



Amelioration of cadmium chloride testicular toxicity by *Plukenetia conophora* aqueous leaves extract in Wistar rats

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ABSTRACT

Cadmium accumulates in different organ and tissues of the body including the testes, impeding normal metabolism. This study aimed at evaluating the effect of *Plukenetia conophora* leaves extract on antioxidants and testicular function indices of cadmium chloride (CdCl₂)-administered Wistar rats. A total of 25 Wistar rats (100–120 g) were randomly divided into 5 groups (A-E) and received orally, once daily for 21 d as follows: A (distilled water); B (6.5 mg/kg body weight of CdCl₂); C (CdCl₂ + 150 mg/kg body weight of extract); D (CdCl₂ + 300 mg/kg body weight extract); and E (CdCl₂ + 600 mg/kg body weight extract). The rats were sacrificed on day 22, serum and tissue homogenates were collected for different biochemical assays. Analysis of data was done using ANOVA and mean value differences were considered significant at $P < 0.05$. CdCl₂ administration resulted in decreased concentrations of testicular protein, glycogen, sialic acid, serum testosterone, glutathione S-transferase, and acid phosphatase activities compared to the control. Catalase CAT and superoxide dismutase SOD activities, malondialdehyde MDA concentration also increased compared with the control. However, co-administration with aqueous leaves extract of *Plukenetia conophora* resulted in restoration of the enzyme activities, glycogen, protein, sialic acid, and serum testosterone concentration compared with control. *Plukenetia conophora* aqueous leaves extract attenuated the toxic effect of CdCl₂ on Wistar rats' testicular function.

Keywords: Antioxidants, CdCl₂, infertility, *Plukenetia conophora*, testes

INTRODUCTION

Infertility is defined as the inability to attain pregnancy after one year or more of constant unprotected sex.^[1] Globally, fertility problems are experienced by one in every four couples and about 50% of these issues stem from men.^[2] Male infertility can result from exposure to environmental contaminants such as heavy metals which are currently a global public health menace in developing and developed nations of the world.^[2,3] One of such heavy metals that is currently a public health menace worldwide is cadmium.

Natural and man-made activities are the major sources of cadmium release into the atmosphere, these include forest

fires, volcanic eruption, batteries production, paints and other coatings, as well as tobacco smoking which is a major source of exposure. Living organisms can be exposed to cadmium toxicity through inhalation, ingestion, and absorption through the skin which is relatively negligible.^[4] Cadmium accumulates in different organ and tissues of the body impeding normal metabolism, some of these organs include liver (Koyu *et al.*, 2007),^[5] kidney (Abdel-Moneim and Said, 2007),^[6] testes (Abdel-Moneim and Said, 2007),^[6] and brain (Vijaya *et al.*, 2020).^[7] It can induce free radicals' generation leading to oxidative damage to the testes.^[8,9] Several antioxidant agents and medicinal plants are therefore being employed to protect and/or improve the toxic effects of cadmium on the

testes and other organs, these agents are currently attracting considerable attention for preventing oxidative stress-related health issues.^[10]

Plukenetia conophora is synonymous with *Tetracarpidium conophorum*, it is commonly called tropical African walnut or Nigerian walnut while locally called “awusa,” “ukpa,” and “gawudi bairi” in Yoruba, Igbo and Hausa-speaking regions of Nigeria.^[10,11] It is of the family “Euphorbiaceae” in the genus “*Plukenetia*.” It is native to western and central Africa which include Central African Republic, Congo, Niger, Togo, Cameroon, and Gabon.^[12] It is a flowering plant of the tropics which is small and climbs up to about 6 to 18 meters in length. Its stem is green when young but dark grey when old and can grow up to 16 cm in width.^[13] The length of the leaf is usually within 10 cm while its breadth is about 5 cm. The leaf has a simple oval shape possessing a saw-like margin. They are commonly arranged singly and pointed. Typically, the walnut is a climber which coils round other plants for support, such as the cocoa tree or kola nut tree.^[12]

In both males and females, *Plukenetia conophora* seeds have been used in the treatment of reproductive health disorders, in treating fibroids, enhancing fertility, regulating menstrual flow, and boosting sperm count in men.^[14,15] Therefore, this current study was aimed at investigating the effect of *Plukenetia conophora* leaves extract on function indices of the testes of CdCl₂-administered Wistar rats.

MATERIALS AND METHODS

Plukenetia conophora leaves were harvested from a cocoyam farmhouse in Ekiti state, Nigeria. The leaves were identified and authenticated at the Faculty of Life Sciences (Plant Biology Department), University of Ilorin, Nigeria. A voucher specimen was dropped at the herbarium with number UILH/001/1366.

Preparation of Extract

Fresh leaves of *Plukenetia conophora* were washed with distilled water to eliminate dust and sand particles, air-dried and blended into powder. The powder obtained (87 g) was extracted in 500 ml of distilled water for 24 h. It was afterward filtered through muslin bag and the filtrate evaporated to a sticky greenish-brown paste. This was subsequently bottled and stored for use. It was later reconstituted in distilled water to give the dosages of 150, 300, and 600 mg/kg body weight of the extract.

Chemical and Reagents

The assay kit used to determine cholesterol was a product of Spectrum Diagnostics Ltd, Egypt while the sex hormone kits used were from Accu-Bind Elisa microwell monobind, USA, lake forest California. CdCl₂ used was a product of Sigma-Aldrich, USA. Analytical grade reagents were used for other preparations which were done in glass wares.

Animal Experiment

Twenty-five male Wistar rats weighing 100–120 g in were obtained from the Animal holding Unit of University of Ilorin, Kwara State, Nigeria. These rats were acclimatized for 14

d prior the start of the experiment. The rats were placed in standard conditions and given water and feed *ad libitum*. Before usage, 6.5 mg/kg body weight of CdCl₂ and 150, 300, and 600 mg/kg body weight of *Plukenetia conophora* extract were dissolved in 1 ml each of distilled water per animal.

The Wistar rats were separated into five random groups (A, B, C, D, and E), each comprising five rats and received as follows:

- Group A - 1 ml of distilled water (control)
- Group B - 6.5 mg/kg body weight CdCl₂ only
- Group C - 6.5 mg/kg body weight CdCl₂ and 150 mg/kg body weight of *Plukenetia conophora* extract
- Group D - 6.5 mg/kg body weight CdCl₂ and 300 mg/kg body weight of *Plukenetia conophora* extract
- Group E - 6.5 mg/kg body weight CdCl₂ and 600 mg/kg body weight of *Plukenetia conophora* extract

Administration was done once daily through oral gavage. On day 22, the animals were sacrificed after being fasted overnight, using diethyl ether as anesthesia. Their testes were removed, weighed and kept in ice-cold 0.25M sucrose solution. Blood samples were collected from the jugular vein into plain sample tubes, left to clot for 10 min and placed in a centrifuge at 224 × g for 15 min (Anke, model TDL-5000B). Sera obtained were stored at 4°C until required for hormonal analysis.

Homogenization of the testes was done in ice-cold 0.25M sucrose solution (ratio 1:10 w/v). Centrifugation of the testes homogenates was carried out at 1398 × g for 15 min (Anke, model TDL-5000B). The supernatants were kept into sample bottles, stored frozen before the biochemical assays were carried out.

Testicular Function, Enzymes and Antioxidant Assays

The testes-bodyweight ratio was calculated using the formula highlighted by De Souza,^[3] testicular protein concentration was assayed using Biuret reagent described by Gornall *et al.*^[16] The glycogen concentration was assayed using the protocol of Kemp *et al.*,^[17] modified by Yakubu *et al.*,^[18] Sialic acid determination was done using the procedure described by Warren,^[19] Testicular cholesterol concentration was assessed using “CHOD PAP” reaction as highlighted by Fredrickson *et al.*^[20]

The methods of Wright *et al.*,^[21] Misra and Fridovich,^[22] Habig *et al.*,^[23] and Aebi,^[24] respectively, were used in assaying for acid phosphatase, SOD (SOD), Glutathione S-transferase (GST), and catalase (CAT) activities, while lipid peroxidation was carried out using the protocol detailed by Varshney and Kale,^[25] result was expressed as MDA produced per mg protein.

Hormonal Assay

The concentration of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and Testosterone was determined by enzyme-linked immunosorbent assay (ELISA) kits. The procedure detailed in the kit inserts was followed.

Animal Ethics

The animals used for this experiment were handled with strict adherence to international and national ethical guidelines.

The protocols used were approved by the departmental animal ethics committee of Landmark University Department of Biochemistry with approval number LUAC-0031A.

Statistical Analysis

The data obtained were represented as (Mean \pm SD) and were analyzed using one-way analysis of variance (ANOVA) with multiple comparisons. Values at $P < 0.05$ were considered significant. Prism 6 GraphPad (GraphPad Software, Inc. San Diego, California) was the statistical package used for analysis.

RESULTS AND DISCUSSION

Testes Body Weight Ratio and Testicular Total Protein

The effect of *Plukenetia conophora* (*P. conophora*) aqueous leaf extract on testes body weight ratio using doses of 150, 300, and 600 mg/kg body weight in CdCl₂ administered rats is shown in Figure 1. There was a significant decrease ($P < 0.05$) in testes-body weight ratio in rats that received CdCl₂ only, which was 0.87% compared to control 1.85%. While an increase was observed across the groups administered with *P. conophora* extract ranging from 1.65 to 1.74% [Figure 1].

The effect of 150, 300, and 600 mg/kg body weight of *P. conophora* aqueous leaves extract on testicular protein concentration in CdCl₂ administered rats is also shown in Figure 1. There was a significant decrease ($P < 0.05$) in testicular total protein concentration in rats that received cadmium only, which was 16.83 mg/ml compared to control 18.38 mg/ml. Significant increase ($P < 0.05$) was, however, noticed in rats that received the highest dose of *P. conophora* extract which was 22.25 mg/ml.

The percentage testes-body weight ratio is regarded as an index of the animals' general health and wellness.^[26] Related findings with this current study were obtained by de Souza et al.,^[3] where there was significant decrease in testes-body weight ratio of Wistar rats administered with cadmium. There was also a decrease in testes-body weight ratio across all the groups administered with *P. conophora* aqueous leaves extract

compared to control, although this was not significant, this could imply that the administration of cadmium may have some detrimental effects on the overall health status of the rats which could not be alleviated by the doses of extract administration used.

Total protein is crucial for sperm morphology and maturation, as well as spermatogenesis.^[27] A significant decrease in protein concentration in the group administered with CdCl₂ only could imply impaired spermatogenesis in these groups of rats. However, the significant increase in protein concentration noted in the group administered with 600 mg/kg body weight of *P. conophora* aqueous leaves extract compared with the control, could be because the dose administered had the ability to prevent the adverse effect of cadmium on protein concentration. Bolatito and Yakubu^[28] also presented similar findings with those of this current study where there was a decrease in total protein level of rats following administration with cadmium, with subsequent increase in the groups administered with *Aspilia Africana* aqueous leaves extract.

Testicular Glycogen and Sialic Acid

Figure 2 displays the effect of *P. conophora* aqueous leaves extract on total glycogen concentration at doses of 150, 300, and 600 mg/kg body weight in CdCl₂ administered rats. There was a significant decrease ($P < 0.05$) in testicular glycogen concentration in rats that received CdCl₂ only, which was 0.02 mg/100 mg glucose compared with the control 0.08 mg/100 mg glucose, whereas there was significant increase in glycogen concentration of rats that received 600 mg/kg body weight of extract compared with the control.

The effect of *P. conophora* aqueous leaves extract on testicular sialic acid content in CdCl₂ administered rats is shown in Figure 2, where there was a significant reduction in the concentration of testicular sialic acid of rats that received CdCl₂ only, which was 1.57 mg/g compared with the control 5.1 mg/g. There was, however, a significant rise in testicular sialic acid concentration in rats that received 600 mg/kg body weight of the extract which was 6.09 mg/g compared with the control [Figure 2].

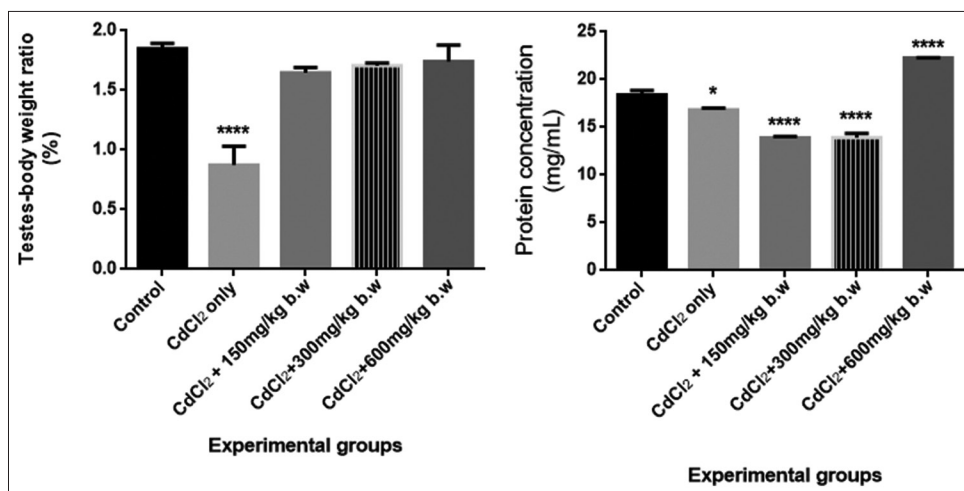


Figure 1: The effect of *P. conophora* aqueous leaves extract on percentage testes-body weight ratio and protein concentration of rats co-administered with CdCl₂. Each value is represented as mean of 5 replicates \pm SD. * represents statistical difference relative to control ($P < 0.05$)

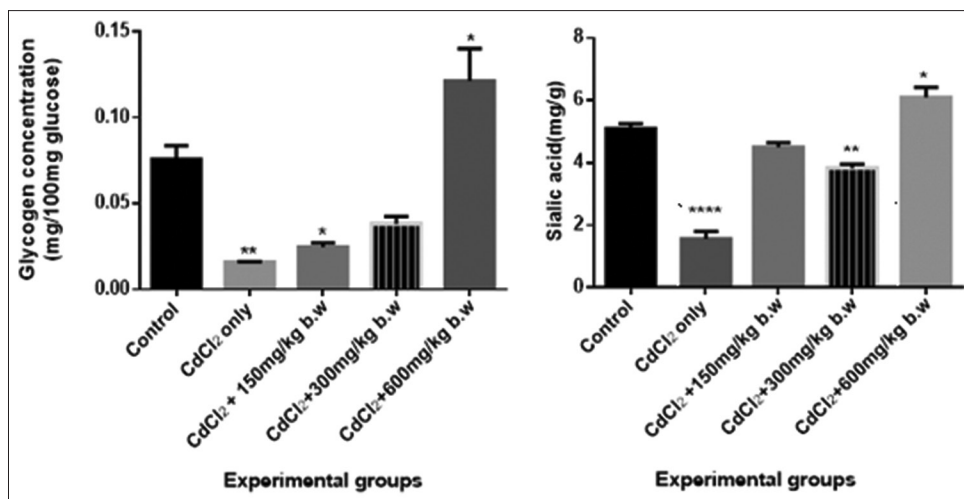


Figure 2: The effect of *P. conophora* aqueous leaves extract on testicular glycogen and sialic acid concentration of rats co-administered with CdCl₂. Each value is represented as mean of 5 replicates \pm SD. * represents statistical difference relative to control ($P < 0.05$)

The significant reduction in glycogen concentration of rats that received CdCl₂ only and rats that received 150 and 300 mg/kg body weight of *P. conophora* aqueous leaves extract compared with the control could mean lack of energy, deficiency of nutrients, and poor sperm maturation.^[29] The increase in testicular glycogen concentration in the group administered with 600 mg/kg body weight of *P. conophora* aqueous leaves extract may mean that this dosage was able to subdue the harmful effect of cadmium on testicular glycogen concentration thereby enhancing provision of nutrients and energy required for sperm development.^[30]

Sialic acid is a neuraminic acid which acts as lubricant by decreasing friction and enhances the upward flow of sperm cells in the testes during transit.^[31] The decrease in sialic acid concentration of rats that received CdCl₂ in this current study shows cadmium's possible detrimental effect on sperm cells movement whereas *P. conophora* aqueous leaves extract was able to attenuate its toxic effect by increasing sialic acid concentration especially in rats that received 600 mg/kg body weight of the extract. A previous study by Bolatito and Yakubu^[28] also reported a significant rise in testicular sialic acid concentration of rats that were treated with *Aspilia africana* leaf after induction with CdCl₂.

Testicular Cholesterol and Acid Phosphatase ACP

The effect of 150, 300, and 600 mg/kg body weight of *P. conophora* aqueous leaves extract on cholesterol concentration of rats administered with CdCl₂ is shown in Figure 3. An insignificant increase in testicular cholesterol was observed in rats that received CdCl₂ only, this was 15.27 mg/dL compared with the control 12.89 mg/dL. There was, however, significant increase in rats that received 150 mg/kg body weight of the extract which was 18.14 mg/dL, whereas compared with the control, there was a decrease in cholesterol concentration of rats that received 300 and 600 mg/kg body weight of *P. conophora* aqueous leaves extract to 12.89 mg/dL.

The effect of *P. conophora* aqueous leaves extract on acid phosphatase activity in CdCl₂ administered rats showed

that acid phosphatase activity significantly decreased in rats administered with CdCl₂ only, this was 3.50 nM/min/mg protein compared with the control of 6.96 nM/min/mg protein. There was, however, significant increase in rats that received the high dosage (600 mg/kg body weight) of the extract to 9.91 nM/min/mg protein.

Cholesterol is an important precursor of testosterone which is involved in steroidogenesis and essential for improved testicular function.^[30] In this current study; however, there was no concomitant increase in testosterone concentration with increasing cholesterol concentration observed in the rats that received CdCl₂ only and CdCl₂ + 150 mg/kg body weight of extract, which suggests negative accumulative effect of cholesterol which did not translate to subsequent testosterone synthesis. The increase in cholesterol concentration observed in the rats that received lead only could be due to cholesterol accumulation in the tissue, because lead limits the activity of cytochrome P450 which can prevent the biosynthesis of the bile acids, the primary route for cholesterol elimination from the body.^[6] Rats that received 300 and 600 mg/kg body weight of extract, however, showed comparable cholesterol level with the control. A similar result was also observed in the studies of Ugwuja *et al.*,^[32] following treatment of Wistar rats with cadmium and spices mixture (including ginger, garlic, and nutmeg).

Acid phosphatase is an abundant lysosomal enzyme that hydrolyses organic phosphates at an acidic pH.^[33] Such significant decrease in the activities of testicular ACP in rats that received CdCl₂ only suggests a risk to the well-being of the testes as it may result in autolysis, as well as cell death.^[34] However, the extract was able to restore ACP activities to levels comparable with the control and even above it especially in rats that received 600 mg/kg body weight of the extract.

SOD and Glutathione S-transferase

The effect of *P. conophora* aqueous leaves extract on the activity of SOD (SOD) in CdCl₂ administered rats is shown in Figure 4. There was a significant increase ($p < 0.05$) in SOD activity in rats that received CdCl₂ only, this was 0.003% inhibition

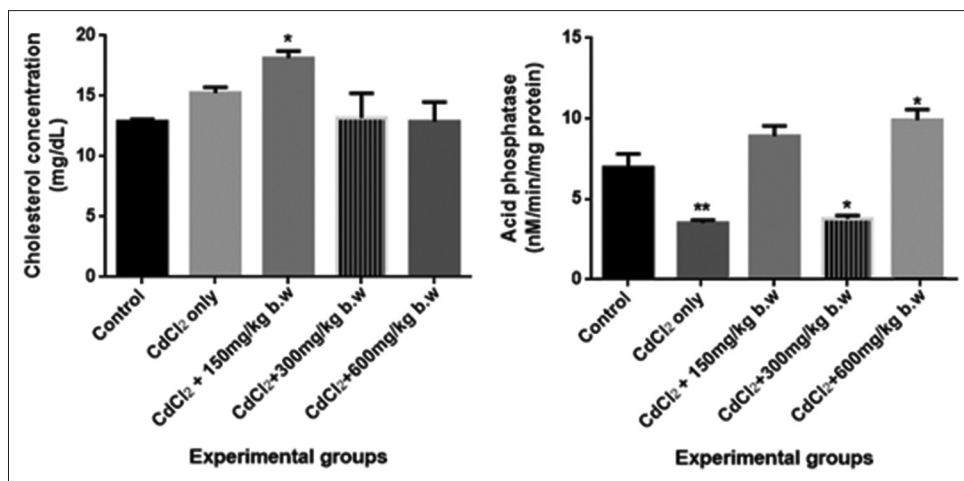


Figure 3: The effect of *P. conophora* aqueous leaves extract on testicular cholesterol level and acid phosphatase activity of rats co-administered with CdCl₂. Each value is represented as mean of 5 replicates \pm SD. * represents statistical difference relative to control ($P < 0.05$)

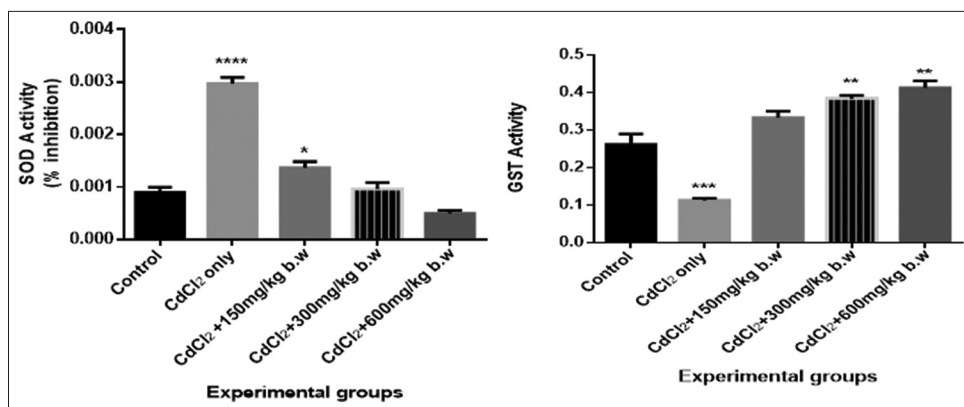


Figure 4: The effect of *P. conophora* aqueous leaves extract on testicular glutathione S-transferase (GST) and SOD (SOD) activities of rats co-administered with CdCl₂. Each value is represented as mean of 5 replicates \pm SD. * represents statistical difference relative to control ($P < 0.05$)

compared with the control of 0.0009% inhibition. SOD activity significantly increased in rats that received 150 mg/kg body weight of *P. conophora* extract but decreased however in rats that received 600 mg/kg body weight of *P. conophora* extracts which was 0.0005% inhibition compared with the control.

Figure 4 also displays the effect of *P. conophora* aqueous leaves extract on glutathione s-transferase (GST) activity in rats administered with CdCl₂. Significant decrease ($P < 0.05$) was observed in rats administered with cadmium only, 0.11 units/mg protein compared with the control of 0.26 units/mg protein. There was, however, a rise in GST activity in rats administered with *P. conophora* aqueous leaves extract across all administered groups from 0.33 to 0.41 units/mg protein.

SOD is reported to be the most powerful antioxidant in the cell and the first detoxification enzyme.^[35] Although studies have shown reduction in SOD activity in rats administered with cadmium,^[36,37] result from this current study contradicts these previous reports. The increase in SOD activity in CdCl₂-administered rats in this current study may be an adaptive reaction by the rats' natural antioxidant system to possible free radicals' generation due to intake of cadmium.^[38] This trend in SOD activity is corroborated by a related significant increase in lipid peroxidation (MDA concentration) in the

rats that received CdCl₂ only and CdCl₂ + 150 mg/kg body weight of extract in this current study. Rats that received 300 and 600 mg/kg body weight of extract, however, exhibited restored SOD activity, comparable with the control.

GST is an enzyme involved in detoxification and it catalyzes the conjugation of reduced glutathione to several range of substrates.^[39] Previously, treatment with cadmium sulfate has been reported to induce significant reduction in the activity of glutathione S-transferase with subsequent increase after treatment with propolis, which is also in line with this present study.^[40] The result of this study agrees with those of Berroukche and colleagues.^[40] The dose-dependent increase in the activity of GST across all extract treated groups shows that the extract was able to restore the diminished GST activity with increasing dose.

Catalase and MDA

The effect of 150, 300, and 600 mg/kg body weight of *P. conophora* aqueous leaves extract on catalase (CAT) activity in CdCl₂ administered rats is shown in Figure 5. There was an increase in catalase activity in rats that received CdCl₂ only; this was 0.02 units/mg protein when compared with

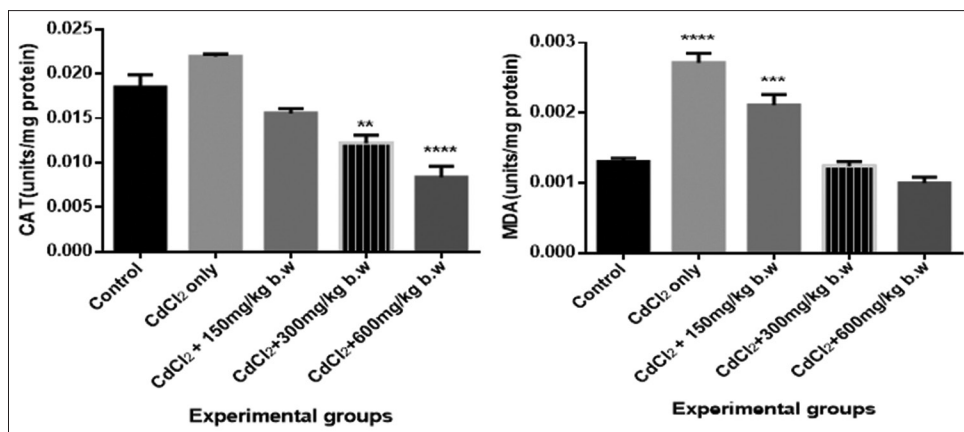


Figure 5: The effect of *P. conophora* aqueous leaves extract on testicular catalase activity of rats co-administered with CdCl₂. Each value is represented as mean of 5 replicates \pm SD. * represents statistical difference relative to control ($P < 0.05$)

the control of 0.0018 units/mg protein. A decrease in catalase activity was, however, observed in rats across all extract-administered groups, in a dose-dependent manner, from 0.0015 to 0.0008 units/mg protein, respectively.

The effect of *P. conophora* aqueous leaves extract on lipid peroxidation (MDA concentration) in CdCl₂ administered rats is shown in Figure 5. Significant increase was observed in rats that received CdCl₂ only, which was 0.0027 units/mg protein when compared with the control of 0.0013 units/mg protein. Whereas there was significant increase in MDA level of rats that received 150 mg/kg body weight of *P. conophora* aqueous leaves extract, which was 0.0021 units/mg protein, there was, however, a reduction in MDA levels of rats administered with 300 and 600 mg/kg body weight of *P. conophora* aqueous leaves extract groups to 0.0012 and 0.0009 units/mg protein, respectively, compared with control.

Catalase is an enzyme that catalyses the decomposition of hydrogen peroxide to molecular oxygen and water, thereby finalizing the process of detoxification started by SOD.^[35] Catalase is also a first line defense antioxidant, in addition to SOD; therefore, the trends in SOD and CAT activities are similar in this current study except that the increase in CAT activity was not significant. Both antioxidant enzymes play protective roles towards wading off attack by free radicals and reactive oxygen species.^[35,38] An increase in the activity of catalase may be due to a response to an increase in free radicals as corroborated by the significant increase in MDA concentration (lipid peroxidation) in this study. There was a restoration in catalase activity in rats that received 150 mg/kg body weight of *P. conophora* extract compared to the control.

MDA concentration was used to determine the level of lipid peroxidation in this current study, MDA is known as an index of lipid peroxidation and oxidative stress.^[41] A significant increase in MDA concentration of rats that received CdCl₂ only and 150 mg/kg body weight of the extract compared with control, could be due to an upsurge in free radicals' generation in the testes of these groups of rats.^[2] A decrease in lipid peroxidation was however observed in rats administered with 300 and 600 mg/kg body weight of *P. conophora* extract compared with the control, which may be due to reduced ROS generation.

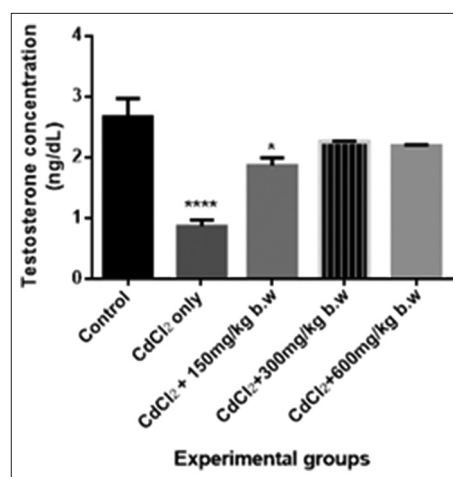


Figure 6: The effect of *P. conophora* aqueous leaves extract on serum testosterone concentration of rats co-administered with CdCl₂. Each value is represented as mean of 5 replicates \pm SD. * represents statistical difference relative to control at $P < 0.05$

Serum Testosterone

From Figure 6, the effect of *P. conophora* aqueous leaves extract on serum testosterone concentration in CdCl₂ administered rats, showed a significant reduction ($P < 0.05$) in level of testosterone in rats that received CdCl₂ only to 0.89 ng/dL compared with the control of 2.67 ng/dL. There was also a reduction in testosterone level of rats across all extract-administered groups, which was only significant ($P < 0.05$) in rats that received 150 mg/kg body weight of *P. conophora* aqueous leaves extract (1.87 ng/dL) compared with the control.

Testosterone, the most important and primary androgen released into the blood, is secreted by the Leydig cells of the testes. The aqueous extract of *P. conophora* leaves was able to restore the concentration of testosterone back to values comparable with the control. This finding is in line with those of previous studies by Olaniyan *et al.*,^[2] which showed an ameliorative effect of *P. conophora* seeds on testosterone concentration in CdCl₂-administered rats.^[2]

CONCLUSION

Although there was a limitation in this study due to lack of histopathological examination, *P. conophora* aqueous leaves extract had beneficial effects on testosterone, glycogen, protein, sialic acid concentrations and the activities of GST, CAT, SOD, and ACP of rats co-administered with CdCl₂ in this study. It was able to restore the enzyme activities and concentration of other assay parameters back to values comparable with the control. Therefore *P. conophora* aqueous leaves extract was able to attenuate the toxic effect of CdCl₂ especially at 600 mg/kg body weight of extract administered after 21 d of co-administration.

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