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Original Article

Ameliorating Effect of Aqueous Leaf Extract of *Citrullus Lanatus* on Lead Acetate-Induced Testicular Damage In Adult Male Wistar Rats

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ABSTRACT

Citrullus lanatus has attracted considerable attention by researchers and has been reported to have strong anti-oxidant activities. This study was aimed at investigating the ameliorative effects of *C. lanatus* leaf extract on lead acetate-induced testicular damage in adult male Wistar rats. Thirty-six Wistar rats were randomly assigned into six groups. Group A served as control, groups B and C were administered 100 and 200 mg/kg body weight, group D was treated with 50mg/kg body weight of lead acetate only while groups E and F were administered 100mg/kg body weight and 50mg/kg body weight of lead acetate and 200mg/kg body weight and 50mg/kg body weight of lead acetate respectively for a period of 56 days. Thereafter, blood sample was collected from the inferior vena cava into plain sterile bottle for antioxidant analysis, testes and epididymes were harvested, weighed and fixed in Bouin's fluid for histopathological analysis. Result showed that the groups treated with low and high doses of the extract in addition to lead acetate showed remarkable antioxidant properties when compared to the group treated with lead acetate only. Histological analysis revealed that the cytoarchitecture of the testis and epididymis in the group treated with the low and high doses of the extract together with lead acetate were less deranged compared to the rats treated with lead acetate only. In conclusion, it was noted that the antioxidant properties of *Citrullus lanatus* is protective of the testes against lead acetate toxicity in Wistar rats.

Keywords: *Citrullus lanatus*, lead acetate, testicular damage, Wistar rats

INTRODUCTION

The name *Citrallus lanatus* is derived from both Greek and Latin origin words. The Greek '*citrus*' refers to fruit; while *lanatus* is of Latin origin, meaning 'being woolly' (Baker *et al.,* 2012). The fruit is about 93% composed of water, so refers to the name 'water melon'. It is a large, round, sweet, pulpy flesh (Van *et al*., 2004).

Watermelon is believed to have originated from Northeast Africa some years ago, where it was found as a wild crippling crop (Renner, 2017). It was introduced to China in the 10th century, and today China is world's largest producer of watermelon (Maynard and Maynard, 2012). In Nigeria, climatic conditions and geographical locations have restricted its production to some regions (Abdel *et al.,* 2005).

Citrullus lanatus is an excellent source of vitamins A, B, C as well as arginine, carotenoids, lycopenes, carbohydrate, sodium, magnesium, potassium and water (Van *et al*., 2004). Its phytoconstituents such as terpenoids, sterols, flavonoids, phenolic compounds are responsible for its laxative effect (Laghetti and Hammer, 2007; Swapnil *et al.,* 2011).

Loiy *et al.,* studied the antimicrobial activities of *Citrullus lanatus* against some bacteria (Escherichia coli, Staphylococcus aureus, Pseudomonas aureginosa and fungi (Aspergillus nigar and Candida albican) using cup-plate diffusion method and disc diffusion method. The results were compared concurrently to standard drugs; clotrimazole and gentamicin and concluded that it exhibited antibacterial and antifungal activities (Loiy *et al.,* 2011).

Co-administration of carbon tetrachloride and watermelon juice was found to significantly reduced serum markers of liver damage, aspartate aminotransferase (AST), alanine aminotransferase (ALT), Total Bilirubin (TB) when compared to the control group (Sevcan *et al.,* 2011). The protective effect of hexane extract of *citrullus lanatus* seed oil against CCl4 induced hepatic damage in rat was studied and the results showed significant decrease in serum ALT, AST and ALP levels in treated groups when compared with the standard drug (Madhavi *et al.,* 2012)

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The potency of anti-inflammatory activity of *Citrullus lanatus* seed oil was demonstrated using carrageenan-induced paw edema in rat model and the oil showed significant decreased edema in carrageenan induced rat paw edema model maximum after 3 hours (Madhavi *et al.,* 2012).

Lead is a useful element but capable of polluting the environment and biological system (Karrari *et al.,* 2012) It is a known toxic metals with harmful effects in humans, animals and plants. It is a known environmental pollutant that may result in deleterious effects in many body organs like kidneys, liver and the male genital system (Assi *et al.,* 2016). Lead may be absorbed into the body via basic physiological processes such as inhalation, ingestion and digestion and becomes toxic when it accumulates in the body (Sharma and Pandey, 2011). Lead acetate exhibit its toxicological effects via the inhibition of antioxidant enzyme activities which causes the generation of reactive oxygen species (ROS) ultimately resulting in lipid peroxidation process (Al-Saady *et al.,* 2011). Thus the mechanism by which lead acetate induce male reproductive damage is through induction of oxidative stress.

MATERIALS AND METHODS

Plastic cage with wire gauze, Grower's mesh, Weighing balance (calibrated in the nearest 5g), Orogastric tube, Fixative (10% buffered formal saline in universal bottles, Lithium heparin sample bottles), plain sample bottles, measuring glass cylinder, 5ml syringes and 1ml syringes, dissecting set, cotton wool pack, disposable hand-gloves, light microscope, thread and masking tape.

Chemicals and reagents: All chemicals used, including those used in the preparation of reagents used were of analytical grade and products of reputable companies. They include the following: Lead acetate, Distilled water, Absolute alcohol, Paraffin wax, Xylene, Heamatoxylin and eosin, choloroform, Normal saline, Bouin's fluid and 100% formal saline.

Plant Material

Citrullus lanatus leaves were obtained from Sakpa Garden in Benin City, Edo State, Nigeria. The leaves were identified as *Citrullus lanatus* by a plant taxonomist in the Department of Plant Biology and Biotechnology, University of Benin, Benin City.

The identified *C. lanatus* leaves were air dried for 72 hours then macerated in a jar containing methanol solvent for 24 hours with constant shaking and stirring, then filtration was done to separate the residue from the filtrate by using a Whatman filter paper, conical flask and funnel. The residue was discarded while the filtrate was concentrated to paste level using water bath and crucible at constant temperature to get the crude extract which was later preserved in a sample bottle and stored in refrigerator for research purpose.

Animals

Thirty (30) male Wistar rats weighing between 200-250g were purchased in the Animal House Unit of the Department of Anatomy, University of Benin, Benin City and used for this study. The animals were fed and cared for in accordance with the guidelines of the College of Medical Sciences Research Ethics Committee and Guide for the Care and Use of Laboratory Animals. They were kept in well ventilated cages and were acclimatized for two weeks before the commencement of the experiment.

Experimental Design

Thirty (30) male Wistar rats weighing between 200-250g were randomly assigned into seven groups (A, B, C, D, E and F) with five rats in each group (n=5). Rats in all groups were allowed access to grower's mash and water *ad' libitum*. Group A served as the control group and was given rat feed and water *ad' libitum.* Group B was treated with100mg/kg body weight of extract only, Group C was treated with 200mg/kg body weight of extract only, Group D was treated with 100mg/kg body weight of extract and 50mg/kg body weight of lead acetate, Group E was treated with 200mg/kg body weight of extract and 50mg/kg body weight of lead acetate while Group F was treated with 50mg/kg body weight of lead acetate only. The Experimental period lasted for a period of 56days and oral administration was carried out via oral of gavage.

At the end of the experimental period, the rats were humanely sacrificed under chloroform anesthesia, blood was collected from the heart via cardiac puncture for oxidative stress assessment. The abdomen was incised to access the testes and epididymis, the testis was excised and fixed in Bouin's fluid for histological examination.

Histological Analysis

The testicular tissues were dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. The deparaffinized sections were stained with Haematoxylin and Eosin

Photomicrography

The sections of the testes were obtained and examined under Leica DM750 research microscope with a digital camera (LeicaICC50) attached. Digital photomicrograph of the tissue sections was taken at 100X and 400X magnifications.

Statistical Analysis

All data collected was expressed in tables as mean ± standard deviation of the mean (SDM) for controls and experimental groups. The data was subjected to analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS), version 17. A value of $p < 0.05$ was taken as statistically significant.

RESULT

Results on oxidative stress markers are shown below in table 1. Plates 1- 12 showed the photomicrographs of both the control and the experimental groups.

Table1: Comparison of oxidantive stress markers in the testicular tissue of control and treatment groups

	Superoxide Dismutase	Catalase	Malondialdehyde
Control	39.13±1.78[a]	42.23±3.57[a]	16.53±0.78[a]
Low Extract Only	39.16±0.87[a]	42.48±3.07[a]	17.67±0.82[a]
High Extract Only	38.73±3.45[a]	38.93±1.95[a]	17.21±1.26[a]
Lead acetate Only	17.19±1.04[b]	18.65±2.38[b]	37.18±3.64[b]
Low Extract+ Lead acetate	35.00±3.82[a]	36.98±2.72[a]	18.49±1.25[a]
High Extract + Lead acetate	32.10±3.06[b]	29.35±1.98[b]	30.03±1.02[b]

Like superscript across a row mean not statistically significant (P>0.05) while unike superscript across a row mean statistically significant (P><.05) while

Histological Findings

Histological result is as shown in plates 1-5 revealed lesion in the testes of the groups treated with lead acetate but showed evidence of reversal in groups treated with different doses of extract.

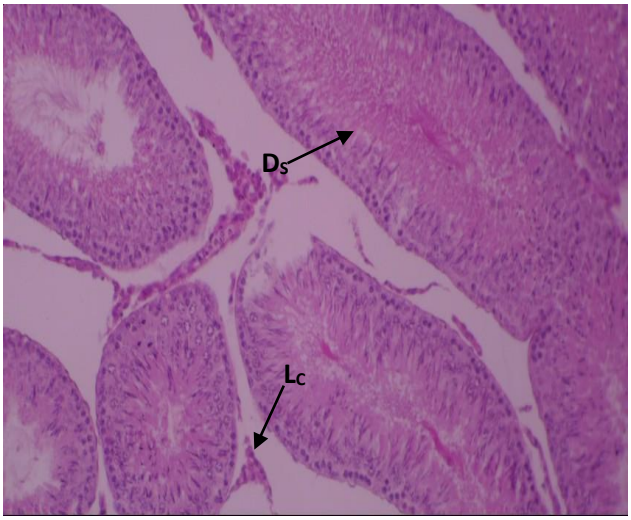

Plate 1: A; control; showing seminiferous tubules containing developing spermatocytes (D_S) and interstitial space containing Leydig cells (L_C).

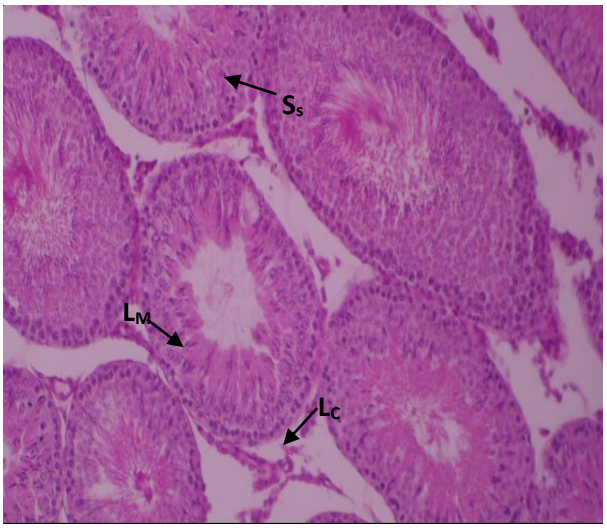

Plate 2: B; low dose extract only; showing normal spermatogenic series (S_S), sperm cells at different levels of maturation (L_M) and Leydig cells (L_C). (H&E: 100X).

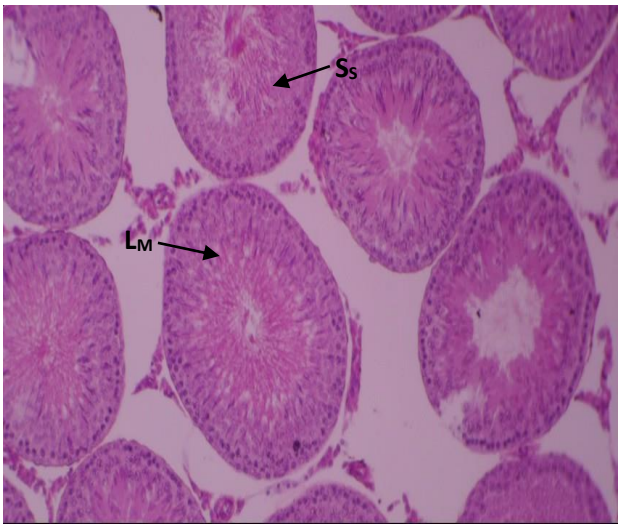

Plate 3: C; high dose extract only; showing normal spermatogenic series (S_S) and sperm cells at different levels of maturation (L_M). (H&E: 100X).

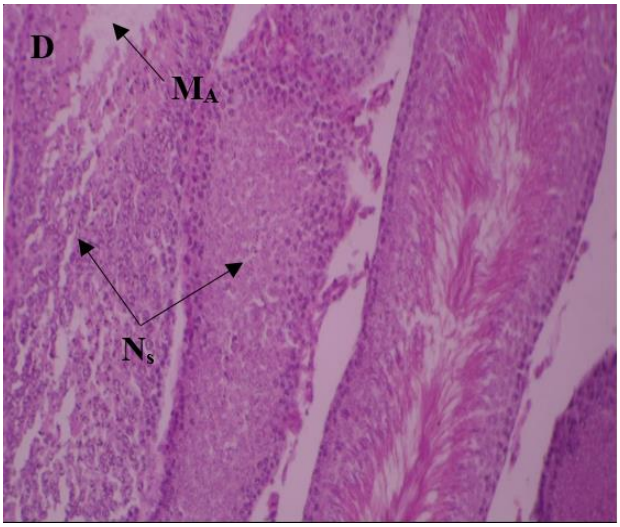

Plate 4: D; lead acetate only; showing maturation arrest (M_A) and necrotic seminiferous tubules (N_S) (H&E: 100X).

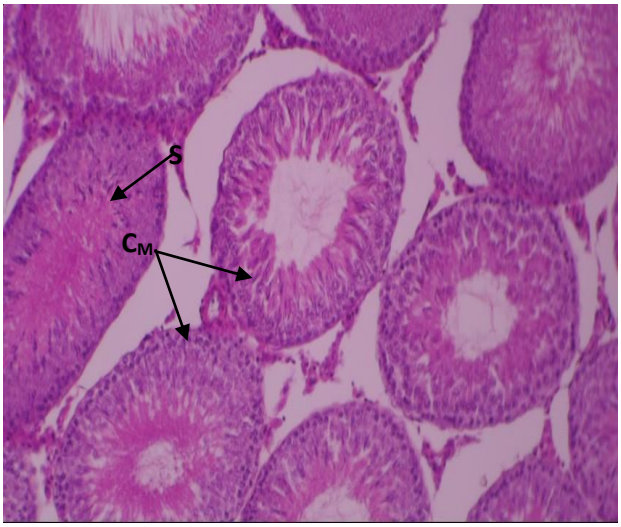

Plate 5: E; low dose + lead acetate, showing normal spermatocytes (S) and normal cell maturation pattern (C_M). (H&E: 100X).

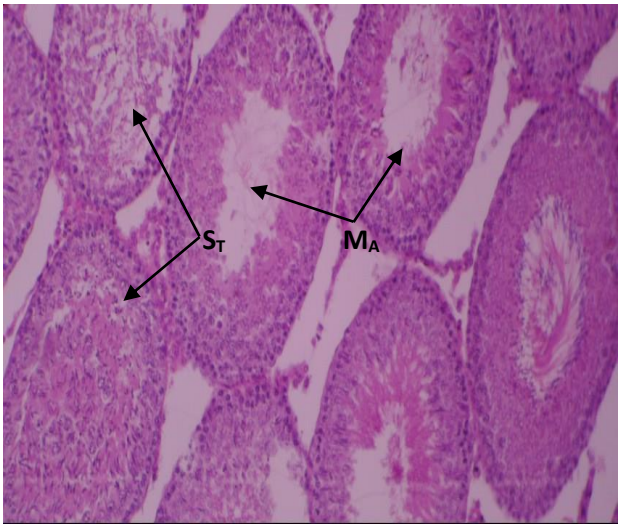

Plate 6: F; high dose + lead acetate showing distorted seminiferous tubules (S_T) and maturation arrest (M_A). (H&E: 100X).

DISCUSSION

Lead acetate is an environmental and industrial pollutants which causes testicular damage (Karrari *et al.,* 2012). Lead acetate accumulates in the testis which impairs the exogenous antioxidant defense system by inhibiting antioxidant enzyme such as superoxide dismutase (Assi *et al.,* 2016). This oxidative damage caused by exogenous substances (lead acetate) was ameliorated to several degrees by phytochemicals present in this plant species (*Citrullus lanatus)* as seen in the result obtained from antioxidant profile which showed that the group administered with lead only and the group co-administered with high dose extract revealed elevated levels of malondialdehyde when compared with the control group, extract only groups and group treated with lead acetate and low dose extract. Just as the testicular levels of superoxide dismutase and catalase were elevated in the control, extract only as well as lead and low of extract treated groups. This could be attributed to the antioxidant properties which protect the body against stress and neutralizes reactive oxygen radical as earlier revealed by Yokota *et al.,* (2002). Also, water melon is a known source of many nutrients like vitamin, minerals and water which are essential for normal body function (Van *et al.*, 2004). The result of extract treatment on testicular tissues revealed the protective effects of *Citrullus lanatus)* against lead toxicity. This reflects in the observed normal histological findings without atrophic damage to the testicular tissues treated with extract only and those co-administered with lead and low dose of extract. The protective effect might be due to its rich antioxidant properties of *Citrullus lanatus)* as evident by the significantly decreased concentration of malondialdehyde (MDA) in the lead treated groups unlike the control and extract only treated groups.

Histological findings further corroborated our findings on oxidative enzymes. Here the testicular tissues showed changes such as necrosis of the spermatogenic cell series in the group treated with lead acetate only, while the group treated with low and high doses of extract showed improvement in the spermatogenic arrangement, and sperm content in the lumen. Although, the testicular tissues appeared better in the groups treated with low dosage of the extract than those treated with high dose and lead acetate from which we can infer that protective potentials of *citrullus lanata* is dose dependent. This finding is similar to research earlier carried out by Baldessarini, 1980.

It could be concluded from this study that *Citrullus lanatus* aqueous leaf extract at low dose could be a more potent natural product providing protective

effects against lead acetate induced testicular damage than higher dosage of the same extract. The study has further revealed the relevance of antioxidants protection against testicular damage when exposed to toxic agents like lead acetate. It is therefore recommended that this modest fruit with valuable phytochemicals should be consumed regularly as it provides the ameliorative effects observed in this study.

REFERENCES

1. Abdel AM, Eldesuki RM, Salman SR and Hussein SD (2005). Performance of some snap bean varieties as affected by different levels of mineral fertilizers. Jour Agro 4: 242-247.
2. Al-Saady MSM, Kamil HZ, Jasim SH (2011). Effect of lead acetate in some physiological genetic parameters in white male rat rattus". Fac. J. Educ. Karbala univ 3 (9): 295-301.
3. Assi MA, Hczmee MN, Haron AW, Sabri MY, Rajion MA (2016). The detrimental effectsof lead on human and animal health. Vet Wor 9 (6): 660-671.
4. Baker TP, Corwin B, Jett LW. (2012). Watermelon Bacterial Fruit Blotch. University of Missouri Extension.
5. Baldessarini RJ. (1980). The Pharmacological basis of therapeutics in drugs and the treatment of psychiatric disorders, eds. By Goodman and Gilman Macmillan Pub. Co. Inc. 301 – 417.
6. Karrari P, Mehrpour O, Abdollahi M (2012). A systematic review on status of lead pollution and toxicity in Iran; guidance for Preventive Measures. Jour phar Sci. 20(1): 2.
7. Laghetti G. and Hammer K. (2007). The Corsican citron melon (Citrullus lanatus (Thunb.) Matsum. et Nakai subsp. lanatus var. citroides (Bailey) Mansf. ex Greb.) a traditional and neglected crop. Gen Res and crop eval vol. 54; 913-916.
8. Loiy E, Ahmed H, Hasnah MS, Sakina M, Ahemd Y, Waleed SK and Siddig IA (2011). In vitro Antimicrobial activities of chloroformic, hexane and ethanolic extracts of Citrullus lanatus var. citroides (Wild melon), Jour of Med Plt Res, 5(8):1338-1344.
9. Madhavi P, Kamala V, Habibur R (2012). Hepatoprotective Activity of Citrullus Lanatus Seed Oil on CCl4 Induced Liver Damage in Rats. Sch Acad Jour Phar. 1(1):30-33.
10. Madhavi P, Maruthi R, Kamala V, Habibur R, Chinna E M (2012). Evaluation of Anti-Inflammatory Activity of Citrullus lanatus Seed Oil by In-vivo and In-vitro Models. Int. Res J Pharm. App Sci. 2(4):104-108.
11. *Maynard, David; Maynard, Donald N. (2012). Cucumbers, melons and watermelons". In Kiple, Kenneth F.; Ornelas, Kriemhild Coneè (eds.). Cambr Wor Histo Food, Part 2. pp. 267–270.*
12. Renner SS (2017). Chromosome numbers, Sudanese wild forms, and classification of the watermelon genus Citrullus, with 50 names allocated to seven biological species Taxon, 66 (6): 1393–1405
13. Sevcan, A., Göksel, K., Murat, K., Aydın, K. and Parvez I. H. (2011). Protective effect of Diyarbakır watermelon juice on carbon tetrachloride-induced toxicity in rats. Food Chem Toxi 49: 243 3–2438
14. Sharma S, Pandey D (2011). Protective role of tinospora ordifolia against lead induced hepatotoxicity". Toxi Inter. 17(1): 12-17.
15. Swapnil Sharma, Sarvesh paliwala, Jaya Dwivedia & Amita Tilakb. (2011). First report on laxative activity of Citrullus lanatus . Pharmacologyonline 2: 790-797
16. Van DV, Denton HAM, El Tahir IM. (2004). Citrullus lanatus (Thunb.) Matsum. & Nakai. [Internet] Record from Protabase. Grubben, G.J.H. & Denton, O.A. (Editors). PROTA (Plt Res of Trop Afri , Wageningen, Netherlands.
17. Yokota A, Kawasaki S, Iwano M, Nakamura C, Miyake C, Akashi K, (2002). Citrulline and DRIP-1 protein in drought tolerance of wild watermelon. Ann. Bot; 89:825-832.