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Effect of Aqueous Extract of *Gnetum Africanum* (Afang) Leave on the Liver and Liver Enzymes of Adult Wistar Rats

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ABSTRACT
Afang (*Gnetum africanum*) leaves are largely consumed as vegetable in Africa. The present study explore the hepatotoxicity of aqueous extract of *Gnetum africanum* on the liver and liver enzymes of adult Wistar rats. Fifteen (15) adult wistar rats weighing between 120 -150g were assigned into 3 groups of 5 rats each. Group 1 was the control group administered with normal rat feed and 0.3ml of normal saline, group 2 was the low dose group treated orally with 0.3ml of *gnetum africanum* and rat feed and group 3 the high dose group was fed with 0.5ml of *gnetum africanum*, normal rat feed with water. The treatment lasted for (14days). After the end of the administration, the weight were taking before sacrificed the next day under chloroform anesthesia. Blood was collected from the left ventricle through cardiac puncture and liver excised and fixed in 10% formal saline, then processed for rapid routine paraffin embedding. Tissues were stained with routine Haematoxylin and Eosin stain, Observed under a digital light microscope and micrograph were taken. Result of the study revealed a dose dependent significant ($p<0.001$) increased of liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT, alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT) in both low dose and high dose in the administered group when compared to the normal control. Histological observations reveal dose dependent distortion in the liver architecture when compared to the normal control group, hence this study is suggestive that administration of *Gnetum africanum* at various dosages may have adverse effect on the liver.

Keywords: *Afang leaf (Gnetum africanum), liver, liver enzymes, aqueous extract, wistar rat.*

INTRODUCTION
Gnetum africanum is one of the numerous plant food widely consumed in various forms in Africa especially Southern Nigeria due to its palatability and taste [1][2]. *Gnetum africanum*, a low genus belonging to the family Gentaceal is a dioecious that grows on trees in humid forests of Africa [3].

The leaves of *Gnetum africanum* are elliptic and lined with reticulate veins, similar to those of dicotyledonous angiosperm [4]. The leaves of *Gnetum africanum* are used in the treatment of enlarged spleen, sore throat and as a cathartic [5]. It is also used to reduce nausea and neutralize some poisons. It can be externally applied to massage boils and warts and is used to reduce pain of childbirth. The leaves of *Gnetum africanum* are commonly eaten rawand used in preparing soups and stew [5] . The medicinal and culinary applications of the leaves of *Gnetum africanum* underscores its importance as major dietary supplement with potential biological effects.

Dietary supplementation with vegetables and other nutrients is employed to study their effects on the physiological and biological characteristics of experimental animals [6] . Studies have showed varying physiological effects associated with the consumption of diets supplemented with leaves of tropical vegetables such as ocimumgratisimum, gongronemalatifolium and *Gnetum africanum* [6][7]. Some of these plants that exhibit medical properties have been known to help in stabilizing different internal organs in animals, while others have side effect on the organs due to varying amount of toxic matter present in such plants [8]. *Gnetum africanumis* a dark green leafy vegetable with a slight bitter taste commonly found in a certain region of Africa, Asia and South America.

In Nigeria, it is commonly used to prepare delicacies such as Okasi and Nsala soup. The term medicinal plants include a various type of plant used in herbalism and some of these plant have a medicinal activities [9] . In many developing countries, traditional medicine is still the mainstay of healthcare, and most of drugs and cure come from natural sources such as plants [10] . The leaves of *Gnetum africanum* can be consumed in its raw state without further processing, and it is used in preparation of soup and salads because of the believe that its leaves are important sources of valuable nutrient [11] .

Medicinal plant contains various pharmacologically active compounds which have useful therapeutic application [12] . Toxic effects due to herbal medicine may manifest in a member of organs such as kidney, liver, stomach, nervous system and blood. The liver is a vital organ for maintaining of metabolic function and

detoxification from exogenous, drugs and viral infections [13].

The liver; the largest gland in the body weighs approximately 1500gm and accounts for approximately one-fortieth of adult body weight. It lies in the right and left upper quadrants, interior to the diapharagm, which separate it from the pleura, lungs, pericardium and the heart. The functions of the liver are vast ranging from metabolism to immune response. Glucose present in the system is converted into glycogen and stored, or into lactate and released into the systemic circulation [14].

MATERIALS AND METHOD

Experimental animals : Fifteen (15) Male albino rats were purchased from the animals' house of the department of Human Anatomy, Cross River University of Technology ,okuku campus. They were acclimatized for weeks prior to the commencement of the experiment, kept at a room temperature, and feed using broiler started. They were weighed prior to the experiment.

Extract preparation: Fresh mature leaves of Afang leaves (*Gnetum africanum*) where purchased from a local market in Ekori in Central Cross River State of Nigeria. The leaves were kept in room temperature to dry. The leaves were crushed first using local grounding stone then blended using an electrical blender. The grinded leaves were kept in an airtight plastic container from where they were used for extraction. Grinded sample of *Gnetum africanum* were weighed into a plastic rubber filled with 250ml of distilled water. It was then filtered using a plain white handkerchief and later filter paper. The filtrate was concentrated under reduced temperature (370c) using water bath for three days, after which the concentrated extract was weighed and ready for use.

Experimental protocol : The fifteen (15) animals were allotted to three groups consisting of five rats each. Animals in group 1 served as the control group, fed with normal rat chew and distilled water, while groups 2 and 3 served as the experimental groups treated with *Gnetum africanum* leaf extract, orally for 21 days. Group 2 (low dose group) animals were treated with 0.3ml/kgBw of *Gnetum africanum* leaf extract, while group 3 (high dose group) animals were treated with 0.5ml/kgBw of the extract.

Termination of experiment: At the end of administration, animals in all the groups were sacrificed under chloroform anesthesia. Blood was collected through cardiac puncture from the left ventricle into labeled specimen bottles. Serum was separated by centrifugation for 5 minute at 100 rpm and used for assay to determine the serum liver enzymes. The liver was harvested and processed through paraffin sections for Heamatoxylin and Eosin (H&E).

Histological procedure: The liver were removed and preserved in labeled bottles containing 10% buffered formalin. These were allowed to stand for 72 hours to achieve good tissue penetration and effective fixation. After this, they were placed in ascending grades of ethanol for dehydration. First they were treated with two changes of 70% ethanol each lasting for one hour followed by 95% ethanol and then absolute alcohol for the same duration. Following dehydration, the tissues were cleared in three changes of xylene each lasting for 15 minutes. Impregnation in molten paraffin wax at 580c was carried out overnight and the following morning, the tissues were imbedded in wax to form blocks. The tissue blocks were trimmed and sectioned at 5µ thickness using a rotary microtome. They were air-dried and stained using the haematoxylin and eosin Harris [15] staining method. Tissue blocks were sectioned at 5µ with a rotary microtome. They were dewaxed in xylene for 20minutes per two changes. Xylene was cleared in 95% alcohol for one minute per two changes and 70% for another minute. The sections were then hydrated in running tap water until sections turned blue. They were thereafter counterstained with 1% alcohol eosin for one minute, followed by rapid dehydration through ascending grades of alcohol, cleared in xylene and mounted with DPX mutant. Stained sections viewed under a light microscope and photo micrograph.

RESULT

Serum aspartate aminotransferase (AST) concentration : The concentration of serum aspartate aminotransferase among the experimental animal, was observed to have a dose dependent significant ($p<0.05$) increase when compared to the control group. The control group recorded 8.77 ± 0.24, while the low and high dose groups had values of 15.3 ± 0.27 and 22.3 ± 0.12 respectively (Figure 1).

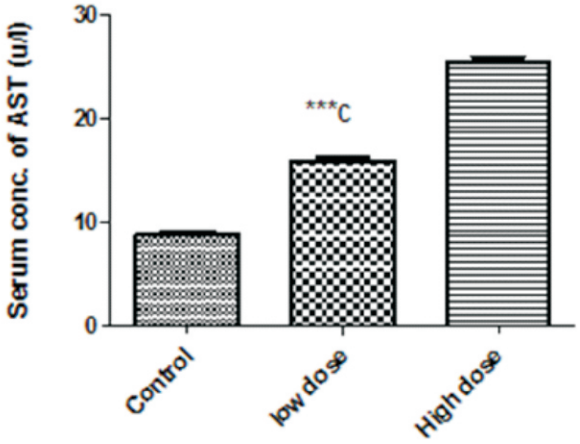

Figure 1: Effect of aqueous extract of *Gnetum africanum* on Aspartate aminotransferase in the treatment groups. Values are expressed in Mean±SEM
n = 3
*** = Significantly different from the control group at p<0.05
c = Significantly different from the high dose at p<0.05

Serum alanine aminotransferase (ALT) concentration: There was a similar dose dependent significant (p<0.05) increase in alanine aminotransferase (ALT) concentration among the entire animals. The high dose (15.0±0.30) was significantly (p<0.05) than the low dose group (7.36±0.21) and the control group (4.83±0.48) (Figure 2).

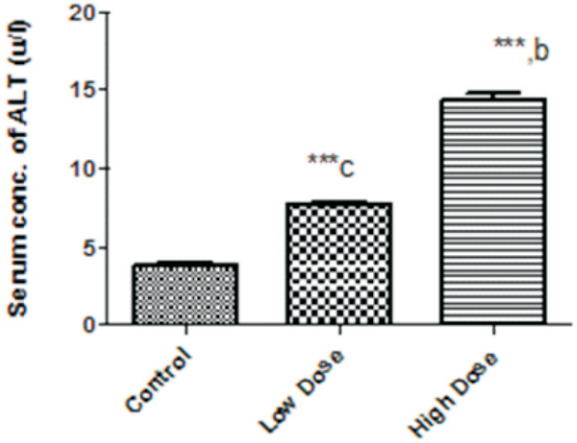

Figure 2: Effect of aqueous extract of *Gnetum africanum* on Alanine aminotransferase in the treatment groups. Values are expressed in Mean±SEM
n = 3
*** = Significantly different from the control group at p<0.05
b = Significantly different from the low dose at p<0.05
c = Significantly different from the high dose at p<0.05

Serum alkaline phosphate (ALP) concentration: The high dose group had the highest significant (p<0.05) concentration of serum alkaline phosphate (108.9±0.10), this was followed by the low dose group which had (88.37±1.09). Both were significantly (p<0.05) higher than the control group (68.63±0.55) (Figure 3).

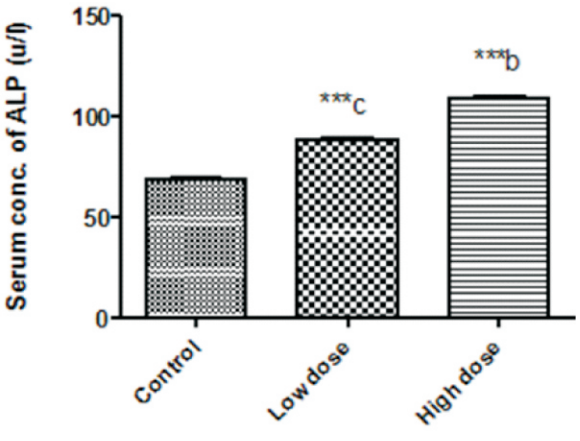

Figure 3: Effect of aqueous extract of *Gnetum africanum* on alkaline phosphate in the treatment groups. Values are expressed in Mean±SEM
n = 3
*** = Significantly different from the control group at p<0.05
b = Significantly different from the low dose at p<0.05
c = Significantly different from the high dose at p<0.05

Serum gamma glutamyl transferase (GGT) concentration: Remarkably, they occurred a dose dependent increase in the concentration of gamma glutamyl transferase in all experimental animals when compared to the control group. The control group, low dose group and high dose group recorded (6.24±0.16), (11.50±0.41) and (13.84±0.22) respectively (Figure 4).

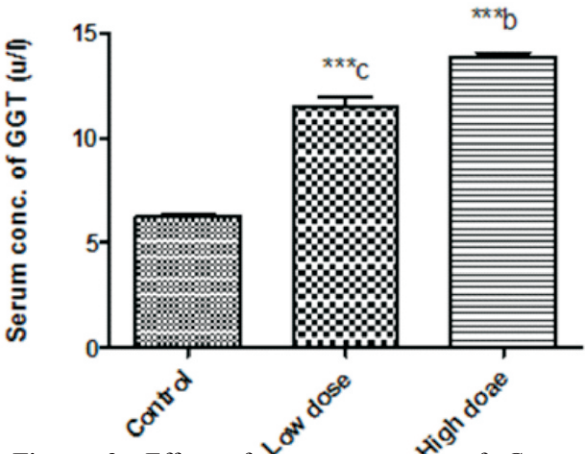

Figure 3: Effect of aqueous extract of *Gnetum africanum* on GGT in the treatment groups. Values are expressed in Mean±SEM
n = 3
*** = Significantly different from the control group at p<0.05
b = Significantly different from the low dose at p<0.05
c = Significantly different from the high dose at p<0.05

Histological observations: Animals in control group showed a normal liver architecture with many sinusoids seen alongside central vein and part of hepatic septa (Plate 1).

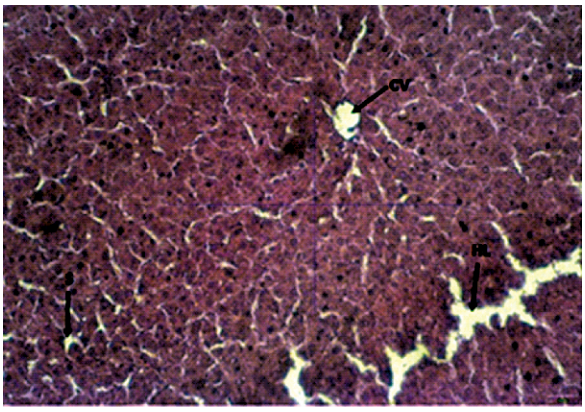

Plate 1: Photomicrograph of the control group of the liver showing many sinusoids (S) spread round the architecture of this micrograph. A central vein (CV) and part of a hepatic septa (HL) can be seen. No pathology seen. X400. H & E

Photomicrograph of animals in the low dose group showed many inflammatory cells which is an indication of a prognosis of semi-lytic necrosis and replacement by inflammatory cells. However the sinusoids appeared normal

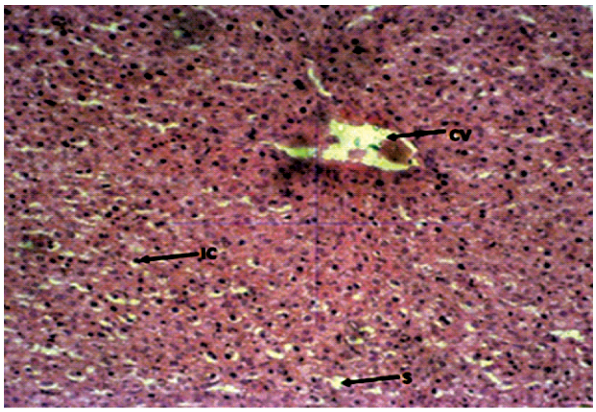

Plate 2: Photomicrograph of the Low dose group of liver showing many inflammatory cells (IC). An indication of a prognosis of semi-lytic necrosis and replacement by inflammatory cells but Sinusoids (S) appear normal. X400 H & E

The high dose group revealed enlarged central vein with enlarged sinusoids. This is a type of focal necrosis in which dead hepatocytes are identifiable as shrunken, a non-specific hepatocellular injury. There was also swelling of hepatocytes which leads to necrosis and replacement by inflammatory cells (Plate 3).

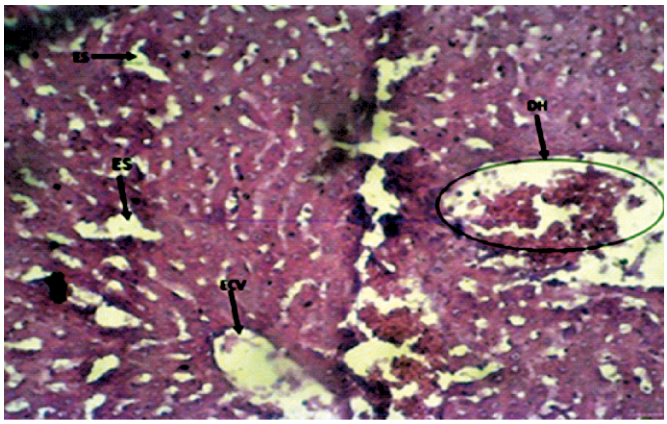

Plate 3: Photomicrograph of the High dose group showing an enlarged central vein (ECV) with enlarged sinusoids (ES). This is a type of focal necrosis in which dead hepatocytes (DH) are identifiable as shrunken, a non-specific hepatocellular injury. There is also swelling of hepatocytes which leads to necrosis and replacement by inflammatory cells.
X400 H & E

DISCUSSION

ALT and AST are important biochemical markers of hepatotoxicity in blood plasma and serum. ALT is a liver enzyme that aids in amino acid metabolism and gluconeogenesis, catalyzing the reductive transfer of an amino group from alaine to ə-ketoglutarate to yield glutamate and pyruvate, AST aids in producing proteins, catalyzing the reductive transfer of an amino group from aspartate to ə-ketoglutarate yielding oxalocatate and glutamate [16].

Result of the study showed a dose dependent significant ($p<0.05$) increase in the level of liver enzymes AST, ALT, ALP and GGT when compared to the control. Usually, increase in these enzymes is associated with hepatocellular damage and leukemia [17]. This result may be accountable for the distortion in the cyto-architecture of the liver as seen in the low and high dose

groups.

CONCLUSION

The results of this experimental work using animal models may not be used to give direct application in man but it gives an insight into the possible toxic effects of the substance. From the study, it could be deduced that the administration of aqueous leaf extract of Gnetum africanum at the doses given induces observable pathological effect on the histology of the liver and may be concluded to have adverse effect on the dosage administered.

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