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Reproductive Analysis of *Bryophyllum pinnatum* Leaf extract against Cadmium Induced Testicular Damage

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ABSTRACT

The purpose of this study is to explore if treatment with aqueous leaf extract of Bryophyllum pinnatum was effective in minimising cadmium induced testicular damage on the testes of male wistar rats. Twenty (20) mature male wistar rats was collected from Animal House of the Faculty of Basic medical Sciences, Nnamdi Azikiwe University, Awka. Four subgroups, each consisting of four rats, were created from them. Group I was the control group and was given unlimited access to food and water for a period of two weeks. Group II was given a two-week treatmet of B. pinnatum together with CdcT + lovv. Group III had B. pinnatum at a medium dosage together with Cdcl for a fortnight. For two weeks, group IV received a high dosage of B. pinnatum along with Cdcl. Upon completion of the final dosage, the rats were sedated, and a blood sample was taken from each one via ocular puncture using a micro capillary tube for biochemical analysis and an epididymis dissection for semen analysis. All particulate parameters were analysed according to standard protocols. The results indicated a significant ($p<0.05$) decrease in the rats' final body weights in groups B and D in comparison to the control group. When the test groups' testicular weight was compared to the control group, it dropped considerably ($p<0.05$). Comparing all test groups to control, there was a significant ($p<0.05$) decrease in both FSH and testosterone. When comparing the test groups to the control, there was a substantial ($p<0.05$) decrease in the motility of active sperm, but there was a significant ($p<0.05$) increase in the motility of sluggish and non-motile sperm. This study shown that Bryophyllum pinnatum leaf extract cannot reverse the reproductive harm caused by cadmium in rats.

Keywords: Testes, Cadmium, *Bryophyllum pinnatum*, Reproductive Analysis, Cadmium and Testicular Damage

INTRODUCTION

There is a lot of promise in using plants to treat and control certain diseases. Many plants have been used for many years to treat and control various ailments by tribal people and folklore in various nations [1], [2]. Numerous plant materials are now being studied for possible medical use. The study of natural materials, particularly plants, as a source of possible drugs has long attracted attention [3], [4], [5]. A common perennial medicinal herb is Bryophyllum pinnatum (Lam.), also known as Bryophyllum calycinum (Salisb.) and syn. Kalanchoe pinnata (Lam.). Although it originated in Madagascar, it has spread to a number of other places, including temperate Asia, Australia, and New Zealand. Common names for B. pinnatum include cathedral bells, mother of thousands, air plant, maternity plant, love plant, miracle leaf, life plant, Lao di Sheng gen, and leaf of resurrection plant. The herb, known as "Never Die" locally in Nigeria, is highly valued in traditional medicine. Many ailments, including rheumatism, bodily discomfort, arthritis, heartburn, skin ulcers, peptic ulcers, diabetes mellitus, microbiological infections, and hypertension, have been treated with it in tropical America, India, China, Australia, and Africa [6]. The plant is especially well-known in Nigeria for its ability to effectively heal wounds and separate an infant's umbilicus. Numerous biological activities, including those that could validate the plant's traditional uses as an immunomodulatory, CNS depressant,

analgesic, anti-inflammatory, antimicrobial, antitumor, anti-ulcer, insecticidal, antidiabetic, anticonvulsant, antioxidant, and antihypertensive, were reported in pharmacological studies on B. pinnatum. Numerous active phytochemicals, including steroids, alkaloids, triterpenes, glycosides, flavonoids, bufadienolides, lipids, and organic acids, have also been reported in studies [7]. The various pharmacological actions of the plant have been attributed to these substances. While many herbal medicines have folkloric claims that have not yet been scientifically verified, B. pinnatum has undergone reasonable research that supports the majority of these claims [8]. This has made it easier to promote the use of B. pinnatum and other plants in place of or in addition to conventional treatments. B. pinnatum leaves were often boiled in water, squeezed, roasted, or soaked in cold water for an entire night. The resulting extracts were then used to treat a variety of ailments, including fevers, headaches, arthritis, joint and body pains, tonsillitis, diarrhoea, and coughs [9]. In addition to the previously mentioned reasons, the local perception that natural extracts have no negative effects makes B. pinnatum a well-liked herbal remedy [10]. This makes it essential to assess the safety of commonly used therapeutic herbs.

Therefore, the purpose of this study was to find out how B. pinnatum leaf extract affected the rats' testicular indices.

MATERIALS AND METHODS

From a farm in Igboukwu town, Aguata Local Government Area, Anambra State, fresh B. pinnatum leaves were gathered. The leaves were allowed to air dry, then ground into 500 g pieces and thoroughly extracted using cold maceration in distilled water for 72 hours. A rotary evaporator was then used to evaporate the filtrate in order to get the dry extract. After 30 g of dry extract were weighed, the yield percentage (7%). Next, using established techniques, the extract was examined for the presence of phytochemicals [11], [12], [13], [14].

Purchase of cadmium chloride

From Franklabdon Co. Nig. (E/583 PS Line, Bridge head Market) in Onitsha, Anambra State, Nigeria, cadmium chloride was acquired.

Experimental Design

For the study, twenty (20) male wistar rats, weighing eleven weeks, were taken from the Nnamdi Azikiwe University's animal house on the Nnewi Campus in Nigeria. Standard rat food and clean drinking water were provided to the animals on an as-needed basis. The animals were housed in a room with adequate ventilation, a 12-hour light/dark cycle, and room temperature. The university's Animal Research Ethics Committee approved all animal research, which followed the guidelines for the use and care of laboratory animals [15].

The animals were divided into four groups at random for the experiment after a week of acclimatisation:

Group A served as the control group and received feed + water *adlibitum* for two weeks.

Group B received CdcT + lovv dose of *B. pinnatum* for two weeks.

Group C received Cdcl + medium dose of *B. pinnatum* for two weeks.

Group D will received Cdcl + high dose of *B. pinnatum* for two weeks.

Collection of Blood Samples

After the rats were given the extract, they were deep-dyed ether anaesthetized and then sacrificed by cervical dislocation. In order to measure hormonal markers, blood samples were obtained. Sperm was taken from the caudal epididymis of dissected animals in order to analyse the sperm. Additionally, the testes were taken out, weighed, and regularly processed in order to undergo a histological assessment.

Hormonal Assay

The enzyme immunoassay method was used to measure the levels of FSH and testosterone in accordance with the manufacturer's instructions.

Sperm analysis

The procedure utilised to extract sperm cells from the epididymis was [16]. In short, the testis was removed, and the caudal epididymis was carefully separated and put in a petri dish with three millilitres of Tyrodes' solution, which had been buffered with sodium bicarbonate (NaHCO3). It was incised several times, measuring 1 mm in diameter. Then, using a plastic transfer pipette, the sperm was carefully extracted and placed into 5 ml test tubes. The cells were then violently agitated to ensure homogeneity and dispersion. Then, using standard procedures, sperm was examined to evaluate its motility, count, percentage of aberrant sperm cells (sperm morphology), and percentage of viable sperm cells (sperm viability) [17].

Histopathological analysis

Testicular tissues were preserved in formalin that had been 10% buffered. Tissue slices (5-7 μm) were stained with hematoxylin and eosin (H and E), fixed in paraffin, and viewed under a light microscope (Nikon Eclipse E400). Every modification from the

typical structures was recorded, and the histopathological differences between the experimental and control rats were observed. An Olympus Model BX51 microscope was used to capture the pictures at a 400x magnification.

Statistical analysis

Testicular tissues were stored in 10% buffered formalin. Hematoxylin and eosin (H and E) was applied to tissue slices (5-7 µm), embedded in paraffin, and examined using a Nikon Eclipse E400 light microscope. All structural deviations from the standard models were documented, and the histological variations between the experimental and control groups were noted. The 400x magnification images were taken with an Olympus Model BX51 microscope.

RESULTS

Table 1: Values of extract on body weight of male Wistar rats

GROUP	WEIGHT(g)	MEAN±SEM	WEIGHT DIFF.	P.VALUE
Group A	Initial	150.00±5.77	50.00	0.038*
	final	200.00±11.54		
Group B	initial	166.66±3.33	-23.33	0.020*
	final	143.33±3.33		
Group C	initial	146.66±6.66	53.33	0.015*
	final	200.00±0.00		
Group D	initial	140.00±11.54	3.33	0.808
	final	143.33±18.55		

Table 2: Values of extract on Testicular weight of male Wistar rats

Organs	Groups	MEAN ±SEM	P-Value
Relative Testicular weight (g)	Group A	0.81±0.05	0.000*
	Group B	0.51±0.00	
	Group C	0.68±0.02	0.008*
	GroupD	0.72±0.04	0.002*

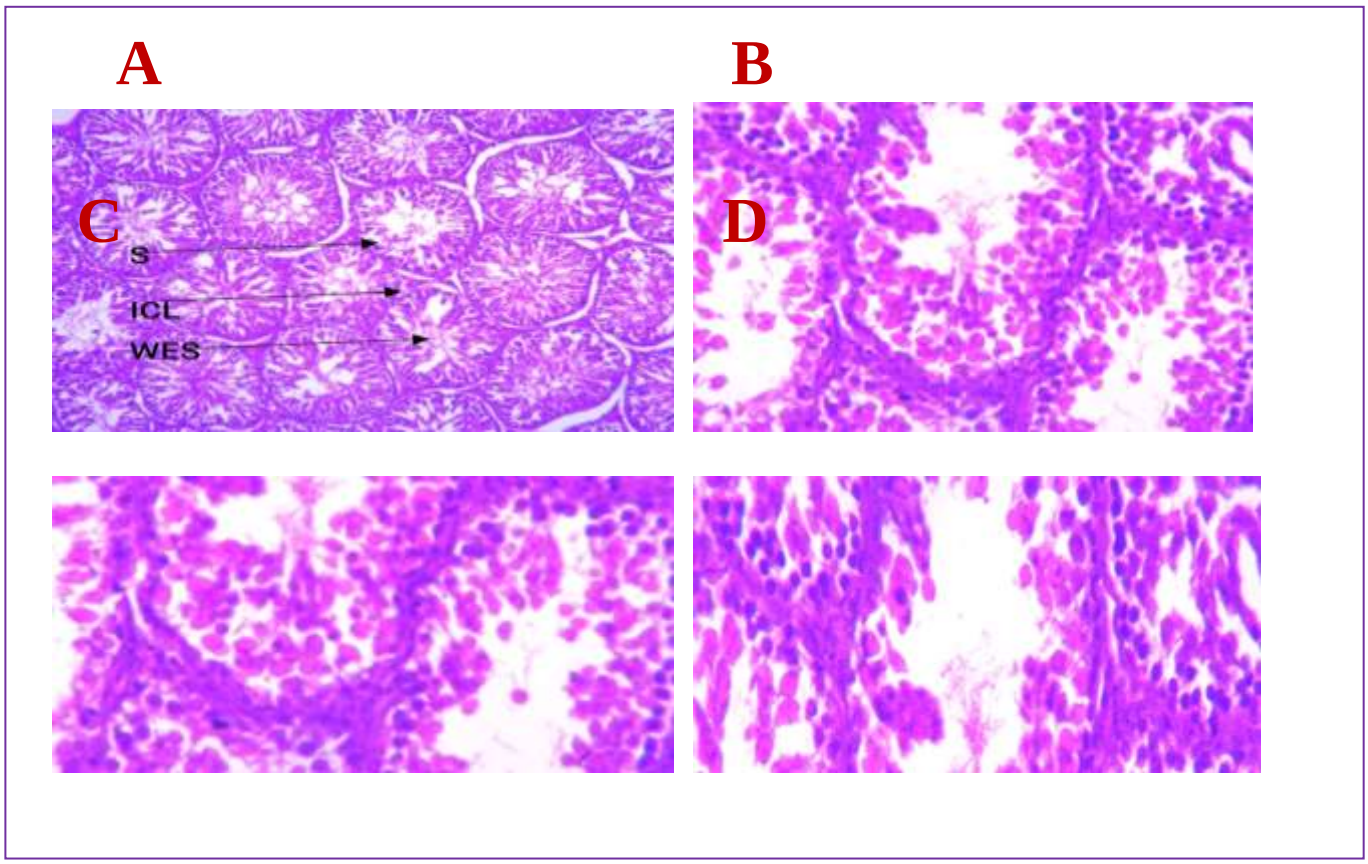
Table 3: Values of extract on hormones of male Wistar rats

Hormone	Groups	MEAN ±SEM	P-Value
Testosterone(ng/ml)	Group A	3.25±0.14	0.171
	Group B	2.45±0.02	
	Group C	2.30±0.11	0.000*
	Group D	1.95±0.08	0.000*
Follicle stimulating hormone (ulu/ml)	Group A	7.75±0.20	0.000*
	Group B	5.50±0.05	
	Group C	5.80±0.11	0.325
	GroupD	6.15±0.31	0.046*

Table 4: Values of extract on sperm indices of male Wistar rats

Sperm motility	Groups	MEAN ±SEM	P-Value
Active motility (%)	Group A	75.00±7.63	0.000*
	Group B	33.33±4.40	
	Group C	31.66±7.26	0.830
	Group D	68.33±4.40	0.001*
Sluggish motility (%)	Group A	16.66±6.66	0.084
	Group B	33.33±10.13	
	Group C	20.00±5.77	0.157
	Group D	13.33±3.33	0.043*
Non-motile Semen	Group A	8.33±1.66	0.008*
	Group B	30.00±10.40	
	Group C	48.33±1.66	0.019*
	Group D	15.00±2.88	

Figure 1: Histology of the testes



DISCUSSION OF FINDINGS

With the express purpose of assessing the impact of the extract's lowest, medium, and maximum biologically active dosages, the doses employed in this investigation were chosen carefully. According to reports, the aqueous extract of B. pinnatum has an LD50 of 1.8 g/kg (i.p.) in rats; however, at the maximum oral dose tested, no overt toxicological symptoms were seen [7], [18]. B. pinnatum has been utilised at doses ranging from 150 mg/kg to 2000 mg/kg in numerous prior animal trials. This paper details how the B. pinnatum leaf extract affected the rat's testicular indices and body weight. When compared to the control, administration of the extract significantly reduced the levels of testosterone and FSH in the serum ($P>0.05$). When compared to control rats, the sperm counts and motility of rats treated with extract were considerably lower ($P>0.05$). Rats treated with extract also showed changes in the percentage of viable sperms (sperm viability) and aberrant sperms (sperm morphology) in comparison to control values. The results showed that administration of B. pinnatum would have a negative effect on the testes across the duration and dose range used in this investigation, since the extract therapy altered the serum levels of FSH and testosterone. A helpful metric that's frequently used in the evaluation of organ toxicity is the organ-to-body weight ratio [19]. When compared to control rats, all extract-treated groups showed a substantial ($P>0.05$) decrease in the testes-to-body weight ratio, which is consistent with changes in the testes' microstructure. Rats given treatment have aberrant architecture and structure in their testicles. This observation implied that the plant extract treatment had a negative impact on the testes, as

evidenced by the positive association seen with the hormonal parameters. Plant extracts have been shown in earlier research to have protective effects against toxicity caused by chemicals [5], [20]. Many medicinal herbs have been shown to counteract the effects of chemicals on testicular function in animals, and the testis is extremely vulnerable to toxicity from these substances [21]. The plant was unable to reduce the impact of cadmium on sperm count, motility, morphology, viability, FSH, or testosterone in the current investigation. Rats given extract also had aberrant histology in their testes, with few seminiferous tubules and fewer spermatozoa in the lumen. The extract also affected the ratio of testicular organ weight to body weight. These findings demonstrated that the plant extract treatment had some negative effects on the fertility and testicular function of the rats. Overall, cadmium injection may have decreased gonadotropin activity in rats; nevertheless, B. pinnatum may have a poor potential for reversibility in relation to the organs examined in this investigation. Previous research [22], [23] found flavonoids, saponins, tannins, and alkaloids as phytoconstituents of the extract. The plant's flavonoids and other significant antioxidant components are well-known free radical scavengers that can lessen oxidative stress and stop oxidative cell damage. Certain cellular components may be poisonous to other components, such as triterpenoids and saponins. Thus, the various impacts that were noted may have been caused by the harmful phytochemical substances that were present in the extract.

REFERENCES

1. Mgbemena Nita , Onah Isegbe Emmanuel. , chidozie vivian (2024): Comparative Effects of Ethanolic Leaf Extracts of Gongronema latifolium Benth and Bryophyllum pinnatum (Lam.) Oken in The Management of Diabetes Mellitus in Albino Rats. *Afghanistan Journal of Basic Medical Sciences* 1(1), 1-10
2. Ifedi I. Charles, Emeka Ugwuishi , Ifedi O. Blessing , Nwafor C. Charles, Okeke C. Jennifer, Okoye O. Fidelis and Ihezuruoha S. Chinyere (2023): Hormonal and Morphological Effects of Averrhoea carambola Fruit Extract on Female Reproduction. *Journal of Advances in Medicine and Medical Research.* 35(19), 305-313; Article no.JAMMR.97671 ISSN: 2456-8899. : https://www.researchgate.net/publication/373011721
3. Amah-Taria F. S., Nwafor C. C., Ajah A. A., Bekinbo M. T (2016). Changes in some female reproductive parameters of Albino wistar rats by hydroethanol leaf extract of fleurya aestuans . International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 5(11):3806-3810. : https://www.researchgate.net/publication/309383399
4. Royère, D., Feuerstein, P., Cadoret, V., Puard, V., & Guérif, F. (2011). Évaluation non invasive de la viabilité de l'embryon humain. In I. C. Poncelet, & C. Sifer (Eds.), Physiologie, pathologie et thérapie de la reproduction chez l'humain (pp. 507– 510).
5. Charles C.N., Amah-Tariah F.S., Dapper D.V. (2021a): Improvements of Male Aphrodisiac Activity and Sexual Parameters By Hydroethanolic Leaf Extract Of Fleurya Aestuans In Wistar Rats. 10(11), 1700-1713. : https://www.researchgate.net/publication/365607389
6. Ghasi S, Egwuib C, Achukwu PU, Onyeanusi JC. Assessment of the medical benefit in the folkloric use of Bryophyllum pinnatum leaf among the Igbos of Nigeria for the treatment of hypertension. Afr J Pharm Pharmacol 2011;5:83-92
7. Aprioku J. S. and Igbe .I (2017): Effects of Aqueous Bryophyllum pinnatum Leaf Extract on Haematological, Renal and Sperm Indices in Wistar Rats. *Indian Journal of Pharmaceutical Sciences.* 79(4):521-526
8. Amabe O, Akpantah KE, Obeten ES, Mokutima, A. E. The effect of ethanolic extract of Bryophyllum pinnatum on the micro anatomy of the testes of adult males Wister rats. European Journal of Biology and Medical Science Research. 2014; 2(2): 37-44.
9. Nagaratna A, Prakash LH. Comprehensive review on Parnabeeja [Bryophyllum pinnatum (Lam.) Oken]. Journal of Medicinal Plants Studies. 2015; 3(5): 166-171.
10. Ojewole JAO. Antihypertension properties of Bryophyllum pinnatum (Lam) (oken) leaf extracts. Am J. Hypert. 2002; 15(4): A34-A39.
11. Jagessar RC, Allen R. Phytochemical screening and atomic absorption spectroscopic studies of solvent type extract from leaves of Terminalia catappa, (Almond). Acad Res In 2012;3:17-26.
12. Kelly DA, Nelson R. (2014): characterization and quantification by gas chromatography of flavonoids. Journal of Braz Chemstry.;25
13. Charles CN, Weleh, II and Green, IK (2021b): Fleurya Aestuans Promotes Oogenesis and Ovulatory Functions In Wistar Rats By Shortening the Estrus Cycle. *World Journal of Pharmaceutical Research.* 10 (13), 1951-1966. https://www.researchgate.net/publication/365607689
14. National Institute of Health Laboratory Animal Welfare. United State Department of Health and Human Services. 1985;14:8.
15. Amelar RD, Dublin L, Schoenfeld C (1973): Semen analysis: An office technique. Urology 1973;2:606-11.

16. Ochei O, Kolhatker A (2002). Medical Laboratory Science, Theory and Practice. 5th ed. New Delhi: Tata McGraw-Hill Publishing Company Limited.
17. Ozolua RI, Idogun SE, Tafamel GE (2010). Acute and subacute toxicological assessment of aqueous leaf extract of Bryophyllum pinnatum (Lam.) in Sprague-Dawley rats. Am J Pharmacol Toxicol;5:145-51.
18. Michael B, Yano B, Sellers RS, Perry R, Morton D, Roome N (2007): Evaluation of organ weights for rodent and nonrodent toxicity studies: A review of regulatory guidelines and a survey of current practices. Toxicol Pathol ;35:742-50.
19. Green I. Kinikanwo , Charles C. Nwafor and Weleh I. Iyke (2021): Attenuation of Reproductive Dysfunctions by Hydroethanolic Leaf Extract of Fleurya aestuans in Diabetic Rats. *Asian Research Journal of Gynaecology and Obstetrics* 6(2): 24-31; Article no.ARJGO.72641. : https://www.researchgate.net/publication/365607700
20. Charles CN, Amah-Tariah FS , Adienbo OM & Dapper DV (2021c): Ameliorative Effects of Fleurya Aestuans Leaves on Mating Behaviours and Reproductive Hormones of Lead Acetate Induced Ovarian Toxicity. *European Journal of Research in Medical Sciences*. . 9 (1), ISSN 2056-600X. https://www.researchgate.net/publication/365374275
21. Charles C. Nwafor, Ifedi I. Charles, Ekikereabasi P. Inyang , Solomon M. Uvoh , Okache B. Ogwihi and Alagha B. Ebiyemzi (2021d): Fleurya Aestuans Leaves and Tetrahydroxyflavone Mitigate Lead Induced Testicular Toxicity in Wistar Rats. *Journal of Advances in Medical and Pharmaceutical Sciences* 23(10): 35-47 ; Article no.JAMPS.77151 ISSN: 2394-1111. t: https://www.researchgate.net/publication/357866009SS
22. Ojewole JAO (2005). Antinociceptive, anti-inflammatory and antidiabetic effects of Bryophyllum pinnatum (Crassulaceae) leaf aqueous extract. J Ethnopharmacol; 99:13-9.
23. Yamagishi T, Haruna M, Yan XZ, Chang JJ, Lee KH (1989). Antitumor agents, 110, Bryophyllin B, a novel potent cytotoxic bufadienoiide from Bryophyllum pinnatum. J Nat Prod ; 52:1071-9.

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