic metal that causes many histological, physiological, and biochemical abnormalities in humans and animals (El-Tantawy, 2016). Lead toxicity remains a common problem in every country due to unavoidable environmental and occupational exposure (Abdel-Zaher *et al.*, 2019). The mechanism of lead (Pb) toxicity involves the induction of oxidative stress (Flora *et al.*, 2012). The mechanism of lead-induced oxidative stress is a result of an imbalance between the generation and removal of Reactive Oxygen Species (ROS) in tissues and cellular components causing damage to DNA, proteins, and membranes (Patra *et al.*, 2001). Lead has been reported to cause oxidative stress by generating the release of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radicals, and lipid peroxides (E-Nekeety *et al.*, 2009). Oxidative stress has a pathological role in the degeneration of tissue in the body (Flora *et al.*, 2012).

Plants have been screened for their medicinal properties (Tamunotonye and Asala, 2007). Most fruits and vegetables are important sources of a balanced diet and nourishment (Ibrahim *et al.*, 2009). *Cyperus esculentus* is an annual or perennial plant that grows up to 90 cm tall (Hassan *et al.*, 2018). Its solitary stems grow from a tuber. Our phytochemical screening of *Cyperus esculentus* revealed that it contains a high percentage of alkaloids, saponins, and tannins (Ekeanyanwu *et al.*, 2010; Hassan *et al.*, 2018). According to Adejuyitan *et al.*, 2009. *Cyperus esculentus* was reported as healthy and helps in preventing heart thrombosis by activating blood circulation and is responsible for preventing and treating urinary tract infections and other bacterial infections. Abano and Amoah (2011) reported that *Cyperus esculentus* oil can be used in the cosmetic industry. As it is an antioxidant (because of its high content in vitamin E), it helps slow down the aging of the body cells. *Cyperus esculentus* has many local names, which include Chufa sedge, Nutgrass, Yellow nut sedge, Tigernut sedge, or Earth almond. In Nigeria, it is locally called Aya by the Hausa tribe, Ofio by the Yoruba tribe, and Aki Hausa by Igbo, respectively (Hassan *et al.*, 2018).

Tannins are water-soluble polyphenolic compounds with high molecular weight. They can bind and precipitate proteins (Dai and Mumper, 2010). Tannins occur in different parts of plants, such as the leaf, root, wood, bark, and fruit. They are divided into condensed tannins (CT) and hydrolyzable tannins (HT). Many studies have reported that tannins are polyphenol compounds that reduce the digestibility and nutritional quality of herbivore diets (Rothman *et al.*, 2009). The presence of tannins in plants enables them to defend themselves from predation and infections, which may be due to the complex formation between tannins and proteins (Barbehenn and Constabel, 2011). The antioxidant (Cosme *et al.*, 2025), anti-inflammatory (Asayesh *et al.*, 2016), anticancer (Cai *et al.*, 2016), and antimicrobial (Doss *et al.*, 2009) activities of tannins from various sources have been reported by many researchers.

This study evaluated the antioxidant potential of tannin fractionate of *Cyperus esculentus* on lead-induced liver damage, using indicators such as alanine transaminase (ALT), aspartate transaminase



(AST), superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) and histology of the liver of adult Wistar rats to study its antioxidant properties on lead-induced hepatotoxicity in Wistar rats.

## MATERIALS AND METHODS

### Ethical Approval

Ethical approval was obtained from the research and ethics committee of the Faculty of Basic Medical Science, University of Nigeria, Enugu Campus, Nigeria with ID UNN/FBMS/20/002181.

### Procurement of *Cyperus esculentus* and Extraction and Isolation of tannin from *C. esculentus*.

*Cyperus esculentus* was procured at Ogbete Market Enugu, Enugu State, Nigeria, and was identified and authenticated at the Department of Plant Science, University of Nigeria, Nsukka, Enugu State, Nigeria, using authentication and identity number UNN131. Fresh *Cyperus esculentus* root tubers were air-dried at room temperature and ground into fine powder using a grinding machine, a micro powder grinding mill, manufactured by TENCAN, into powder form. About 80g of dried and powdered *Cyperus esculentus* root tuber was defatted with petroleum ether in a mechanical shaker manufactured by DECENT for 48 hours at room temperature. Then, it was extracted with aqueous acetone (70% acetone) for 60 minutes at 60°C in a water bath with constant stirring. The mixture was then filtered and centrifuged at 3000 rpm for 10 minutes. The supernatant was allowed to evaporate at room temperature. The acetone-free extract was lyophilized (Lark, Penguin Classic Plus, Chennai). The powder was collected, weighed, and stored in sterile bottles at 4°C in a refrigerator (Makkar, 2003).

### Quantitative analysis of isolated tannin

Tannin content was measured by the Folin-Denis method (Polshettiwar *et al.*, 2007), and this method was based on the reducing power of the phenolic hydroxyl group of tannins. An equal volume of Folin-Denis reagent was added to the 2mls extract. The contents were mixed thoroughly, and after 3 minutes, 2ml of 1N sodium carbonate was equally added. The mixture was mixed well and kept at room temperature for about 1 hour for colour development. The optical density of the resultant solution was read at 700 nm.

### Experimental animals

Twenty (20) Wistar rats with a mean weight of 120g were used for the experiment. The rats were caged and fed with grower's mash. They were kept for fourteen (14) days for acclimatization before the experiment.



## Experimental Designs and Experimental Animals

Twenty adult male Wistar rats were divided into four (4) groups, (A-D) (n=5). Group A was administered normal saline only. Groups B, C, and D were fed 30mg/kg body weight of lead. Additionally, groups C and D were administered with 50mg/kg body weight and 100mg/kg body weight of tannin fraction of *Cyperus esculentus* respectively orally for 28 days. The following day after the last administration, the animals were sacrificed by cervical dislocation and blood samples were obtained through the intra-cardiac puncture to assay the levels of liver enzymes [Aspartate transaminase (AST) and Alanine transaminase (ALT)] and oxidative stress markers [malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT)]. The dose was evaluated after the LD50 test.

## Histological Study

The animals in all groups were sacrificed under 50 mg/kg body weight of thiopental anesthesia. The abdominal cavities were opened up through the midline incision to expose the liver. The livers were immediately removed, weighed, and fixed in 10% formal saline for histological analysis. After 24 hours, the livers were dehydrated in ascending grades of alcohol, cleared in xylene, and embedded in paraffin wax. The tissue blocks were subsequently sectioned with a rotary microtome at 5  $\mu$ m thickness. The tissue sections were picked up with albumenized slides and allowed to dry on hot plates for 2 minutes. The slides were dewaxed with xylene and rehydrated in descending grades of alcohol and then water for 5 min. The slides were then stained with hematoxylin and eosin. The slides were mounted in DPX. Photomicrographs were taken at a magnification of 160x on a Leica DM750 microscope.

## Malondialdehyde (MDA) estimations

Lipid peroxidation products were estimated by measuring TBARS antioxidants in the livers as determined according to the method described by Wheel *et al.* (Wheel, 1990).

## Quantification of Glutathione (GSH) activities

Glutathione activity was quantified using a modified protocol based on the methodology described by Giustarini *et al.*, 2014. This colorimetric assay involves the reaction of reduced glutathione (GSH) with DTNB (Ellman's reagent), forming a chromogenic product with maximal absorbance at 412 nm. The concentration of glutathione was subsequently determined through calibration against a standard curve.

## Quantification of Catalase (CAT) activities

Catalase activity was calculated through analysis of extinction coefficient changes over a defined time interval, a protocol adopted from Trawczyńska *et al.*, 2020. The spectrophotometric assay quantified hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) decomposition by tracking the reduction in absorbance at wavelengths commencing near 240 nm.



### Quantification of Superoxide Dismutase (SOD) activities

Superoxide dismutase (SOD) activity was determined using the nitroblue tetrazolium (NBT) reduction method, as described by Sanyal *et al.*, 2022. This assay quantifies SOD activity by generating superoxide radicals via the xanthine-xanthine oxidase system and measuring the extent of NBT reduction inhibition.

### Estimation of liver enzymes

Aspartate transaminase (AST) and Alanine transaminase (ALT) levels were estimated automatically using an auto-analyser with model BA-A-280 manufactured by Infitek at Anatomy Department, University of Nigeria. The results were expressed in U/L (Unity International/L).

### Data presentation and statistical analysis

Graphpad Software Inc. was used to analyze the data. Results obtained were expressed as Mean  $\pm$  SEM. Statistical differences between the groups were evaluated by one-way analysis of variance (ANOVA). Differences yielding  $p < 0.05$  were considered statistically significant.

## RESULTS

To study the effect of lead on liver function, serum ALT (Alanine Amino-Transferase enzyme) and AST (Aspartate Amino-Transferase enzyme) activities were investigated. According to our result, there was a statistically significant increase in AST and ALT levels ( $P < 0.05$ ) following exposure of rats to the Lead group as compared with the control group as indicated in Table 1 below.

### Effects of tannin fractionate of *C. esulentus* on Liver enzymes (ALT and AST) in lead-treated rats

Table 1 shows the effects of tannin fractionate of *C. esulentus* on Liver enzymes (ALT and AST) in lead-treated rats. Results showed a significant ( $p < 0.05$ ) increase in levels of ALT and AST in rats treated with Lead alone (group B) when compared to the control group A. All the groups that received tannin fractionate of *C. esulentus* and lead treatment had statistically significant ( $p < 0.05$ ) decreased levels of ALT and AST compared to group B (lead-treated group alone).

| Groups                               | ALT (U/L)                      | AST (U/L)                      |
|--------------------------------------|--------------------------------|--------------------------------|
| A. Control                           | 30.01 $\pm$ 0.04               | 50.05 $\pm$ 0.01               |
| B. Pb                                | 60.00 $\pm$ 0.03 <sup>a</sup>  | 80.01 $\pm$ 0.04 <sup>a</sup>  |
| C. Pb+C. <i>esulentus</i><br>(50mg)  | 45.05 $\pm$ 0.05 <sup>ab</sup> | 60.00 $\pm$ 0.02 <sup>ab</sup> |
| D. Pb+C. <i>esulentus</i><br>(100mg) | 35.00 $\pm$ 0.02 <sup>ab</sup> | 55.05 $\pm$ 0.04 <sup>ab</sup> |



Data presented as Mean  $\pm$  standard error of mean (SEM). <sup>a</sup>p< 0.05 compared to the vehicle (group A), <sup>b</sup>represents p<0.05 significant difference compared to lead-treated group B. Each group has 5 rats, *C. esulentus*: *Cyperus esulentus*, Pb: Lead.

#### Effects of tannin fractionate of *C. esulentus* on Oxidative stress Makers on lead-treated rats.

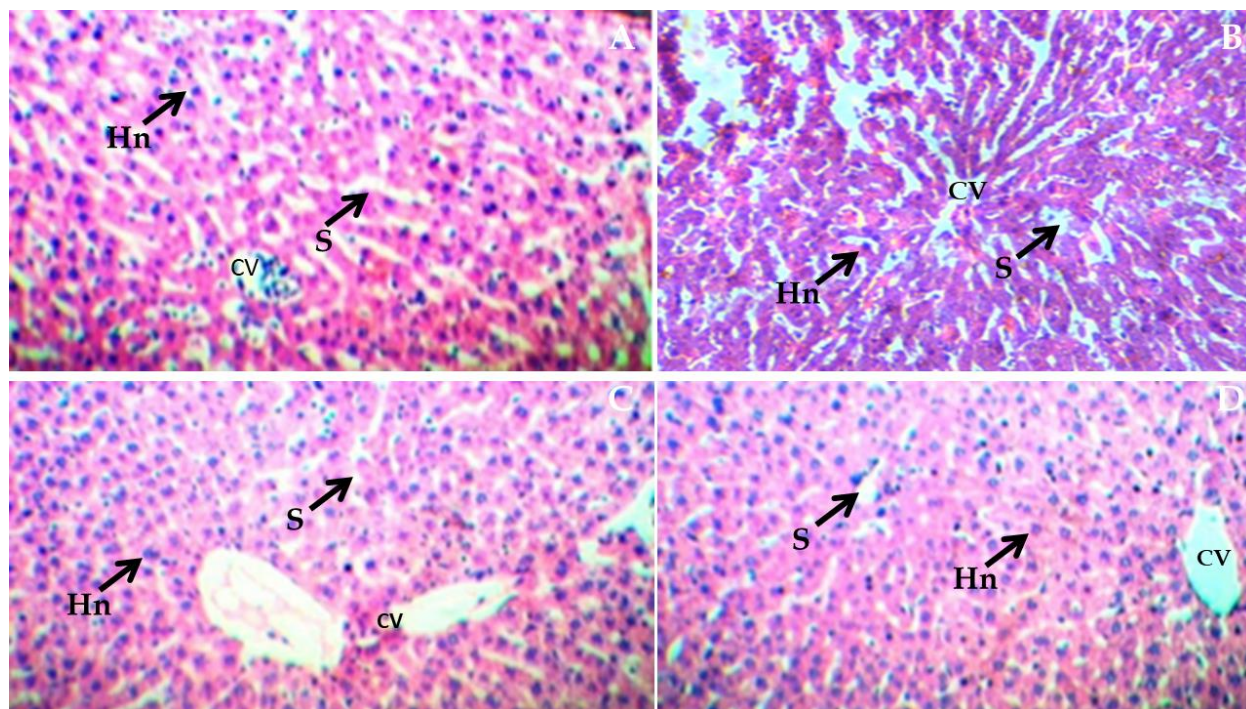
Table 2 shows the effects of tannin and lead on the levels of Superoxide Dismutase, Catalase, Malondialdehyde, and glutathione. Results showed a significant (p<0.05) decrease in the activity level of SOD, CAT, and GSH and a significant (p<0.05) increase in the level of MDA in rats treated with Lead alone (group B) when compared to the control group A. All the groups that received tannin fractionate of *C. esulentus* and lead treatment had a statistically significant (p<0.05) increase in the activity level of SOD, and CAT, but a decrease in MDA.

| Groups                               | SOD<br>(U/mg pro)               | CAT<br>(U/mg pro)               | MDA<br>(nmol/mg pro)         | GSH<br>(nmol/mg protein)       |
|--------------------------------------|---------------------------------|---------------------------------|------------------------------|--------------------------------|
| <b>A. (Control)</b>                  | 26.10 $\pm$ 0.04                | 13.30 $\pm$ 0.02                | 0.52 $\pm$ 0.04              | 2.30 $\pm$ 0.03                |
| <b>B. Pb</b>                         | 12.60 $\pm$ 0.03 <sup>a</sup>   | 8.30 $\pm$ 0.01 <sup>a</sup>    | 3.40 $\pm$ 0.02 <sup>a</sup> | 1.10 $\pm$ 0.02 <sup>a</sup>   |
| <b>C. Pb+C.esulentus<br/>(50mg)</b>  | 18.20 $\pm$ 0.02 <sup>a,b</sup> | 14.20 $\pm$ 0.03 <sup>a,b</sup> | 1.20 $\pm$ 0.01 <sup>b</sup> | 2.90 $\pm$ 0.04 <sup>a,b</sup> |
| <b>D. Pb+C.esulentus<br/>(100mg)</b> | 23.30 $\pm$ 0.03 <sup>a,b</sup> | 9.30 $\pm$ 0.02 <sup>a,b</sup>  | 0.90 $\pm$ 0.03 <sup>b</sup> | 2.40 $\pm$ 0.02 <sup>b</sup>   |

Data presented as Mean  $\pm$  standard error of mean (SEM). <sup>a</sup>p< 0.05 compared to the vehicle (group A), <sup>b</sup>represents p<0.05 significant difference compared to lead-treated group B. each groups has 5 rats, *C. esulentus*: *Cyperus esulentus*, Pb: Lead.



## Histological Results



**Figure 1 (A-D):** photomicrograph showing a section of rat liver, central vein (CV) appeared lined by flat endothelial cells, surrounded by cords of polygonal hepatocytes with granular cytoplasm central, rounded, vesicular nuclei. Blood sinusoids(s) are lined by flat endothelial cells and Kupffer cells. Group A (control group), H&E x 160 Stain: haematoxylin and eosin. **Fig. (1b):** Showing swollen hepatocytes with vacuolated cytoplasm and rounded vesicular nuclei. Some nuclei are darkly stained. Other hepatocytes appear with acidophilic cytoplasm and vesicular nucleus. Cellular infiltration is seen near the congested central vein. Group B, H&E 160x. **Fig. (1c):** A photomicrograph showing a section of rat liver, central vein appeared lined by flat endothelial cells. The tissue looks normal compared to the controls. Group D, H&E Stain: haematoxylin and eosin, 160x.



## DISCUSSION

Since time immemorial, various parts of plants have been used in the treatment and prevention of many diseases (Ayyanar and Ignacimuthus, 2009). Herbal medicine has been around for centuries and a precursor to modern medicine (Burke *et al.*, 2006). Herbal medicine can be derived from different parts of plants like leaves, bark, flowers, and roots (Khaki *et al.*, 2011). Tannin has been reported to have antioxidant properties (Cosme *et al.*, 2025). In this study, the tannin fraction of *Cyperus esculentus* tuber increased tissue antioxidant enzymes and decreased MDA levels in groups C and D compared with group B. In this study, rats who received tannin extract showed a significant increase in GSH and catalase activity and significant decreases in MDA level compared to that of the control. This was under some investigators (Ayyanar and Ignacimuthus, 2009).

These clarified that Lead increased generation of reactive oxygen species (ROS). The high level of ROS is known to cause impairment of antioxidant enzyme activities resulting in oxidative stress. Oxidative stress in turn induces various actions including lipid peroxidation, which is characterized by oxidative degeneration of membrane phospholipids. Malondialdehyde (MDA) level reflects the degree of lipid peroxidation in tissues especially hepatocytes and elevated MDA product coincides with hepatotoxicity seen with administration of Lead (Ibrahim *et al.*, 2012).

Hassan *et al.* in their study have previously reported that *C. esculentus* contains significant amounts of antioxidants (Hassan *et al.*, 2018). Therefore, this study revealed tannin as one of the phytochemicals of *C. esculentus* responsible for its antioxidant properties

The activities of serum AST and ALT were investigated in order to determine the effect of Lead on liver functions and ameliorative potential of tannin fraction of *C. esculentus*. Treatment with lead in this study showed increase in ALT and AST activities when compared with control groups.

Tannin fraction of *C. esculentus* from this study maintained the normal histological architecture of the liver when compared to lead group (B). Our findings shows that tannin protected the liver from the harmful effects of Lead. In the present study, rats treated with tannin fractionate of *C. esculentus* plus lead showed hepatic architecture nearly similar to that of the negative group (A) as no hepatocyte injury was observed.

The light microscopic examination of liver sections of rats treated with Lead alone (Group B), in this study showed most of the hepatocytes appeared swollen with vacuolated cytoplasm and irregular and darkly stained nuclei as compared to the control. Cellular infiltration and congestion in central and portal veins were also observed in this work.

The appearance of congestion was attributed to the induction of morphologic changes and to alterations in the relationship between hepatocytes and sinusoidal lining cells as a result of Lead toxicity. An apparent increase in the number of the lining cells of bile ductules was also noticed in Lead treated group of this work.

In this study, administration of tannin fractionate of *Cyperus esculentus* extract in groups C and D mitigates the toxic effect of Lead on the liver. The hepatocytes appeared less vacuolated and with vesicular nuclei compared to that of the Lead treated group (Group B).





## CONCLUSION

It was discovered that the tannin fraction of *Cyperus esculentus* effectively reversed the ALT and AST levels significantly compared to control group B. The tannin fraction of *Cyperus esculentus* also protected the histological architecture of the liver. Findings from this study suggest that the tannin fraction of *C. esculentus* could protect the structure and function of the liver against the effects of Lead. The hepatoprotective effect of *C. esculentus* in this study makes it a probable natural product that could be effectively employed in the management of liver injury and maintenance of liver health. The exact therapeutic threshold in humans would require further research.

## AUTHOR CONTRIBUTIONS

Hassan L.A. and Ojo F.O formulated and designed the study. Adamu S.P and Lawal R.T performed the experiments, Hasiya S.B and Abdul D.I analysed the data. F.O. Ojo and L.A. Hassan drafted the manuscript. All authors read and approve the manuscript.

## CONFLICTS OF INTEREST

Authors declare no conflicts of interest

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