

Antioxidant Effects of Ethanolic Extract of *Piper guineense* (Uziza) Leaves on Lead-Induced Testicular Toxicity in Wistar Rats

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Abstract

Piper guineense (uziza) leaves may have therapeutic input on male infertility caused by Lead exposure. This study investigated the antioxidant activities of the ethanolic extract of *Piper guineense* leaves (EEPL) on lead-induced testicular toxicity in rats. Twenty male wistar rats (150-200g) were divided into 4 groups consisting of 5 rats each. Control received 1ml of distilled water, lead only received 150mg/kg of lead, low dose received 150mg/kg of lead + 300mg/kg of EEPL, high dose received 150mg/kg of lead + 600mg/kg of EEPL. Administration was done orally for 14 days and then sacrificed. Blood was collected and analysed for serum testosterone and testes were harvested and homogenised for testicular antioxidant enzymes and zinc levels. Data was analysed for ANOVA using Graph Pad Prism 5. Lead exposure negatively affected the antioxidant enzymes, testosterone and zinc levels. Administration of EEPL significantly ($p < 0.05$) increased the testosterone and zinc levels in a dose dependent manner. EEPL also significantly ($p < 0.05$) increased the testicular Superoxide dismutase, Catalase, and Glutathione peroxidase levels and significantly decreased the testicular Malondialdehyde levels. *Piper guineense* leaves ameliorate testicular oxidative stress damage induced by lead toxicity.

Keywords: *Piper guineense*, Uziza, lead induced, testicular toxicity, ethanolic extract, antioxidant.

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INTRODUCTION

There are several reasons for infertility; approximately 50% of such cases are due to the male factor [1-3]. Lifestyle, environment and occupation contribute to the deterioration of semen quality which has been implicated in declining male fertility [4]. The negative impact on the semen quality could be attributed to oxidative stress which destroys DNA mitochondria and inhibits ATP production which in turn affects sperm function [5].

Lead acetate: an environmental pollutant accumulates in body tissues including reproductive organs [6]. Lead exerts its effects by causing oxidative stress to tissues and organs, leading to disruption of the cell defence system. Antioxidant nutrients (vitamins E, C, B₆ and zinc) alleviate oxidative stress induced by lead [7].

Piper guineense, commonly called Ashanti pepper, is a spice plant which comes from the *Piperaceae* family and the *piper* genus. It is called different local names like 'Uziza' in Igbo, 'Iyere' in Yoruba, 'Ebe-ahinhi akpoke' in Edo and 'Etinkene' in Efik [8]. *P. guineense* is native to the tropics of Western and Central Africa and cultivated in Nigeria, commonly in Southern Nigeria [9]. *P. guineense* leaves are commonly used as leafy vegetables in most Nigerian soups while the fruits are used as flavour in most dishes. The leaves of *P. guineense* are known to contain phytochemicals like alkaloids, flavonoids, saponins, phenols and tannins [10] and minerals like zinc, iron and potassium [11, 12]. The plant has been shown to have positive effects on liver [8, 13], kidney [14], female reproductive system [15], diabetes [16], against ulcer [17] male libido enhancement [18] and male reproductive parameters [19, 20].

Since lead causes oxidative stress and study have shown *Piper guineense* to contain antioxidant activities [21], this study investigated the antioxidant effects of ethanolic leaf extract of *Piper guineense* (uziza) on lead-induced testicular toxicity in male rats.

MATERIALS AND METHODS

Plant extraction

Fresh leaves of *Piper guineense* (uziza) was purchased from Ukwunwangu market, Uturu Abia State, Nigeria, and was identified and authenticated at the Department of Agriculture, Gregory Univeristy Uturu, Abia State. The leaves were thoroughly washed and oven dried. The dried leaves where blended into fine powder and extracted using cold maceration. Extraction was done using ethanol with 200g of the powder soaked in 200ml of 80% ethanol for about 72 hours with regular intermittent agitation and then filtered with filter paper. The extract was then concentrated and the mixture stored in a refrigerator until further use.

Chemical

The laboratory grade lead acetate was obtained from the Department of Biochemistry laboratory, Gregory University Uturu.

Experimental animals

A total number of thirty (30) male wistar rats weighing 150-200g were used in this study. The rats were procured from the animal house of the Faculty of Basic Medical Sciences, Gregory University, Uturu. The rats were fed with standard growers feed (pelletized) and water *ad libitum*. They were kept in cages in a clean and comfortable environment under a dark/ light cycle. The animals were acclimatized for a period of 14 days.

Ethics

This study was approved by the Ethics Review Committee, Department of Anatomy Gregory University Uturu. Animals received humane care, and procedures were in accordance with the Ethics and Regulation Guiding the Use of Research Animals as approved by Gregory University, Uturu Nigeria.

Experimental Design

After acclimatization, 20 rats were randomly distributed into 4 experimental groups (n=5). Control (Group A): given 1ml of distilled water. Group B: given 150mg/kg of pure lead acetate. Group C: given 150mg/kg of lead acetate + 300mg/kg of *P. guineense* ethanolic leaf extract. Group D: given 150mg/kg of lead acetate + 600mg/kg of *P. guineense* ethanolic leaf extract according to the modifications of Aribo and co [22]. Administration was done orally by oral gavage for 14 days.

Animal Euthanasia and Sample Collection

At the end of administration, the animals were euthanized using cervical dislocation. Blood was collected via cardiac puncture, put into a sample bottle and centrifuged at 3000rpm for 15 minutes and the serum used for hormonal assay of testosterone using ELISA kit obtained from Accubind Inc (USA) according to manufacturer's instructions. The left testis was excised out and homogenized in cold phosphate buffer and centrifuged at 3000rpm. The supernatant was collected into plain sample bottle and stored at -20C for oxidative stress studies and testicular zinc parameters.

Determination of Antioxidant Enzymes activities

Malondialdehyde (MDA) was determined using the method of Mihara and Uchiyama [23] based on the interaction of MDA with thiobarbituric acid (TBA). Activity of Superoxide Dismutase (SOD) was determined using the method of Sun and Zigman [24] by its ability to inhibit the auto-oxidation of epinephrine. Activities of Catalase (CAT) were determined according to method of Aebi [25] based on the exponential disappearance of H₂O₂ by the action of catalase in the tissue sample. Glutathione peroxidase (GPx) level was determined according to the method of Flohe and Gunzler [26].

Determination of Zinc levels

Zinc was assay with Zinc kit (Centronic GmbH, Switzerland). Supernatants obtained following homogenized testicular tissue were brought to room temperature. Standards were prepared according to manufacturer instructions.

Statistical Analysis

All parametric values were expressed as means ± standard error of mean (SEM). To determine the difference among various treatments groups, one way analysis of variance was estimated by using the Graph Pad Prism 5. Multiple comparisons among various treatments groups were determined by using Boneferroni post hoc comparison test. Values were considered significant at p<0.05.

RESULTS

Serum Testosterone levels

Fig 1 showed a statistically significant decrease in serum testosterone levels in the lead only group and a statistically significant increase in the lead+ high dose extract group when compared with control. However, there was a non-significant decrease in the serum testosterone levels in the lead+ low dose extract when compared with the control group. The result also showed a statistically significant increase in the serum testosterone levels of the treatment groups when compared with the lead only group.

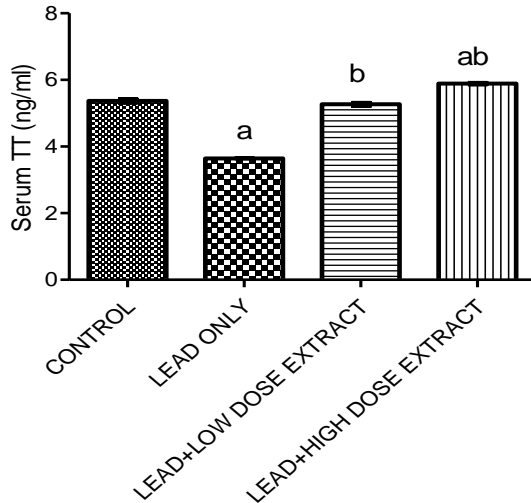


Fig 1

Fig 1 Serum Testosterone levels of lead-induced rats with or without treatment with ethanol extract of *P. guineense*. ‘a’ indicates statistically significant to control group; ‘b’ indicates statistically significant to lead only group (P<0.05)

Testicular Malondialdehyde (MDA) levels

Fig 2 showed a statistically significant increase in testicular MDA levels in the lead only group when compared with control. Also, when compared with the control group, there was a non-significant difference in the testicular MDA levels of the treatment group. However, there was a statistically significant decrease in the testicular MDA levels of the treatment group when compared with the lead only group.

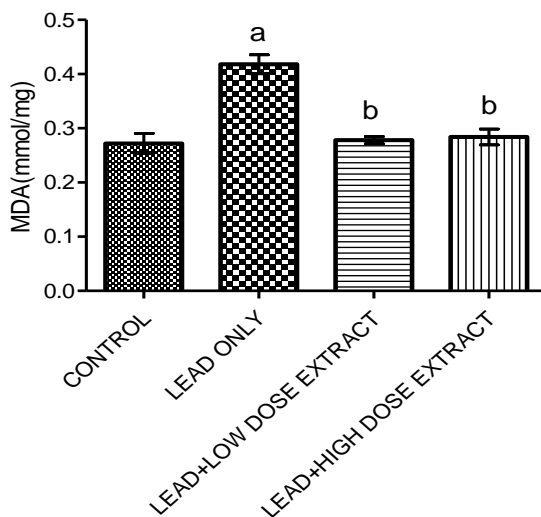


Fig 2

Fig 2 Testicular Malondialdehyde (MDA) levels of lead-induced rats with or without treatment with ethanol extract of *P. guineense*. ‘a’ indicates

statistically significant to control group; ‘b’ indicates statistically significant to lead only group (P<0.05)

Testicular Superoxide Dismutase (SOD) levels

In Fig 3, the result showed a statistically significant decrease in the testicular SOD levels in the lead only group and treatment groups when compared with the control group. Also, the result showed a statistically significant increase in the testicular SOD levels in the treatment groups when compared with that of the lead only group.

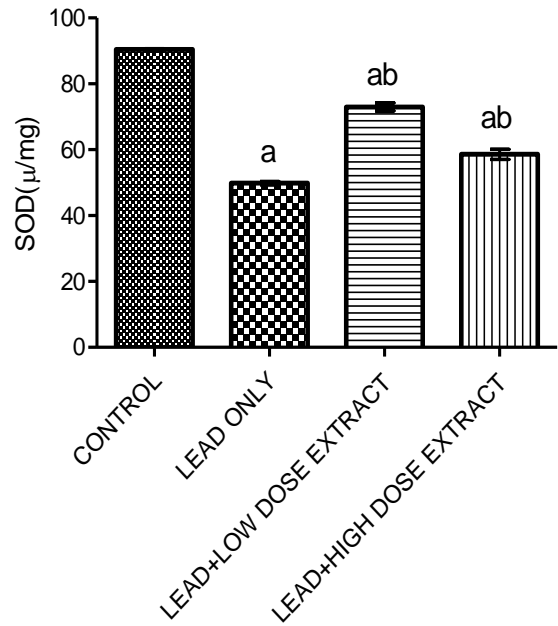


Fig 3

Fig 3 Testicular Superoxide Dismutase (SOD) levels of lead-induced rats with or without treatment with ethanol extract of *P. guineense*. ‘a’ indicates statistically significant to control group; ‘b’ indicates statistically significant to lead only group (P<0.05)

Testicular Catalase (CAT) levels

In Fig 4, when compared with the control group, the result showed a statistically significant decrease in the testicular CAT levels in the lead only group and a statistically significant increase in the treatment group. Also, there was a statistically significant increase in the testicular CAT levels in the treatment groups when compared with the lead only group.

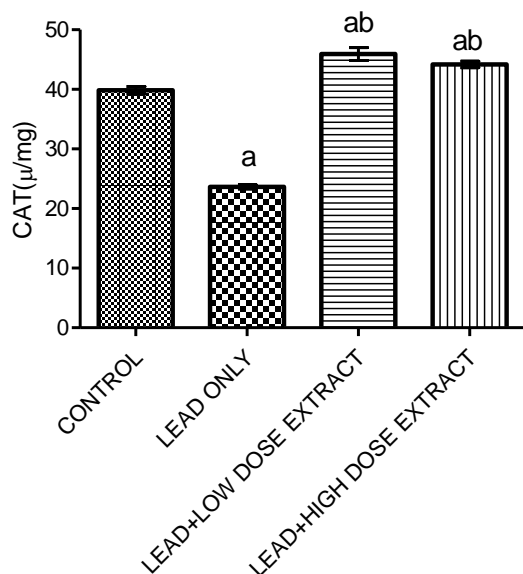


Fig 4

Fig 4 Testicular Catalase (CAT) levels of lead-induced rats with or without treatment with ethanol extract of *P. guineense*. 'a' indicates statistically significant to control group; 'b' indicates statistically significant to lead only group (P<0.05).

Testicular Glutathione Peroxidase (GPx) levels

In Fig 5, the result showed a statistically significant decrease in the testicular GPx levels in the lead only group and a statistically significant increase in the treatment groups when compared with that of the control group. The result also showed a statistically significant increase in the testicular GPx levels in the treatment groups when compared with that of the lead only group.

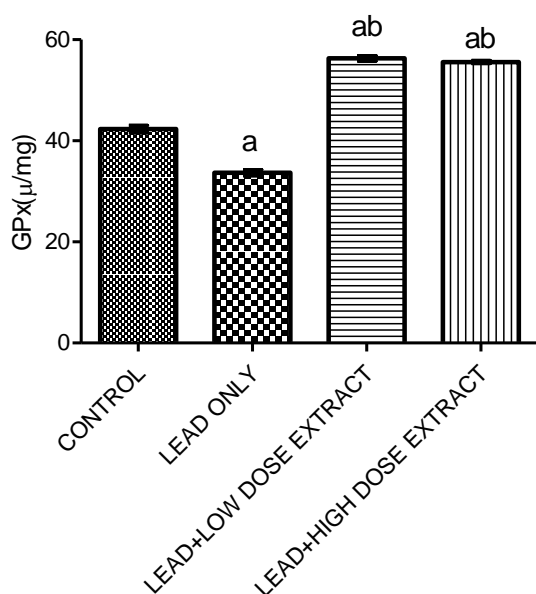


Fig 5

Fig 5 Testicular Glutathione Peroxidase (GPx) levels of lead-induced rats with or without treatment with ethanol extract of *P. guineense*. 'a' indicates statistically significant to control group; 'b' indicates statistically significant to lead only group (P<0.05).

Testicular Zinc levels

Fig 6 showed a statistically significant decrease in the testicular zinc levels in the lead only group and a statistically non-significant increase in the lead+ high dose extract group when compared with the control group. However, when compared to the control group, there was no significant difference in the testicular zinc levels of the lead+ low dose extract group. The result also showed a statistically significant increase in the testicular zinc levels in the treatment groups when compared with the lead only group

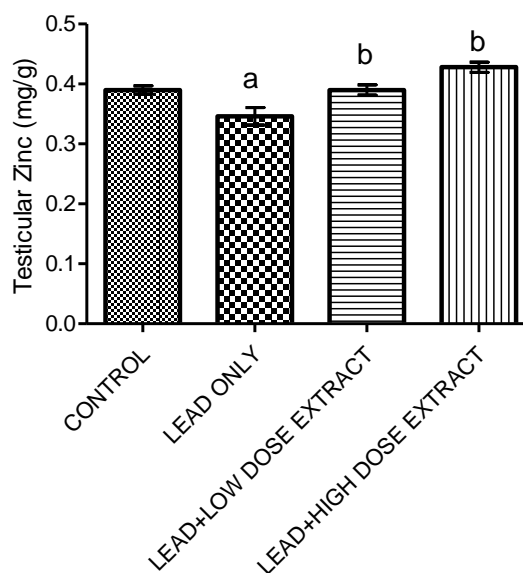


Fig 6

Fig 6 Testicular Zinc levels of lead-induced rats with or without treatment with ethanol extract of *P. guineense*. 'a' indicates statistically significant to control group; 'b' indicates statistically significant to lead only group (P<0.05)

DISCUSSION

This study showed that leaves of *P. guineense* have antioxidant properties as there was positive effect on the antioxidant enzymes after there were significant adverse effects following exposure to lead as seen in figures 2-5. High ROS concentrations activates lipid peroxidation which ultimately produces hydrogen peroxide in the form of Malondialdehyde and testicular MDA level has been used as a biomarker to assess the degree of oxidative stress and to describe testicular damage [27] as confirmed in this study by the increase in MDA levels in the rats exposed to lead (Fig 2). However, administration of the *P. guineense* leaves significantly decreased the MDA level: This is an

indicator of its antioxidant activity in conformity with the report of [21]. The antioxidant property of *P. guineense* leaves is also evident in the activities of CAT, SOD and GPx as it increased the antioxidant activities which were initially reduced by exposure to lead.

This study also showed dose-dependent increase in the testicular zinc levels after administration of *P. guineense* as shown in fig 6 and this is evident of the zinc content of *P. guineense* as reported by previous studies [11, 12]. This may have contributed to the positive impact of *P. guineense* leaves on antioxidant levels reported in this study, since zinc is a known antioxidant. Zinc deficiency is known to contribute to the onset of the dysfunctioning of male reproduction through the reduction of circulating testosterone [28]. Result from this study (Fig 1) showed supplementation of Zinc through the administration of *P. guineense* increased the levels of circulating testosterone initially reduced by lead toxicity.

CONCLUSION

In conclusion, this study showed that lead exposure causes testicular toxicity and that supplementation with the leaves of *P. guineense* (uziza) has antioxidant role which ameliorated the lead-induced testicular toxicity in rats.

Conflict of Interest: None

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