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Aqueous extract of *Adansonia digitata* prevents Cadmium Chloride-induced testicular damage in Wistar Rats

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Abstract

Objectives: The role of aqueous extract of *Adansonia digitata* was investigated against cadmium chloride-induced testicular damage in Wistar Rats.

Methods: Thirty (30) male Wistar Rats weighing (150–170) were divided into six groups (n=5). Group A served as control and received oral administration of phosphate buffer saline; group B received 800 mg/kg *A. digitata* only; group C were injected intraperitoneally with single dose 2 mg/kg b.w cadmium chloride; group D were injected intraperitoneally with single dose 2 mg/kg b.w cadmium chloride and treated with 800 mg/kg aqueous extract of *A. digitata*; group E received 300 mg/kg vitamin E only; group F were injected intraperitoneally with single dose 2 mg/kg b.w cadmium chloride and treated with 300 mg/kg vitamin E. After 21 days, the animals were sacrificed by cervical dislocation, the testes were excised fixed in Bouins fluids for histological analysis and the other homogenized in 5% sucrose solution for determination of tissue malondialdehyde (MDA) and antioxidant enzyme activity, biochemical assay.

Results: The group treated with cadmium chloride plus *A. digitata* caused significant decrease in MDA levels with significant increase ($p<0.05$) in antioxidant activities and biochemical enzymes when compared to cadmium chloride only group.

Conclusions: Aqueous extract of *A. digitata* appears to have ameliorative effect against cadmium chloride-induced testicular damage. This could be attributed to the presence of polyphenolic compound.

Keywords: *Adansonia digitata*; antioxidant; Cadmium Chloride; testes; Wistar Rats.

Introduction

Cadmium is regarded as a toxic, non-essential compound with diverse industrial use. It is generally utilized in various industrial processes such as in textile and dyes, electrochemical and batteries industry which has caused its accumulation in soils, water and the environment [1]. Different organs accumulate cadmium in the body such as the testes, liver, kidneys and brain, by binding to metallothionein protein [1] thereby causing injury to hepatocytes, degeneration in renal proximal tubule cells, impairments in testes and various disorders in erythrocytes. Oxidative stress is regarded as one of the physiological mechanism by which cadmium induces it detrimental effect causing peroxidation of membrane lipids, release of cytochrome C from the mitochondrial and disturbances in the normal redox state of cells [2]. Cadmium also acts as a catalyst in the formation of reactive oxygen species like hydrogen peroxide, hydroxyl radicals, and superoxide radical ions, depletion of glutathione and protein-bound sulfhydryl groups, hence promoting the production of inflammatory cytokines, change intercellular stability, damage to DNA and ultimately resulting in cell death [3].

The testes have several protective mechanisms that minimize the toxic potential of these reactive oxygen species

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to ensure that the twin spermatogenic and steroidogenic functions of this organ are not impacted by oxidative stress [4]. Reactive oxygen species are formed continuously as by-products of aerobic metabolism, through reactions with drugs and environmental toxins, or when the level of antioxidants is diminished, all creating the condition of oxidative stress [5]. The biochemical damages caused by free radicals to cells and tissues, lead to the development of diseases such as atherosclerosis, high blood pressure, renal failure, cancer, inflammation, and infertility [6].

Medicinal plants are largely used either for the prevention, or curative treatment of several diseases because of their active constituents and biological activity [7, 8]. Plants used in traditional medicine may constitute an important source of new biologically active compounds and huge economy for pharmaceutical industries if properly harnessed. It is estimated that there are about 2,500,000 species of higher plants and majority of these plants have not been studied for their pharmacological role in the management of various ailments [9, 10]. In recent years, there has been increased interest from scientists to evaluate the biological role of some plants components in an attempt to counteract the inflammatory and oxidative stress damages through the use of diet/supplements with antioxidant properties, especially those derived from natural sources [11]. In this regards, several studies have been directed toward the evaluation of several antioxidant properties of many herbs and plants, potentially useful as nutrition supplements [12].

Adansonia digitata is an African plant known as baobab tree. Leaves, bark and fruits of this tree are traditionally employed in several African countries as food and for medicinal purposes, and for these reasons baobab is named as "the small pharmacy" or "chemist tree" [13]. It is well established for its antioxidant properties [14], anti-inflammatory [15, 16], antipyretic activity [17], analgesic property [15]; Bamidele and Ahmed, [18], Hepatoprotective properties [19], antimicrobial activity [20]; antiviral activity [21], antitrypanosomal activity [22], antidiarrheal activity [23]. The pulp is therapeutically utilized as analgesic, antidiarrhoea/antidysentery and for treatment of smallpox and measles [24]. It is confirmed that the health benefits of fruits and vegetables of *A. digitata* are mediated through its active constituents, it seems reasonable to consider the baobab fruit pulp as new valuable ingredient for food and/or nutritional application in the promotion of health [14]. As a result of its high content in vitamin C, polysaccharides triterpenes, flavonoids, polyphenols, β-carotene, magnesium and sterols, baobab fruit pulp have a well-documented antioxidant capability [25]. Hence, the study aimed at investigating the role of aqueous extract of *A. digitata* on cadmium chloride-induced testicular damage in adult Wistar Rats.

Methods

Thirty (30) male Wistar Rats weighing between (150–170 g) were obtained from National Veterinary Research Institute (NVRI) Vom, Plateau State and were divided into six groups (n=5). The rats were housed in a well-ventilated room at Bingham University animal house facility and acclimatized for two weeks before the experiment commenced. They were provided with rat pellets (growers mash) and water *ad libitum*. The ethical approval on animal right was obtained from the Institutional Animal Care Committee of the same Institution with assigned number ERC/2016/06/04. All the experimental procedures were done following the experimental guidelines of the Institutional Animal Ethics Committee (IAEC) of Bingham University, Karu, Nasarawa State, Nigeria.

Sample collection and extract preparation

A. digitata fruit was obtain from Mararaba market in Nasarawa state, Nigeria and authenticated at the Department of Biological Science, Bingham University, Nasarawa state. The fruits were split open and the inner pulp was removed. 2,500 g of the pulp was soaked in 25,000 mL of distilled water for 48 h. It was then filtered and the filtrate was concentrated to a semi solid form using beakers and water bath at 50 °C. Hundred and forty grams of the concentrate was obtained and dissolved in phosphate buffer solution for dosage preparation.

Experimental animals and handling

The rats were grouped into six (6) groups of five rats each;

- Group A received 0.5 mL of phosphate buffer orally,
- Group B received 800 mg/kg b.w dose of *A. digitata* extract orally,
- Group C received 2.0 mg/kg single dose cadmium chloride intraperitoneally,
- Group D received 2.0 mg/kg single dose cadmium chloride intraperitoneally and 800 mg/kg b.w of *A. digitata* extract orally,
- Group E received 300 mg/kg dose of Vitamin E orally,
- Group F received 2.0 mg/kg single dose cadmium chloride intraperitoneally and 300 mg/kg dose of vitamin E orally.

All administration lasted for a period of Vitamin E and 21 days. The rats were sacrificed by cervical dislocation and the testes were excised following abdominal incision, fixed in 10% formo saline for histological analysis and some

were homogenized in 5% sucrose solution for biochemical enzymes assay.

Experimental protocol

Routine histological preparation

The histology of the testes was done by modification of method described by Akpantah et al. [26]. The organs were cut into slabs of about 0.5 cm thick transversely and fixed in 10% buffered formalin for a day after which it was transferred to 70% alcohol for dehydration. The tissues were passed through 90% and absolute alcohol and xylene for differentiations before they were transferred into two changes of molten paraffin wax for 1 h each in an oven at 65 °C for infiltration. They were subsequently embedded and serial sections cut using rotatory microtome at six microns (6 µm). The tissues were transferred onto albumenised slides and allowed to dry on hot plate for 2 min. The slides were dewaxed with xylene and passed through absolute alcohol (two changes); 70% alcohol, 50% alcohol and then water for 5 min. The slides were then stained with hematoxylin and eosin. The slides were mounted in Canada balsam. Photomicrographs were taken using ×400 objectives lens with the microscope for each group.

Enzyme histochemistry

Excised testicular tissues were put in Lao style mortar containing 1 mL of 0.25 mm (5%) sucrose solution and were homogenized thoroughly. Tissue homogenate were collected in 5 mL plain serum bottle for enzyme assay; Glucose 6 phosphate dehydrogenase (G6PD), Malondialdehyde (MDA), Glutathione Peroxidase (GPx), Superoxide Dismutase (SOD) and Lactate Dehydrogenase (LDH).

Determination of Malondialdehyde (MDA) level in tissue homogenates

MDA levels in tissues were measured according to the protocol outlined by Srour et al. [27]; as shown below.

A 0.1 mL of homogenate was pipetted into a plastic test tube. Onw milliliter of 20% Trichloroacetic was added to it. The mixture was mixed and centrifuged at 2,000 g for 5 min 0.5 mL of the supernatant was pipetted into a pyrex test tube. 0.05 mL of 10.0 µmol/L of 1, 1, 3, 3-tetramethoxylpropane was pipette into another pyrex test tube (std). A 0.5 mL of trichoroacetic acid solution and 1.0 ml of thiobarbituric acid were pipetted into a 3rd pyrex test tube (blank). All tubes were stoppered tightly. The test tubes were heated in a water bath at 100 °C for 20 min. All tubes were cooled in water. The spectrophotometer was blanked using the reagent blank at 532 nm. Absorbance of tests and standards were read.

$$\text{Tissue Malondialdehyde} = \frac{(\text{Absolute of test} \times 10)}{\text{Absolute of standard}} \times 10\ \mu\text{mol/L}$$

Determination of glucose 6 phosphate dehydrogenase (G-6-PDH) activity in tissue homogenate

Activity of G6PD in homogenate was measured using the method of Lohr and Walker 1974 [28]. The procedure is as shown in the reactive table below.

Reagent R2 (buffer)	1.0 mL
Reagent R2 (NAOP)	30 µL
Homogenate	15 µL
Mix, incubate for 5 mins at 37 °C	
R3 (Glucose 6 phosphate)	15 µL
Read initial absorbance at 365 nm and start timer simultaneously (against air)	
Read the absorbance again after 1, 2 and 3 min	

Calculation

G6PDH in homogenate activity = 60,571 × rate of change in absorbance per min (mµ/mL)

G6PDH in U/L homogenate = 60,571 × rate of change in absorbance per min × 1,000.

Determination of lactate dehydrogenase (LDH) activity in tissue homogenate

Activity of LDH in the homogenate was measured according to method in Jeyaraman et al. [29]. The homogenate was centrifuged at 10,000x*g* for 10 min at 4 °C. The clear supernatant obtained was used for the measurement of LDH activity.

Determination of Glutathione Peroxidase and superoxide dismutase activities in tissue homogenate

GPx and SOD activities in homogenate were determined using the method of [30].

Statistical analysis

Data were statistically evaluated using the One-way ANOVA (Analysis of Variance) with SPSS/17.0 software (SPSS Inc, Chicago, USA) Tukey post-hoc analysis and were expressed as mean ± standard deviation. p Value<0.05 was considered statistically significant.

Results

Effects aqueous extract of *A. digitata* treatment on G6PDH and LDH activities in cadmium chloride-induced testicular damage in Wistar Rats.

From the results obtained from (Table 1), the enzyme activities (G6PDH and LDH) was significantly reduced (p<0.05) in the group administered with cadmium chloride only as compared to the control group. However, the enzyme activity in the group treated with both cadmium chloride plus *A. digitata* significantly increased (p<0.05).

Effects aqueous extract of *A. digitata* treatment on lipid peroxidation level and antioxidant activity in cadmium chloride-induced testicular damage in Wistar Rats.

Table 2 showed that the group treated with cadmium chloride caused a significant increase (p<0.05) in MDA level and significant decrease (p<0.05) in antioxidant activity (SOD and GPx) as compared with control. The group that received both cadmium chloride plus *A. digitata* caused a significant reduction (p<0.05) in MDA level and significant increase (p<0.05) in antioxidant activity.

Table 1: Effects aqueous extract of *Adansonia digitata* treatment on G6PDH and LDH activities in Cadmium Chloride-induced Testicular Damage in Wistar Rats.

Groups	G6PDH, IU/L	LDH, IU/L
A	5,620 ± 40	3,640 ± 46
B	5,389 ± 39	4,126 ± 37
C	4,584 ± 17^{+}	2,915 ± 12^{+}
D	5,035 ± 23*	3,953 ± 12*
E	5,024 ± 12	3,020 ± 9
F	4,928 ± 14*	3,122 ± 17*

Values are represented as mean ± SEM, n=5, $^{+}$represent significant difference from the control at p<0.05, *represent significantly difference from cadmium chloride group only at p<0.05. G6DPH, Glucose-6-phosphate dehydrogenase; LDH, Lactate dehydrogenase.

Table 2: Effects aqueous extract of *Adansonia digitata* treatment on lipid peroxidation level and antioxidant activity in cadmium chloride-induced testicular damage in Wistar Rats.

Groups	MDA, µmol/L	SOD, IU/L	GPx, IU/L
A	24 ± 1	890 ± 40	710 ± 29
B	22 ± 1	663 ± 16	715 ± 9
C	30 ± 2^{+}	549 ± 12^{+}	495 ± 9^{+}
D	27 ± 1*	635 ± 9*	625 ± 12*
E	32 ± 1	532 ± 18	502 ± 12
F	28 ± 1*	691 ± 23*	635 ± 9*

Values are represented as mean ± SEM, n=5, $^{+}$represent significant difference from the control at p<0.05, *represent significantly difference from cadmium chloride group only at p<0.05. MDA, Malondialdehyde; SOD, Superoxide dismutase; GPx, Glutathione peroxidas

Effects aqueous extract of *A. digitata* treatment on the histology in cadmium chloride-induced testicular damage in Wistar Rats.

As revealed from the photomicrograph of the control animals with the detail normal testicular architecture; the seminiferous tubules contain the three typical cells, spermatogonia, sertoli and the interstitial cells. All stages of spermatogonia were expressed across the seminiferous tubules diameter. The basal membrane integrity and the interstitial spaces define the basic histologic structure of the testes; thus the spermatogonia population and the testicular integrity seen. Similarly, the testicular architecture in *A. digitata* group and the Vitamin E treated, present features in relation to the control groups as stated in Figure 1. However, the administration of cadmium chloride enhances the widening of the interstitial spaces, loss of the interstitial cells and the degeneration in the spermatogonia population. The basal membrane and the regeneration of the interstitial cells and maintenance of the spermatogonia population was preserved in the $CdCl_2$ group that was treated with the Vitamin E and *A. digitata*.

Expression of the continuous evidences of cell divisions that characterized the high region of cell proliferations and the cell migration demonstrated by the use of Feugen DNA were noticed. The characteristic dividing cells defines the histological observation in the control animals and the animals that were treated with Vitamin E and *A. digitata*. However, the spermatogonia population expressed but with no evidences of cell proliferation occasioned by cell division in $Cdcl_2$ treated animals. *Adasonia digitata* and Vitamin E in $Cdcl_2$ treated animals expressed their fertility enhancing effects with the continuous spermatogonia proliferation that

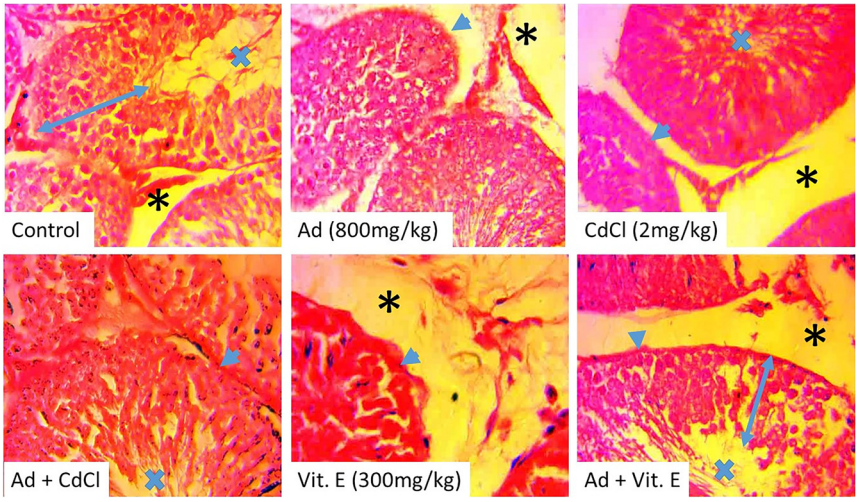


Figure 1: The control animals showed the lumen laden with spermatocytes, the spermatogonia population across the tubule diameter and the black star indicating the leydig cells of the interstitial spaces. Slight increase in the interstitial space observed in the ad (800 mg/kg), spermatogonia stages expressed. $Cdcl_2$ treated with the black star indicating the abnormal widening of the spaces and reduced or loss of leydig cells. In Vitamin E treated animals, though interstitial space widening noticed, the spermatogonia integrity preserved across the tubule diameters. H/E stains ×400.

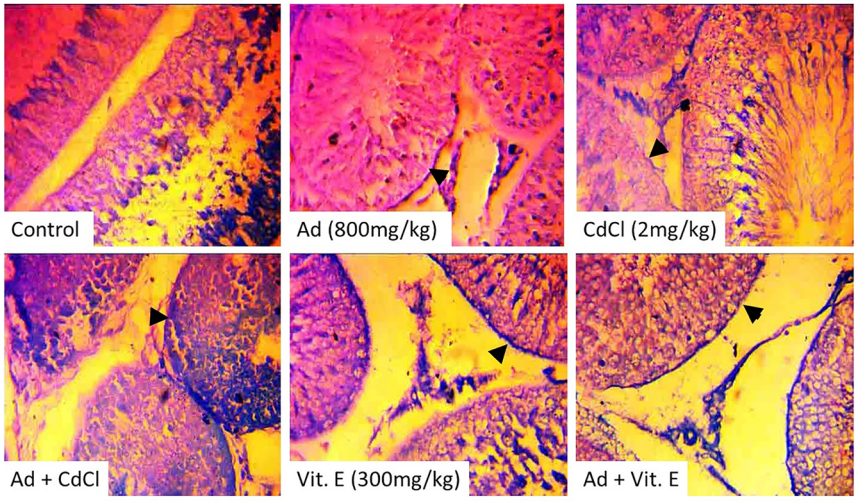


Figure 2: Testicular seminiferous tubules with the detail dividing spermatogonia across the diameters; cell division evident in the control, *Adasonia digitata* and the Vitamin E groups. The administration of $Cdcl_2$ arrest the division with trace evidence of division across the tubular diameters, tough widening of the interstitial spaces in the $Cdcl_2$ groups treated with Vitamin E and Adansonia was observed.

determine the quality and quantity of spermatogonia characteristics as shown in Figure 2.

Discussion

A. digitata fruit pulp has a well-documented antioxidant capability. The protective role of aqueous extract of *A. digitata* was investigated in cadmium chloride-induced testicular damage in Wistar Rats due to its high content in vitamin C, polyphenolic compounds like proanthocyanidins, triterpenes, β-carotene, magnesium and flavonoids. Studies have shown that the antioxidants in *A. digitata* could help prevent oxidative stress related diseases such as aging, cancer, inflammation, cardiometabolic diseases and infertility as they may eliminate free radicals which contribute to these chronic diseases [31]. It is generally known that cadmium chloride induces oxidative stress as a result of increase in peroxidation of membrane lipids in the cell where it accumulates, decrease in glutathione and of protein-binding sulfidryl groups which causes an increase in reactive oxygen species like hydrogen peroxide, hydroxyl radicals and superoxide radical ions which increase lipid peroxidation, change intercellular stability, damage deoxyribonucleic acid (DNA) and membranes, and cell death [1]. The present study demonstrated that administration of *A. digitata* in cadmium chloride-induced damage of the testes of adult Wistar Rats caused significant improvement in biochemical parameters and reversal in the alterations of the histological morphology of the testis.

Analysis of carbohydrate metabolic enzymes revealed an increase in glucose-6-phosphate dehydrogenase (G-6-PDH and lactate dehydrogenase LDH) activity in the group treated with cadmium chloride plus *A. digitata*. LDH is an enzyme found mainly in different tissues that is released from the lysosomes of the degenerating cells and rapid catabolism of the injured cells into the blood stream when

cellular damage occurs. The decreased LDH activity in cadmium chloride-treated rats denotes its cytotoxic effect on testicular cells. Estimation of end products of lipid peroxidation such as MDA is an index of oxidative damage to cellular structures [32]. There was a significant decrease in MDA level in the cadmium chloride plus *A. digitata* group compared to groups treated with cadmium chloride only [33] noted significant increase in level of peroxidation of the lipid layer of cell membrane as revealed by the significant increase in MDA activities in the groups administered gossypol (antifertility agent in cotton seed oil). MDA has been considered the biomarker of lipid peroxidation, which leads to impairments of the cell's functions as well as genotoxicity and carcinogenesis. They also noted SOD as the most predominant enzymatic antioxidant found in sperm cells significantly lower in the gossypol treated groups compared to the group treated with date palm (acting as the antioxidant substances.

There was also a significant increase in antioxidant activities in cadmium chloride plus *A. digitata*-treated group as compared to group treated with cadmium chloride only. These results are in accordance with previous study that cadmium chloride depletes glutathione and protein bound sulfhydryl groups in the testes, resulting in enhanced production of reactive oxygen species such as superoxide ion, hydroxyl radicals and hydrogen peroxide [34, 35]. *A. digitata* is able to reverse these detrimental effects due to the presence of high amount of flavonoids, β-carotene, magnesium, sterols, polysaccharides, vitamin C, and polyphenolic compound such as proanthocyanidins capable of scavenging free radicals as noted by Silvia et al. [14].

The photomicrograph section of the testes of rats in group A (control) showed normal histology of the testes with the testicular lobules, seminiferous epithelium and its constituent cells. The photomicrograph of the testes of rats in group B (*Adansonia* only) also showed a close resemblance to the control group; normal histology of the testes with the testicular lobules, seminiferous epithelium and its constituent cells. The testicular integrity of the rats treated with the extract only was maintained in close proximity with the control rats; an indication of fertility enhancing ability of the aqueous extract of *A. digitata*. The photomicrograph of the testes of rats in group E (Vitamin E only), also showed a resemblance to the control group; normal histology of the testes with the testicular lobules, seminiferous epithelium and its constituent cells. The photomicrograph of the testis of the animals in group C (cadmium chloride only) showed that cadmium chloride disrupts spermatogenesis by destroying the spermatids and spermatogonia. Photomicrograph in this group showed degeneration of seminiferous epithelium, abnormal widening of interstitial space. This is in accordance with previous studies which claimed that even low doses of 1 mg/kg cadmium chloride-induced lack of spermatogenesis and severe necrosis of the testes of rats [36]. The photomicrograph of the testis of the animals in Group D (cadmium chloride plus *A. digitata*) and Group F (cadmium chloride plus Vitamin E) both showed regeneration in the seminiferous epithelium and interstitial space; however, spermatogonia and Leydig cells are fairly observable. This in consistence with previous studies confirms the antioxidant effect of *A. digitata* extract due to its active constituents [14].

Conclusion

This study showed that *A. digitata* has active components particularly polyphenolic proanthocyanidins that give it the ability to play a protective role against cadmium chloride-induced testicular damage. It is therefore recommended that more studies should be done to elucidate the molecular mechanism of the antioxidant property.

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Competing interests: The authors wish to declare that there is no competing interest.
Informed consent: Not applicable.
Ethical approval: The ethical approval on animal right was obtained from the Institutional Animal Care Committee of the same Institution with assigned number ERC/2016/06/04. All the experimental procedures were done following the experimental guidelines of the Institutional Animal Ethics Committee (IAEC) of Bingham University, Karu, Nasarawa State, Nigeria.
Availability of data and material: The material used during the review work was made available from the corresponding author on request.

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