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The Histological Effects of Ethanolic Leaf Extract of Annona muricata on the Testes of Adult Wistar Rats

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ABSTRACT

This study was carried out to evaluate the effects of *Annona muricata* ethanolic leaf extract on the testes of adult wistar rats. Twenty wistar rats weighing between 180 – 205g were used for the study. They were designated into four groups (A, B, C & D) of five animals each. Group A animals served as the control and were orally administered 0.3ml of distilled water; the experimental groups B, C & D were orally administered 0.2 ml, 0.4ml and 0.6ml of *Annona muricata* ethanolic leaf extract respectively for twenty eight days. Twenty four hours after the last administration, the animals were weighed, sacrificed under the influence of chloroform vapour and dissected. Testes tissue were removed, weighed and fixed in 10% formalin for histological studies. The final body weight of groups C and D decreased significantly ($P<0.001$) compared with the control. The relative organ weight of groups C and D increased significantly ($P<0.001$) compared with the control. The mean relative organ weight of group B was statistically similar with the control. Histological findings revealed necrotic changes in the interstitial tissue, loss of spermatide changes and multinucleated giant cells in group C and D. From these findings, *Annona muricata* ethanolic leaf extract administered in high doses may cause histopathological alterations/lesions in the testicular cells.

Keywords: *Annona muricata*, Body weight, Organ weight, Wistar rats, Spermatide.

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INTRODUCTION

Plant and plant based medications are used as the basis of many of the modern pharmaceuticals that we use today in order to treat our various ailments [1]. The better understanding of the plant derived medicine depends mainly on two factors that have gone hand in hand. One criterion involves the proof to show that the formulated medicine does what it is claimed to do and other is the identification of the active compound by means of the chemical analysis [2]. According to the world health organization (WHO), greater than 80% of the total world's population depends on the traditional medicines in order to satisfy their primary health care needs. It also suggested in improving the technologies for cultivation of medicinal plants [3]. *Annona muricata* is one of the medicinal plants which is also called as the soup-sop, sir sak or guanabana has been named as a popular fruit tree that is cultivated throughout the tropical regions of the world. The seeds and leaves of this specie were found to contain more than 50 mono-THF acetogenins [4]. *Annona muricata* is found to grow more in many of the regions of the tropical world was extensively studies for the exploration of the new Annonaceous acetogenins from its bark, seeds and leaves which posses much of the diverse biological activities. Studies done on the leaves of *Annona muricata* has been resulted in the isolation of eight cytotoxic acetogenins [5]. Phytochemical analysis of the leaf extract of *Annona muricata* revealed the presence of secondary metabolites like tannins, steroids; cardiac glycosides were present in very trace amounts. Secondary plant metabolites which are called as the phytochemicals possess some of unknown pharmacological activities. Phytochemicals with adequate antibacterial efficacy can be used for the treatment of bacterial infections [6]. In view that *Annona muricata* has medicinal properties, there is need to investigate its protective efficiency or toxic effects on testicular cells for future knowledge of its usefulness in protecting or its toxic effects on reproductive organs.

MATERIALS AND METHOD

Breeding of Animals

Twenty wistar rats were purchased from the animal house of Department of Pharmacy, Nnamdi Azikiwe University, Agulu, Anambra State. The ethical committee permission was gotten from Faculty of Basic Medical Sciences, Nnamdi Azikiwe University Ethical Committee. They were allowed to acclimatize in the animal house of department of Anatomy, Nnamdi Azikiwe University, Nnewi Campus under normal temperature (27℃-30℃). They were fed ad-libitum with water and guinea feed pallets from Agro feed Mill Nigeria Ltd.

Drug Preparation

Annona muricata leaves were plugged from Okofia, Nnewi, Anambra State. It was identified at herbarium unit, Botany department, Nnamdi Azikiwe University. It was dried in an oven at a temperature of 50℃ and crushed using laboratory blender. Extraction was done using ethanol. 200mg of this extract/kg body weight was dissolved in 10mls of distilled water and administered to the animals.

Experimental protocols

The twenty wistar rats were weighed and allocated into four groups (A, B, C & D) of five animals each. Group A served as the control and administered 0.3ml of distilled water; the experiment groups B, C & D were orally administered 0.2ml, 0.4ml, and 0.6ml of *Annona muricata* leaf extract for twenty eight days. Twenty four hours after animals were weighed and there weights recorded. The animals were then anaesthetized under the influence of chloroform vapour and dissected. The testes tissues were removed weighed and fixed in 10% formaline for four hours for histological studies.

Tissue Processing

For easy study of sections under microscope, the tissues passed through processes of fixation, dehydration, clearing, infiltration, embedding, sectioning and staining. Fixation was carried out in 10% formal saline for 10 hours. After fixation, the tissues were washed in stream tap water. Dehydration of the fixed tissues was done using ascending grade of alcohol, 50%, 70%, 90% and absolute. The tissues were cleared in xylene after which infiltration was done in a molten paraffin wax at 60℃ for two hours each in two changes. The embedding of the tissues was done in molten paraffin wax and was sectioned afterwards. Haematoxylin and eosine method was used in staining.

RESULTS AND DISCUSSION

Morphometric Analysis of Body Weight

Table 1: Comparison of mean initial, final body weight and weight change in all the groups (A, B, C & D) before and after administration of the extract.

	Group A	Group B	Group C	Group D	F-Ratio	Prob. of Sig
Initial body weight	180.10±2.50	183.20±3.10	186.50±2.80	190.40±2.40	60.140	<0.001
Final body weight	200.10±3.40	196.30±2.90	172.20±4.20	170.30±1.40	40.100	<0.001
Weight change	20.00±0.90	13.10±0.20	14.30±2.60	20.10±1.00	7.140	<0.001

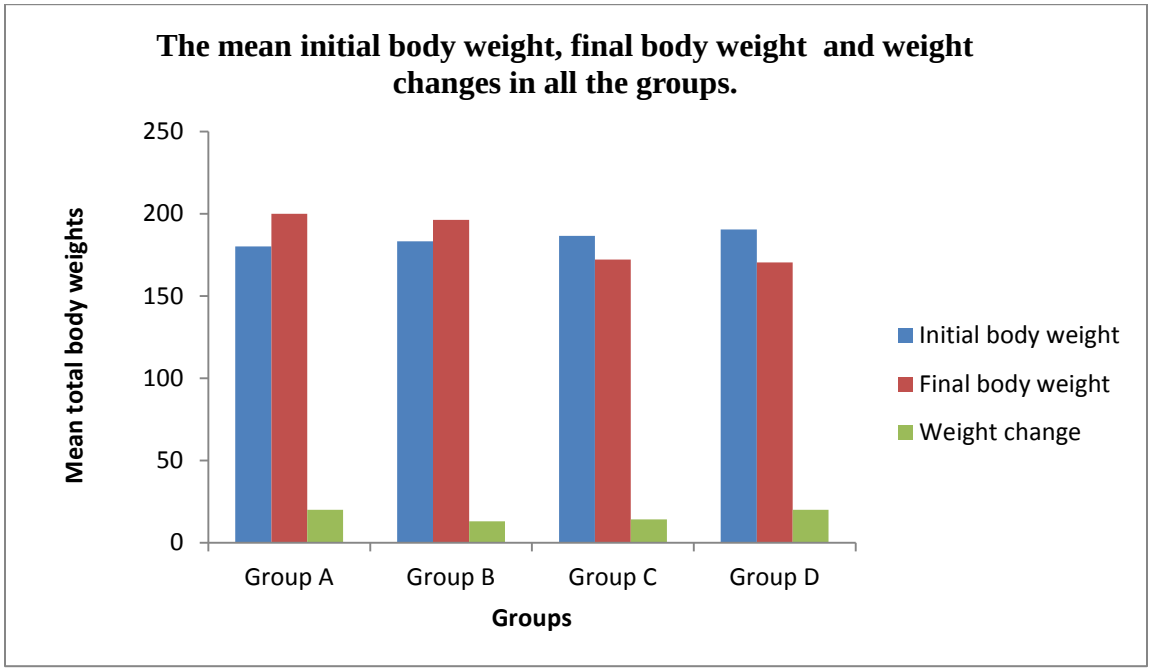

Figure 1: Bar chart representation of the mean initial body weight, final body weight and weight changes in all the groups before and after administration of the extract.

Morphometric Analysis of Testes Weight

Table 2: Comparison of mean relative testes weight of all the groups (A, B, C & D)

	Group A	Group B	Group C	Group D	F-Ratio	Prob. of Sig
Testicular weight	1.32±0.01	1.35±0.12	1.48±0.26	1.55±0.40	0.540	<0.001

(Mean ± SEM given for each measurement)

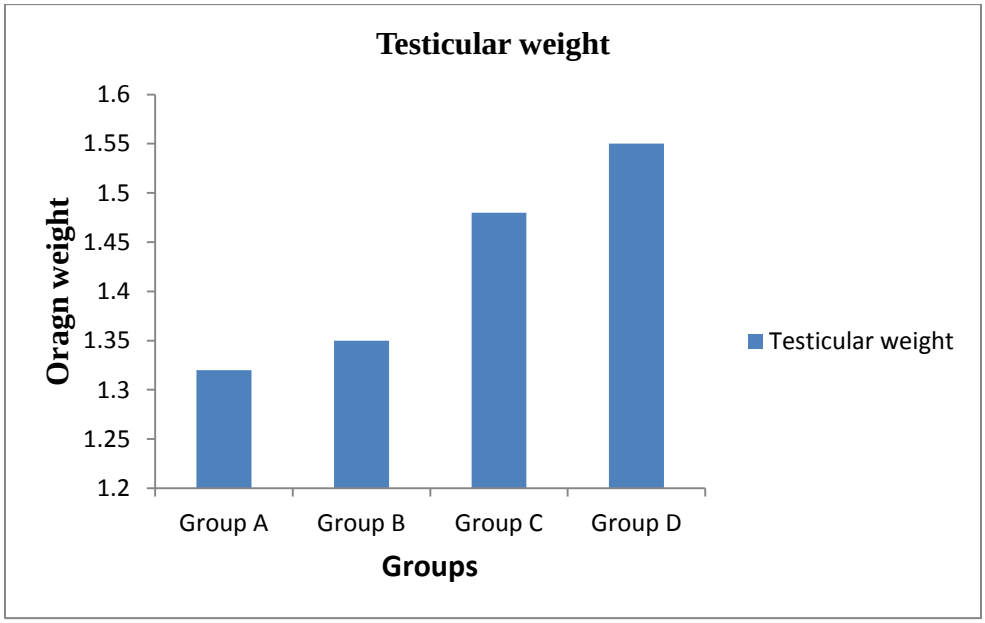

Figure 2: Bar chart showing the organ weights of all the groups

Histological Findings

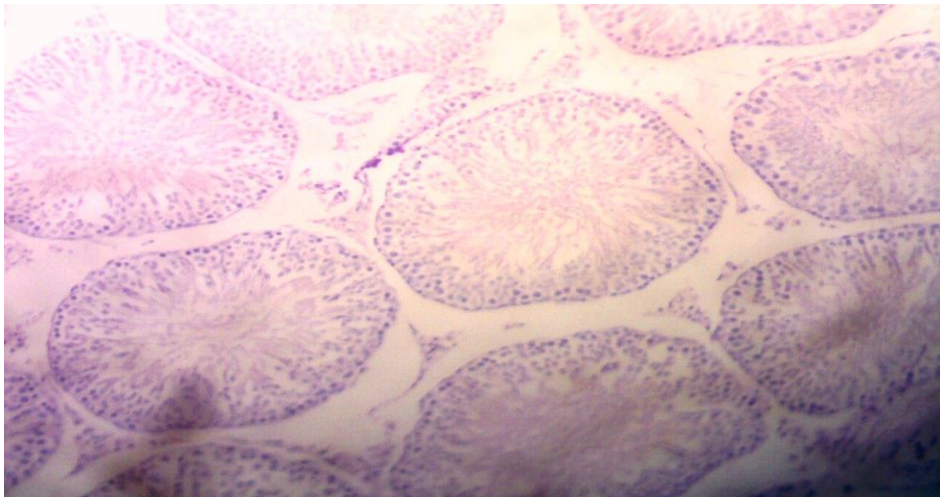

Figure 3: Micrograph 1 (control) showing normal histological structure.

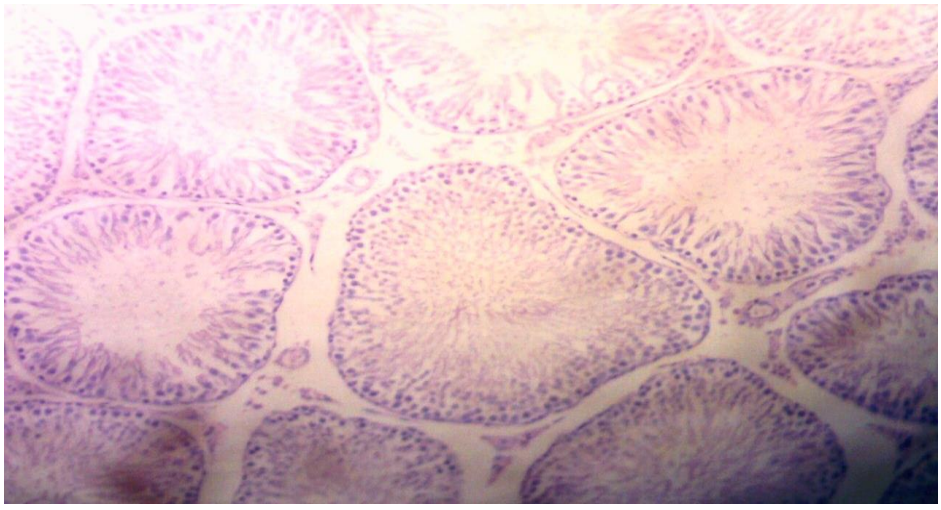

Figure 4: Micrograph 2 (treated with 0.2ml of *Annona muricata* ethanolic leaf extract) showing mild alteration of the spermatid cells.

The wound healing activity of alcoholic extract of stem and bark of *Annona muricata* was found to show marked reduction in area of the wound which was tested in the albino rats which proves their possible use in the healing of wounds [7]. Leaf extract of *Annona muricata* is used in the treatment of various bacterial infectious diseases such as pneumonia, diarrhea, urinary tract infection and even some skin disease. *Annona muricata* extract contains a wide spectrum of activity against a group of bacterial that are responsible for the most common bacterial disease. Thus, the plant possesses an abundant of the antibacterial compounds [8]. *Annona muricata* extract was screened against Herpes simplex virus-1 (HSV-1) and clinical isolate in order to check whether they inhibit the cytopathic effect of HSV-1 on vero cells which is the indicative of anti-HSV-1 Potential. The minimum inhibitory concentration of ethanolic extract of *Annona muricata*

was found to be 1mg/ml which shows that the *Annona muricata* could be used as the potential antiherptic drugs [9]. In the present study, the final body weight for groups C and D decreased significantly ($P < 0.001$) when compared with the control. The final body weight of group B animals increased significantly with the control. The comparison of mean relative organ weight of groups C and D increased significantly ($P < 0.001$) when compared with the control, while group B mean relative organ weight was statistically similar with the control group A. The histological findings indicated histological lesions in groups C and D treated with high doses of *Annona muricata* ethanolic leaf extract.

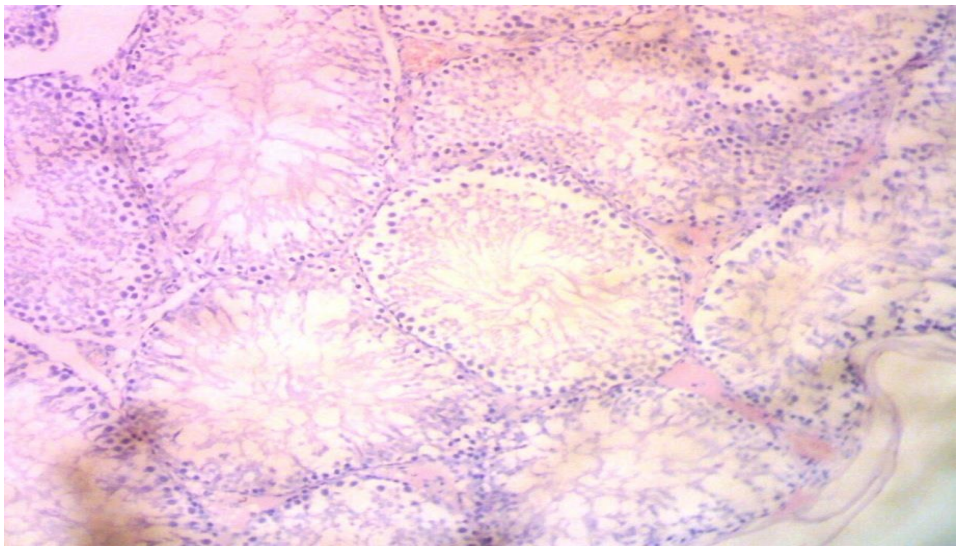

Figure 5: Micrograph 3 (treated with 0.4ml of *Annona muricata* ethanolic leaf extract) showing distortion of the spermatid cells with nercotic changes in seminiferous tubules.

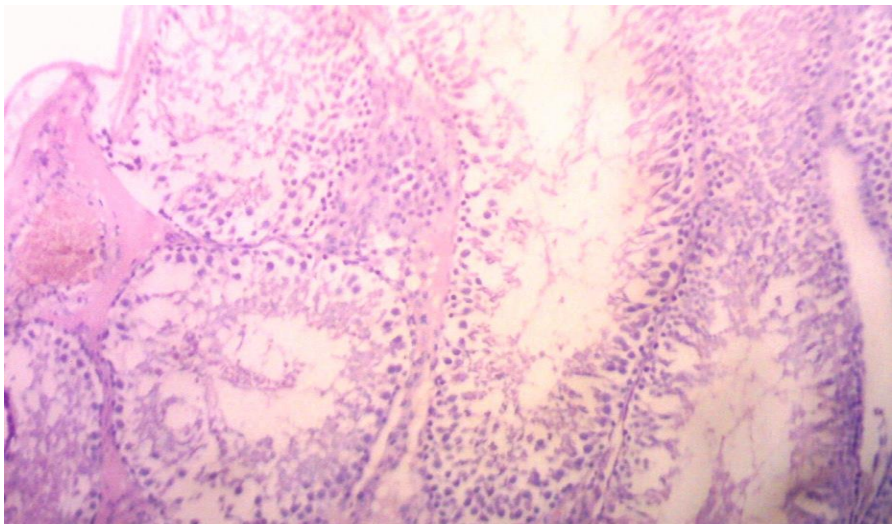

Figure 6: Micrograph 4 (treated with 0.6ml of *Annona muricata* ethanolic leaf extract) showing nercotic changes in the intestinal tissues, loss of spermatids and multinucleated giant cells.

CONCLUSION

From the present study, we inferred that *Annona muricata* leaf extract administered in high doses could be unhealthy to the testicular cells.

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