

HEALING POTENTIALS OF ANNONA MURICATA LEAVES EXTRACT AGAINST HYOSCINE INDUCED OXIDATIVE STRESSED WISTA RATS AND ITS EFFECT ON LIVER ENZYMES

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ABSTRACT

Annona muricata is a plant that has several medicinal properties; however, there are dearths of scientific information on the healing activities. This study therefore focused on the healing effects of the methanolic leaves extract of *A. muricata* against Hyoscine induced oxidative stress and liver morphology in Wister rats. Twenty five rats divided into five research subgroups were used for this study. Group A was the control group Group B received Hyoscine only while Groups C,D and E received 1ml/kg of Hyoscine and 0.15, 0.30 and 0.65ml/kg of MAM respectively. The result revealed a significant decrease of SOD in Groups B when compared to the control group, while groups C-E had a significant increase when compared to group A. Also there was a significant increase in the MDA in Group B when compared to A while groups C-E had a significant decrease when compared to group B. Lastly there was a significant decrease in GSH in group B when compared to control group while a significant decrease in group C-E when compared to group B. The results, therefore, sour sop is antioxidants plants that can be used to treat oxidative stress in the female Wister rats.

KEYWORDS: *Methanolic Annona muricata, Hyoscine, Oxidative Stress.*

INTRODUCTION

Oxidative stress is the destruction by free radical molecules in the living system. This results in an imbalance between the production of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage.^[1] Disturbances in the normal redox state of tissues can cause toxic effects through the production of peroxide & free radicals that damage components of cells including proteins, lipids & DNA

Block, (2003) Sources of oxidative stress include exogenous factors such as cigarette smoke, prescription drugs and toxic chemical such as pesticides and endogenous factors such as the oxidative burst from activated macrophages.^[2] Hence oxidative stress results when oxidative force exceeds the antioxidant system due to loss of the imbalance between them. Among the antioxidant include vitamin C (ascorbate) vitamin E (tocopherol) selenium, lycopene, enzymes, reduced glutathione and carotenoids.^[1] The global concept of

“Oxidative Stress” is defined as “an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage.^[10] It has developed from its initial formulation in 1985 to incorporate new knowledge on the role of redox signaling.^[9]

A. muricata is an evergreen, terrestrial, erect tree of about 5–8 meters in height and detailed an open, roundish canopy with large, glossy, dark green leaves. The edible fruits of the tree are large heart-shaped, and green in color, and the diameter varies between 15 and 20 cm.^[3] The tree and fruits of *Annona muricata* bloom yearly based on the altitude with more defined seasons.^[17]

Each fruit of *A. muricata* does contain approximately 55–170 black seeds when fresh, and they turn light brown when dry.^[17] The sour sop fruit is an edible collective ovoid berry, dark green in color, with dimensions of 15–35 cm×10–15 cm.^[19] Its average weight is 4 kg and ranges between 0.9 and 10 kg. It has a prickly nature with a sour-sweet taste, and when unripe, its color is dark green and light-green when ripe. The fruit of Sour sop constitute several fruitlets fusion, with a fibrous juicy white segment with a sour-sweet acid taste, and its aroma is made up of 80%esters.^[4,5]

MATERIALS AND METHOD

LOCATIONS

This study was carried out in the animal house, department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Nnewi campus, Nnamdi Azikiwe University.

ETHICAL APPROVAL

Ethical approval was obtained from the faculty of basic medical science, college of health sciences Nnewi campus Nnamdi Azikiwe University.

PREPARATION OF THE SOURSOP LEAF EXTRACT

The *Annona muricata* leaves were harvested from a local farm in Okofia, Nnewi Local Government Area, Anambra State. The *Annona muricata* leaves were dried in an ambient temperature. The dried *Annona muricata* leaves were milled into a coarse form using a local grinder. Two hundred and fifty-(250) gram of the

Annona muricata leaf was macerated in 1000mls of 95% absolute ethanol (JHD Chemicals, Guangdong, china) for 48-hours. It was then filtered using a clean porcelain cloth and further filtered using Filter paper (Whatman Qualitative Filter paper, No. 1, Sigma Aldrich; WHA1001042, USA). The filtrate obtained was concentrated using a rotatory evaporator (Digital TT-52; Techmel & Techmel, USA), which was further dried using a laboratory oven (DGH-9023A, PEC MEDICAL, USA) at 45oC into a gel-like form. The extract was preserved in a refrigerator (Haier Thermo cool) for further usage.

EXPERIMENTAL ANIMALS

Experimental rats were purchased from the animal house in the department of Veterinary Medicine in the University of Nigeria (UNN) Nsukka. The animals weighed between 160-300g, and were randomly grouped and housed in steel cages and kept at room temperature. The rats had no history of drug consumption, that is; they had not been used for any investigation. The rats were put on standard rat's pelleted (feed) and pure drinking water and allowed to get acclimatized for 21 days before the start of the experiment. The study was done in accordance with the guidelines for animal use of the Faculty of Basic Medical Sciences, Nnamdi Azikiwe University.

EXPERIMENTAL PROCEDURES

INDUCTION OF AMNESIA USING HYOSCINE BUTYL BROMIDE (SCOPOLAMINE)

Hyoscine butyl bromide was obtained from Christ the king pharmacy Nnewi. It was dissolved in ethanol for about 10mins and was administered to the animals intraperitoneally. It interferes with memory and cognitive function in human beings and experimental animals by blocking muscarinic receptors along the impairment of long term potentiation and the central cholinergic system.^[23]

GROUPING OF ANIMALS

Twenty-five (25) female Wister rats were divided into five (5) different research group containing 5 animals in each group. Experimental animals grouping and treatment procedures. Hyoscine was administered once daily for 14 days which *Annona muricata* extract was given for 14 days.

RESULTS

TABLE 1.

Groups	Treatment	Duration
Group I (control).	Rats pellets and water (1.5ml/kg).	14 days
Group II (Hyoscine butyl bromide)	Rat pellets and water + Hyoscine injection. Administered Induced amnesia (Intraperitoneally).	Once daily
Group III (Hyoscine butyl bromide +.	Rat pellets and water + Hyoscine injection (1ml/kg) + <i>Annona Muricata</i> (0.15ml/kg). Low dose	Administered with sour sop and Hyoscine
Group IV	Rats pellets and water + Hyoscine	Administered with

(Hyoscine butyl bromide + injection (1ml/kg) + Annomia M (0.30ml/kg) medium dose	sour sop and Hyoscine
Group V (Hyoscine butyl bromide + Rats pellets and water + Hyoscine injection (1ml/kg) + Annomia M (0.60ml/kg) High dose	Administered with sour sop and Hyoscine

Table 2: Effect of methanolic extract of *Annona muricata* on SOD level following hyoscine butyl bromide induced dementia.

Groups	SOD (U/ml) MEAN_SEM
Group A (control)	11.76_0.52
Group B (1 mg/kg of HBB)	9.51_0.54*
Group C (1 mg/kg of HBB + 100 mg/kg of MAM)	13.17_0.29 a
Group D (1 mg/kg of HBB + 200 mg/kg of MAM)	13.42_0.28 a
Group E (1 mg/kg of HBB + 400 mg/kg of MAM)	16.53_0.57 a
F-ratio	30.646

Data was analyzed using ANOVA followed by post Hoc LSD comparison and values were considered significant at $p < 0.05$, *: significant, #: not significant compared to group A. a: significant, b: not significant compared to group B. HBB: hyoscine butyl bromide, MAM: methanolic extract of *Annona muricata*.

Table 2 result revealed a significant decrease in the SOD in Group B when compared to A ($p = 0.006$), when group C, D, and E ($p = 0.000$, $p = 0.000$, $p = 0.001$) was compared to group B, there was a significant increase.

Table 3: Effect of methanolic extract of *Annona muricata* on MDA level following hyoscine butyl bromide induced dementia.

Groups	MDA (nmol/ml) MEAN_SEM
Group A (control)	0.52_0.04
Group B (1mg/kg of HBB)	0.74_0.04*
Group C (1 mg/kg of HBB + 100 mg/kg of MAM)	0.39_0.00 a
Group D (1 mg/kg of HBB + 200 mg/kg of MAM)	0.47_0.05 a
Group E (1 mg/kg of HBB + 400 mg/kg of MAM)	0.33_0.03 a
F-ratio	14.475

Data was analyzed using ANOVA followed by post Hoc LSD comparison and values were considered Significant at $p < 0.05$, *: significant, #: not significant compared to group A. a: significant, b: not significant Compared to group B. HBB: hyoscine butyl bromide, MAM: methanolic extract of *Annona muricata*.

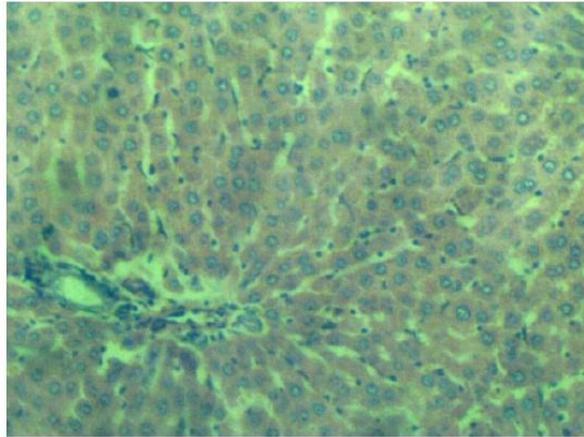
Table 3 result revealed a significant increase in the MDA in Group B when compared to A ($p = 0.004$), when group C, D, and E ($p = 0.000$, $p = 0.001$, $p = 0.001$) was compared to group B, there was a significant increase.

Table 4: Effect of methanolic extract of *Annona muricata* on GSH level following hyoscine butyl bromide induced dementia.

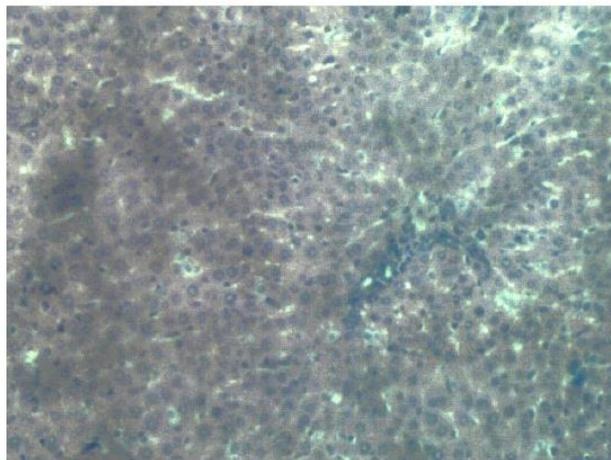
GROUPS	GSH (u/ml) MEAN_SEM
Group A (control)	2.59_0.23
Group B (1mg/kg of HBB)	1.65_0.11*
Group C (1 mg/kg of HBB + 100 mg/kg of MAM)	3.06_0.05 a
Group D (1 mg/kg of HBB + 200 mg/kg of MAM)	2.55_0.31 a
Group E (1 mg/kg of HBB + 400 mg/kg of MAM)	5.04_0.19 a
F-ratio	38.864

Data was analyzed using ANOVA followed by post Hoc LSD comparison and values were considered Significant at $p < 0.05$, *: significant, #: not significant compared to group A. a: significant, b: not significant Compared to group B. HBB: hyoscine butyl bromide, MAM: methanolic extract of *Annona muricata*

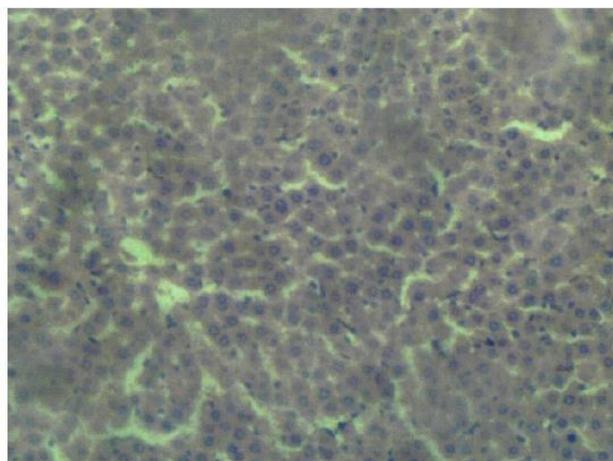
Table 4 result revealed a significant decrease in the GSH in Group B when compared to A ($p = 0.008$), when group C, D, and E ($p = 0.001$, $p = 0.000$, $p = 0.000$) was compared to group B, there was a significant increase.

HISTOLOGY FINDINGS OF THE LIVER

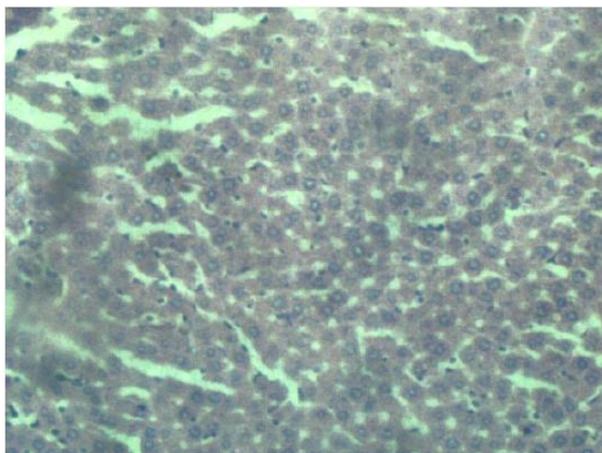
Control: Photomicrograph of liver tissue shows morphology consistent with liver histology. The hepatocytes (arrow) are normal with no obvious sign of injury (H&E x 400).



A: Photomicrograph of liver tissue shows morphology consistent with liver histology but with mild inflammatory background. The hepatocytes (arrow) are normal with no obvious sign of injury (H&E x 400).



B: Photomicrograph of liver tissue shows morphology consistent with liver histology. The hepatocytes (arrow) are normal with no obvious sign of injury (H&E x 400).



C: Photomicrograph of liver tissue shows morphology consistent with liver histology. The hepatocytes (arrow) are normal with no obvious sign of injury (H&E x 400).

DISCUSSION AND CONCLUSION

The study finding shows that an administration of the drug Hyoscine butyl Bromide (HBB), there was a decrease in physical activity and food intake in the female Wister rats.^[24] HBB can affect neurotransmitters related to food intake. It has been found that HBB can potentially decrease the amounts of food consumed during a meal without affecting the general incentive properties of food. HBB acts by antagonizing muscarinic receptors which can increase extra cellular levels of acts and induce sanity following the administration of ethanolic *Annona muricata* extract there was a significant weight gain in the treated group of animal induced with HBB.^[23] *Annona muricata* has compounds that interact with serotonin neurotransmitters responsible for mood regulation.^[2] This interaction can improve mood, stress and anxiety which may indirectly increase appetite. The compound inhibits the neurotransmitters that prevent food intake and reduced metabolism.^[5] The compounds like flavonoids, in sour sop leaves can potentially help regulate serotonin leaves in the brain.^[15]

CONCLUSION

In the female Wister rat there was increased oxidative stress which was indicated in the MDA level and decreased in SOD & GSH. However, treatment with *Annona Muricata* improved antioxidants activity which resulted in decreased MDA activity and increased antioxidants activity of SOD & GSH. Therefore sour sop is antioxidants plants that can be used to treat oxidative stress in the female Wister rats.

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