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Research Article

The histological effects of *Annona Muricata* (Soursop) on the Adrenal Gland of Adult Wistar Rats

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Abstract

This study was undertaken to evaluate the histological effects of ethanolic extract of *Annona muricata* on the adrenal gland of adult wistar rats. Twenty wistar rats weighing between 180 – 210g were used for the study. They were divided into four groups (A, B, C & D) of five animals each. Group A animals served as the control and received 0.3 ml of distilled water; the experimental groups B, C & D were orally administered 0.2 ml, 0.4 ml and 0.6 ml of ethanolic extract of *Annona muricata* respectively for twenty eight days. Immediately after the last administration, the animals were weighed, sacrificed under the influence of chloroform vapour inhalation method and dissected. Adrenal gland tissues 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 mean relative organ weight result revealed significant increase ($P<0.001$) in groups C and D while B organ weight is statistically similar with the control group A. Histopathological findings showed distortion of adrenal gland tissues in groups C and D while group B was not distorted compared with the control. From these findings, *Annona muricata* consumed in high doses may distort cytoarchetecture of adrenal gland.

Keywords: Adrenal gland, *Annona muricata*, Body weight, Organ weight, Cytoarchetecture

1. Introduction

The use of herbs to treat diseases is almost universal among non-industrialized societies and is often more affordable than purchasing expensive modern pharmaceuticals. The world Health Organization estimates that 80 percent of the population of some Asian and African countries presently uses herbal medicine for some aspect of primary health care. Studies in the United State and Europe have shown that their use is less common in clinical settings but has become increasingly more in recent years as scientific evidence about the effectiveness of herbal medicine has become more widely available. The annual global export value of pharmaceutical plants in 2011 accounted for over US $2.2 billion[1-3].

In India, Ayurveda medicine has used many herbs such as turmeric possibly as early as 1900 BC [4,5].
Earliest sanskrit writings such as the Rig Veda, and Atharva Veda are some of the earliest available documents detailing the medical knowledge that formed the basis of the Ayurdeda system [6].

The use of and search for drugs and dietary supplents derived from plants have accelerated in recent years. Pharmacologists, microbiologists, botanists and natural products chemists are combing the earth for phytochemicals and leads that could be developed for treatment of various diseases. In fact, according to the World Health Organization, approximately 25% of modern drugs used in the United States have been derived from plants[7].

A number of herbs are thought to be likely to cause adverse effects[8]. Furthermore, adulteration, inappropriate formulation or lack of understanding of plant and drug interactions have led to adverse reactions that are sometimes life threatening or lethal[9].
Annona muricata is a flowering evergreen tree native to Mexico, South America and produced in some parts of Africa especially in Eastern Nigeria and in Southeast Asia[10].

Annona Muricata is widely promoted as an alternative cancer treatment. There is however no medical evidence that it is effective[11]. The Federal Trade commission in the United State determined that there was no credible scientific evidence that extract of *Annona muricata* sold by Bioque Technology can prevent, cure or treat cancer of any kind[12].

With the unscientific proofs on medicinal properties of *Annona muricata*, this scenario call for research on the histological effects of *Annona muricata* ethanolic leaf extract on the adrenal gland using adult wistar rats.

2. Materials and Methods

2.1 Breeding of Animals

Twenty wistar rats were purchased from the animal house of Faculty of Pharmacy, Nnamdi Azikiwe University, Agulu, Anambra State. 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.

2.2 Drug Preparation

Annona Muricata leaves were plugged from Okofia, Nnewi, Anambra State and 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.

2.3 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 the last administration, the animals were weighed and there weights recorded. The animals were then anaesthetized under the influence of chloroform vapor and dissected. Adrenal gland tissues were removed and weighed. The tissues were then fixed in 10% formaline for four hours for histological studies.

2.4 Tissue Processing

For easy study of sections under microscope, the tissues passed through several processes of fixation, dehydration, clearing, infiltration, embedding, sectioning and stained using haematoxylene and eosine method.

3. Results

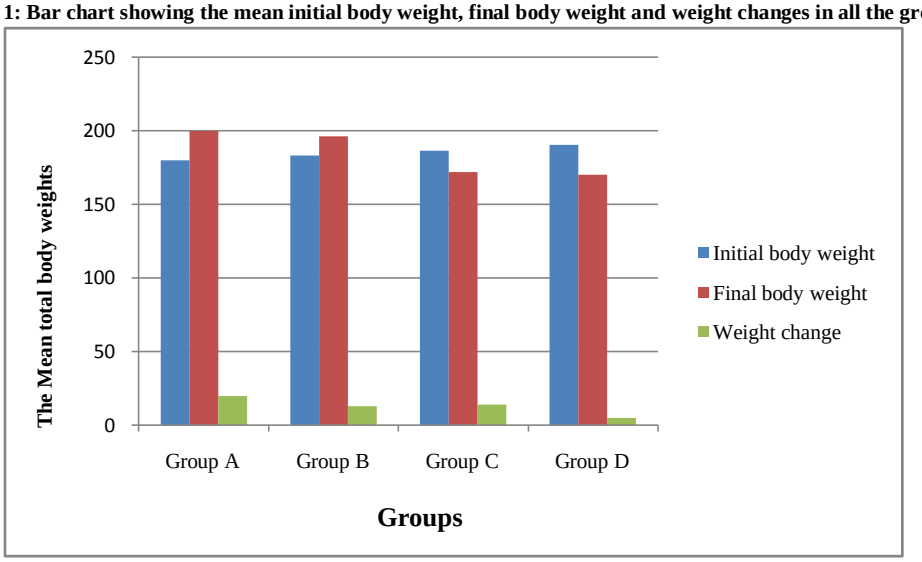
3.1 Morphometric Analysis of Body Weight

Table 1: Comparison of Mean initial and final body and weight change in all the groups (A, B, C & D)

	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

(Mean ± SEM given for each measurement)

Figure 1: Bar chart showing the mean initial body weight, final body weight and weight changes in all the groups.



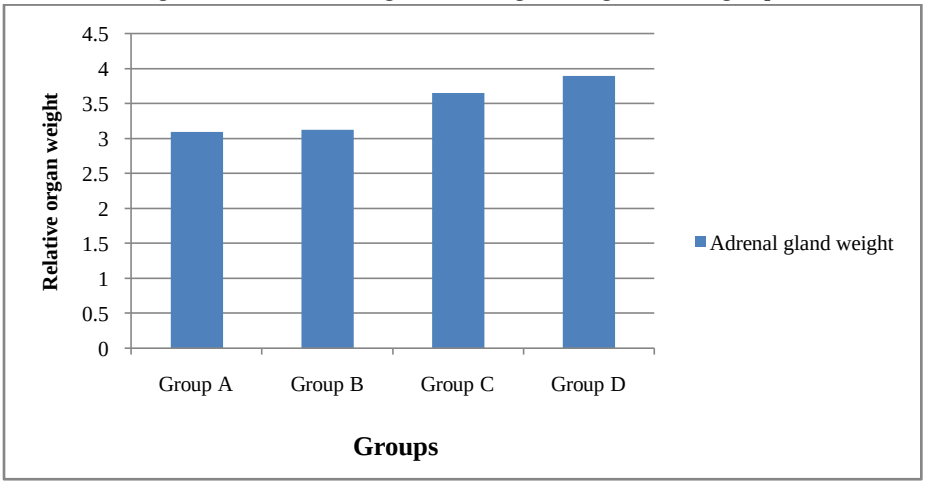
3.2 Morphometric Analysis of Organ (Adrenal gland) Weight

Table 2: Comparison of Mean relative Organ (Adrenal gland) weight of all the groups (A, B, C & D)

	Group A	Group B	Group C	Group D	F-Ratio	Prob. of Sig
Adrenal Gland Wt.	3.10±0.400	3.13±0.200	3.65±0.120	3.90±0.210	3.40±0.320	<0.001

(Mean ± SEM given for each measurement)

Figure 2: Bar chart showing the Adrenal gland weight of all the groups



3.3 Histopathological findings

Figure 4: Micrograph (control) showing normal histoarchetecture of the adrenal gland.

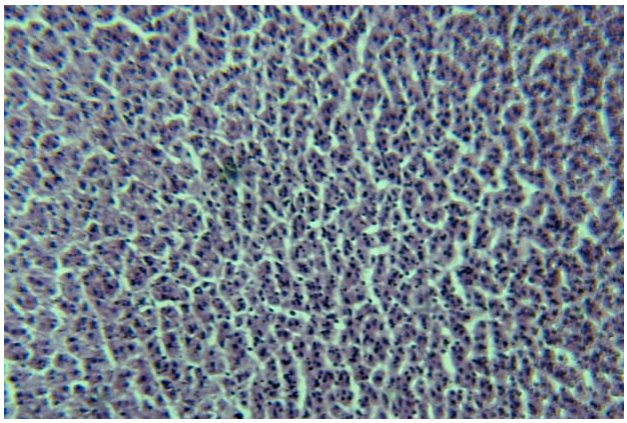


Figure 5: Micrograph 2 (Group B treated with 0.2ml of ethanolic leaf extract of *Annona muricata*) showing normal histology of the adrenal gland.

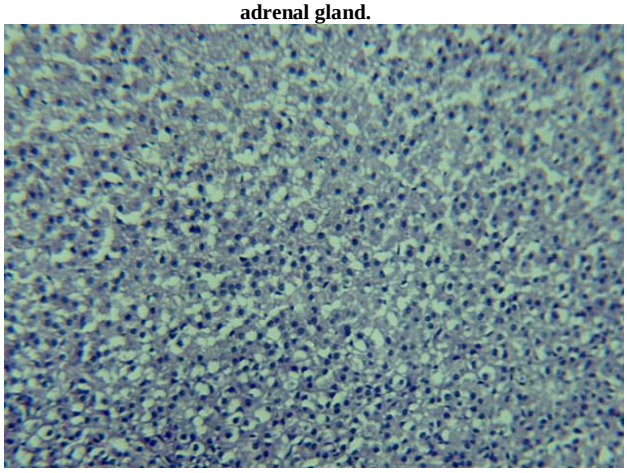


Figure 6: Micrograph 3 (Group C treated with 0.4ml of ethanolic leaf extract of *Annona muricata*) showing abnormalities in zona glomerulosa and zona reticularis

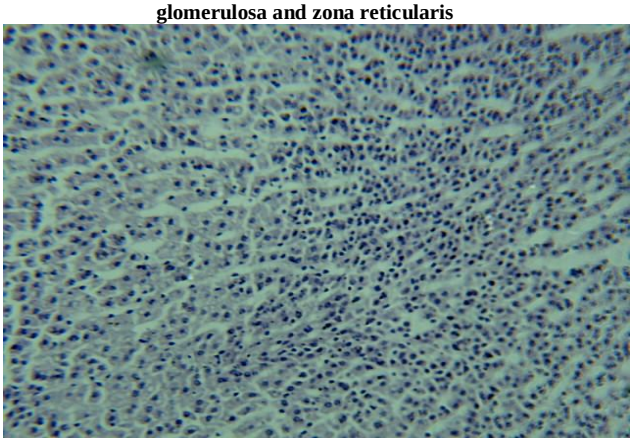
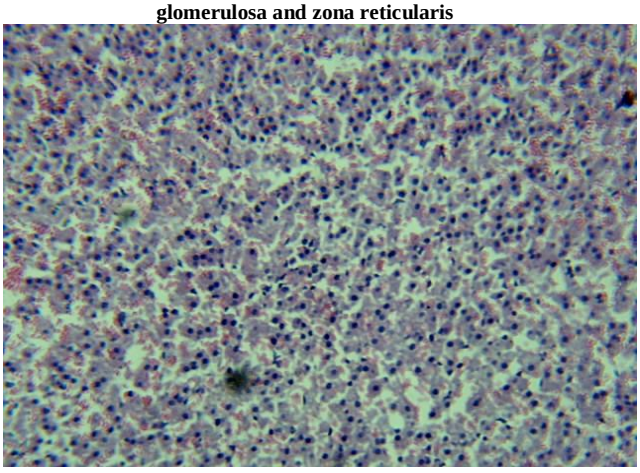


Figure 7: Micrograph 4 (Group D treated with 0.6ml of ethanolic leaf extract of *Annona muricata*) showing distortion in zona glomerulosa and zona reticularis



4. Discussion

A number of herbs are thought to be likely to cause adverse effects [8]. Furthermore, adulteration, inappropriate formulation or lack of understanding of plant and drug interactions has led to adverse reactions that are sometimes life threatening [9].

Proper double-blind clinical trials are needed to determine the safety and efficacy of each plant before they can be recommended for medical use [13].

Although many consumer's believes that herbal medicines are safe because they are natural, herbal medicines and synthetic drugs may interact, causing toxicity to the patient. Herbal remedies can also be dangerously contaminated and herbal medicines without established efficacy may unknowingly be used to replace medicines that do not have corroborated efficacy [14].

Many herbs have shown positive result in-vitro, animals model or small-scale clinical tests while studies on some herbal treatments have found negative result[15,16].

The memorial Sloan-Kettering Cancer Center Cautions, alkaloids extracted from graviola may cause neuronal dysfunction and degeneration leading to symptoms of parkinsons disease[17].

The compound annonacin which is contained in the seeds of *Annona Muricata* is a neurotoxin associated with neurodegenerative disease and research has suggested a connection between consumption of *Annona Muricata* and atypical forms of Parkinson's diseases due to high consumption of annonacin[18,19].

In the present study, the mean final body weight result showed that groups C and D treated with high doses of *Annona Muricata* ethanolic extract had decreased body weight while group B, treated with low dose increased significantly ($P<0.001$) with the control group A.

The mean relative organ weight result showed that groups C and D had significant increase ($P<0.001$) compared with the control while group B was statistically similar with the control group A.

Also, the histopathological result revealed distortion of the adrenal gland tissues in groups C and D while group B showed no abnormality.

The present study, agrees with the studies by the Memorial Sloan-Kettering Cancer Center of side effects of high consumption of annonacin which is an alkaloid from *Annona Muricata*.

5. Conclusion

The present study suggests that high consumption of *Annona Muricata* extract may distort the histology of adrenal gland tissues.

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