Hepatic Assessment of Ethanol and Aqueous Extracts of *Gnetum Africanum* Root on Wistar Alibino Rats

Akporhono Onyedikachi Joannah

Biochemistry Department, Federal University of Technology, Owerri, Nigeria

Abstract*: The effect of ethanol and aqueous extracts of *Gnetum africanum* root on hepatic biomarkers of wistar albino rats was determined. Samples of *Gnetum africanum* root obtained from Obokwe Ngor Okpala in Imo State were milled, homogenized and extracted with ethanol and aqueous solvents respectively. The lethal dose (LD_{50}) of the crude samples were determined and found not to be toxic after acute and sub-chronic determination. 35 rats divided into seven groups of five rats each were used. The first (control) group received 1ml normal saline daily, the 2nd, 3rd and 4th groups received 250mg/kg, 500mg/kg and 1000mg/kg body weight of aqueous extract, while groups 5, 6 and 7 received 250mg/kg, 500mg/kg and 1000mg/kg body weight ethanol extract for 14 days duration of the research. The animals were sacrificed after 14 days, blood and liver organs were collected. The result revealed a significant increase in alanine transferase (ALT), aspartate transferase (AST), alkaline phosphatase (ALP) activity and bilirubin concentration with ethanol extract and little or no significant change with aqueous extract of the *Gnetum africanum* root. Histopathology examination of the liver sections of the rats treated with ethanol extract revealed some abnormal morphology characteristics such as hypercellularity and slight haemorrhagic necrosis in all the treated groups. In conclusion, the aqueous extracts of *Gnetum africanum* root at 250mg/kg and 500mg/kg body weight may possibly be safe for consumption without any significant toxic effect on the liver of the rats. It is recommended that further studies be done on additional biomarkers such as genetics, proteomics, metabolomics and MicroRNAs of hepatotoxicity in the serum; this can be measured in conjunction with ALT, with respect to specificity of liver injury

***Keywords*: *Gnetum africanum* root, liver markers, histopathology and toxicity.**

I. INTRODUCTION

Gnetum africanum is considered to be a wild vegetable, a perennial that grows approximately 10 metres long, with thick papery-like leaves growing in groups of three. The leaves may grow approximately 8 cm long and at maturity, the vine produces small flowers. The seeds of the vine resemble a fleshy fruit (drupe), sized 10-15 mm x 4-8 mm, and are red-orange in colour when fully ripe (Chindong, 2011).

Gnetum africanum leaves are used as vegetable for soup and stew, commonly called Eru soup or afang soup. The leaves may also be used as a remedy for nausea, sore throat, or as a dressing for warts. The stem of the plant may be eaten for medicinal purposes, including the reduction of pain during childbirth (Styslinger, 2000). The seeds of the vine may also be eaten cooked. The leaves of *Gnetum africanum* is a good source of protein and is rich in essential and non-essential amino acids (glutamic acid, leucine and aspartic acid, with low levels of histidine, cysteine and trace amounts of trytophan). The amino acid content of *Gnetum africanum* is comparable to recommended levels by the FAO (Ali, *et al.*, 2011). It is also rich in iodine. Fibre levels average approximately 33.4 g/100 g of dried okazi leaves (Tekwe, *et al.*, 2003); while recommended daily intake of fibre is 30 g. Okazi has been noted to have anti-inflammatory, anti-carcinogenic and antioxidant effect (CNDEP, 2009).

This study is aimed to assess the effect of ethanol and aqueous extracts of *Gnetum africanum* root on hepatic biomarker of albino rats.

II. MATERIALS AND METHODS

2.1. Sample collection and preparation

Roots of *gnetum africanum* were purchased from obokwe in ngor opkala Imo state Nigeria. The samples were washed under running tap water and rinsed with distilled water and allowed to air dried under room temperature. The dried roots were milled to fine powder and stored in a clean bottle.

2.2. Extraction

300g powdered sample was placed in a stoppered container and 1200mls of the solvents (ethanol and distilled water) was added respectively. They were allowed to stand at room temperature to 48hrs, with frequent agitation. The extract was filtered with a fine cloth and then re-filtered using whatman filter. The filtrate was poured into a clean round bottom flask at a volume that will not allow the filtrate to siphon into the extraction chamber. The temperature is adjusted in accordance with the boiling point of ethanol and water ($78.4^{O}C$ and $100^{O}C$) respectively. The solvent evaporates and drip into the extraction chamber where is collected. The active component left behind in the flask is dried in water bath to completely evaporate the remaining solvent. The condensed extracts were preserved in tightly corked labeled bottles ans stored in a refrigerator until required.

2.3. Experimental animals

Thirty-seven rats (male albino rats) were divided into seven groups of five rats each, according to range of their body weight and each group labeled. The mean body weights were calculated and different concentrations of the extract were prepared based on the mean body weight. The extracts were given orally daily for 14 days. All the rats had free access to food and water throughout the time of the experiment and

they were observed daily for symptoms of toxicity and mortality. The weight of the rats in each group was taken on weekly basis.

2.4. Biochemical analysis

Blood sanples were taken through cardiac puncture into plain bottles. The blood was centrifuged for 10mins at 3000rpm and the serum was use to examine AST, ALT, ALP and Total bilirubin. Randox kit was used for the procedure according to the manufacturer's protocol.

2.5. Histopathology examination

The liver of rats were fixed in 10% neutral buffered formalin solution for 24 hours and cleared in xylol solution followed by embedding on the paraffin block. The tissues were then cut using rotary microtome into 4µm thick, mounted on glass slides, stained with hematoxylin and eosin, and examined under a high microscope at magnification ×400

2.6. Statistical analysis

All data were expressed as mean+SD and the data analyzed using one-way analysis of variance (ANOVA) values of $p<0.05$ were considered as statistically significant.

III. RESULTS

3.1. The ALT, AST, ALP and TBil of albino rats with aqueous (Aqu.) and ethanol (Eth.) extracts (mg/kg body weight) of Gnetum africanum root.

ALT activity (fig1) of the rats treated with aqueous and ethanol extracts of *gnetum africanum* root showed that all the treated groups were significantly higher than the control. There was no significant difference between groups treated with 250mg/kg and 500mg/kg aqueous when compared with the group treated with 1000mg/kg aqueous. Except for group treated with 250mg/kg ethanol, there was no significant difference between groups given 50mg/kg and 1000mg/kg ethanol. AST activity (fig 2) revealed no significant difference in all the groups treated with aqueous extract and 250mg/kg ethanol test group when compared to control and to one another except for the groups treated with 500mg/kg and 1000mg/kg ethanol. ALP activity (fig3) revealed that all groups increased significantly when compared to the control. There was no significant difference in the activity of ALP in all the groups given different concentrations of aqueous extract as well as 500mg/kg and 1000mg/kg ethanol extract. The total bilirubin (fig4) revealed that except for the groups given 250mg/kg aqueous extract which had no significant difference, bilirubin concentration of all other groups were significantly different from the concentration obtained from the control. No significant ($p<0.05$) was observed in all the groups treated with different concentrations of aqueous extract as well.

Figures (1-4) showing ALT, AST, ALP and Total bilirubin (T.Bil) of albino rats treated with aqueous (Aqu.) and ethanol (Eth.) extracts (mg/kg body weight) of Gnetum africanum root.

Bars are mean ± standard deviation. Bars bearing different alphabet better(s) are statistically significant ($p<0.05$).

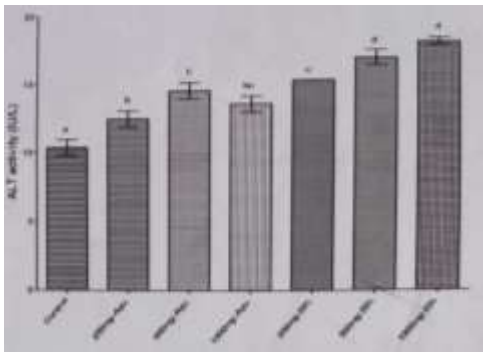
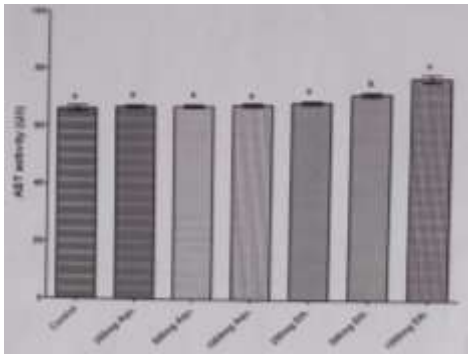


fig1



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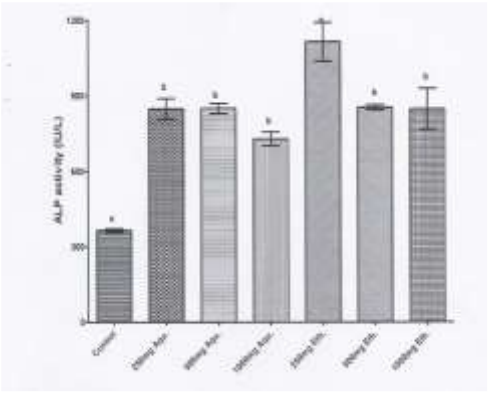

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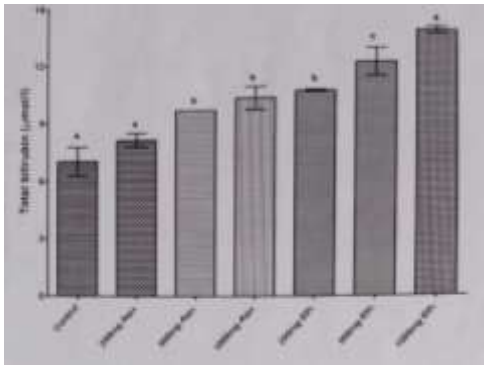
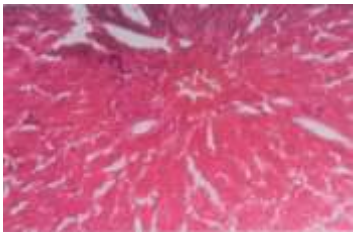


fig4

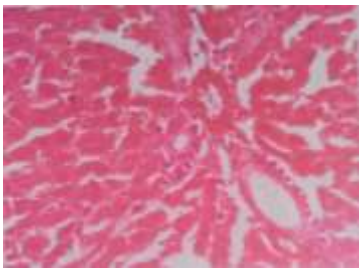
3.2. The Liver Histopathology

Figure5(a-g). The liver histology (H/E×400) *of albino rats with aqueous (Aqu.) and ethanol (Eth.) extracts (mg/kg body weight) of Gnetum africanum root.*

Figure 5(a) is characterized by pleomorphic nuclei, hepatocytes are separated by narrow sinusoids with prominent kipffer cells. No histopathological lesion seen. Figure 5(b) revealed a cystically dilated sinusoids and bi-nucleated hepatocytes amidst a balance nucleo-cytoplasmic morphology. Figure 5(c) showed a pleomorphic, cystically dilated sinusoids, eccentric nuclei and prominent nucleoli. Figure 5(d) revealed hypercellularity, pleomorphism and insignificant heamorrhagic necrosis. No lesion seen.



A



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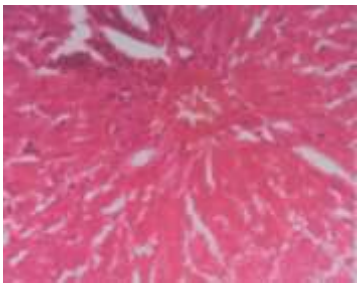

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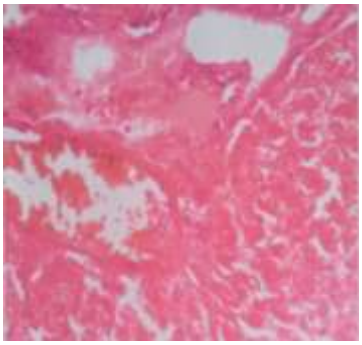
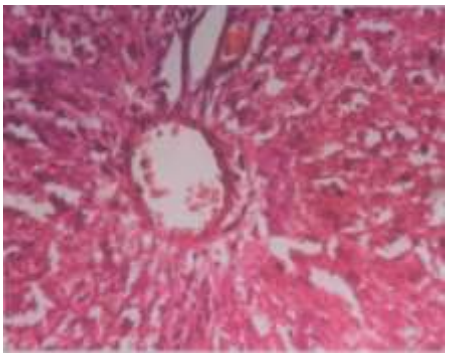


D

The result of figure 5(e) revealed pleomorphic nuclei. The sinusoids appear dilated while the hepatocytes showed slightly unremarkable morphology. Figure 5(f) showed eccentrically positioned nuclei, prominent nucleotide with slight insignificant heamorrhagic necrosis. Figure 5(g) revealed a cystically dilated sinusoids and hypercellularity.



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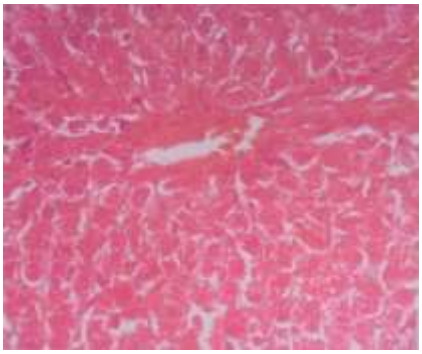

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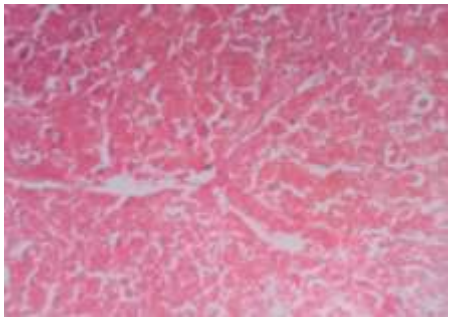

G

IV. DISCUSSION

Lethal Dose (LD_{50})

The result of the lethal dose test of ethanol and aqueous extracts of *Gnetum africanum* root showed no toxicity on the albino rats, even when the concentration on both extracts were increased to 5000mg/kg body weight. However, the animals showed some signs of weakness at 1600mg/kg. 3000mg/kg and 5000mg/kg body weight, after which they recovered, gained weight and become more active.

Owing to the fact that the liver is the first internal organ to encounter a number of insults including ingested metals, drugs

and environmental toxicants (Klaseen, 2007), it is consequently exposed to significant concentrations of these chemicals and its functions can be adversely exposed to significant concentrations of these chemicals and its functions can be adversely affected by the acute or chronic exposure (Hinson, *et al*., 2010). The liver function biomarkers studied in this research are useful to indicate the alterations caused by the extract of *Gnetum africanum* root on the hepatic integrity of the rats and can be used to monitor the level of damage by the extract of *Gnetum africanum* root before biopsy (Tandy and Person, 1984).

The effects of extracts from *Gnetum africanum* root on normal albino rats were clearly expressed by the ethanol extract group. The result from figure 1 suggest that the aqueous extract *Gnetum africanum* root had little effect on the serum ALT level when compared with the control, while the ethanol extract group also shown in figure 1 revealed an appreciable increase in serum ALT level compared with the control.

The result shown in figure 2 revealed the aqueous extract of *Gnetum africanum* root had no effect on the serum AST levels while the ethanol extract of *Gnetum africanum* root revealed little increase on the level of serum AST of the rats when compared with the control.

This increase seen in ethanol extracts indicates a cellular leakage and loss of functionality of membrane integrity of the liver (Saraswat, *et al.,* 2010). Xenobiotic in form of extracts in experimental animals could cause derangement of biochemical processes (Uboh*et al.*, 2010), thus increasing or decreasing the activities of ALT and AST which are indicators of liver damage (Edet, *et al.,* 2011). These injuries may have been caused by free radicals and peroxidants (Jalalpure, *et al.*, 2003) generated during the metabolism of some active components of the extract (Burns *et al.,* 2000), which then cause the compromise of the membranes of hepatocytes leading to leakage of enzymes and increase in the serum liver biomarkers. The type of liver damage owing to increase in ALT level may be hepatocellular necrosis while AST increase could be cirrhosis or acute alcoholic hepatitis (Xi, *et al*., 2014).

The effect of ethanol and aqueous extracts of *Gnetum africnum* root revealed an appreciable increase in ALP activity in the serum of the albino rats, with 250mg/kg body weight of the ethanol extract showing the highest increase in ALP activity. Alkaline phosphatase is an enzyme located in the liver, and its elevation in serum increases when bile ducts are blocked (Xi. *Et al., 2014)* Alkaline phosphatase is a diagnostic biomarker of cholestatic drug induced liver injury (DILI) (Ramachandran and Kaka, 2009).

However, it is worthy of note that conditions other than DILI, such as bone disease, formation and pregnancy are also associated with ALP elevation (Reust and Hall, 2001), thus ALP is not regarded as a specific biomarker of cholestic DILI but is at least partially predictive of biliary obstructive types of liver injury when used together with other DILI biomarkers (Xi, *et al*., 2014: Aghara, 2014). Therefore, the sharp increase in ALP activity observed in 250mg/kg body weight ethanol extract does not necessarily indicate hepatotoxicity.

The influence (figure 4) of aqueous extracts of *Gnetum africanum*root showed little increase in bilirubin concentration and the ethanol extract revealed a significant increase in the concentration of bilirubin level when compared with the control.

Total bilirubin (TBL) which constitute of unconjugated (extra-hepatic) and conjugated (hepatic) bilirubin. Increases TBL causes jaundice and this suggests metabolism problems in the liver such reduced hepatocyte uptake, impaired bilirubin conjugation or reduced bilirubin secretion (Xi. *et al.,* 2014).

These metabolism problems in the liver may be due to damage of the liver cell which is seen by increase in ALT activity, as such altering the numerous functions of the liver which include bilirubin conjugation amongst others.

Serum bilirubin concentration is a real liver function biomarker, which measures the ability of the liver to clear bilirubin from the blood as it circulates through the liver (Xi, *et al.,* 2014; Senior, 2006).

The histopathological examination of the liver section in the control albino rat group showed a normal histological feature. The nuclei lie at the center of the hepatocyte with prominent kupffer cells. No histopathological lesion seen. The liver section of the rats treated with ethanol extract revealed some abnormal morphology characteristics such as hypercellularity and insignificant haemorrhagic necrosis in all treatment groups including the lowest dose of treatment (250mg/kg) and the group treated with 1000mg/kg aqueous.

Sinusoidal dilation found in the absence of an impaired sinusoidal outflow has been far of unclear significance (Chiara, *et al.,* 2015). Sinusoidal dilation is characterized by widening of hepatic capillaries which may involve the entire lobule or predominately in the central, periportal, or medial area, can be encountered in different situations (Degott and Potect, 1984). It is commonly found in the vicinity of hepatic tumors or heart failure, hepatic venous outflow block, veno-occlusive disease, granulomatous disorders, infectious conditions, or infiltration of sinusoids by various types of benign or malignant cells (Laffon*et al.,* 1989): and in the clinicopathological entity non-cirrhotic intrahepatic portal hypertension, which consists of various types of architectural alteration. These alterations include modular regenerative hyperplasia, perisinusoidal fibrosis, hepatoportal selerosis or incomplete septal cirrhosis (Hillaire, et al., 2002): have been related to several casual factors, including xenobiotics (thorium salts, plant extracts, arsenicals, vinyl chloride, vitamin A or azathioprine) and thrombophilia. All of these factors have been postulated to act by inducing portal or sinusoidal obstruction (Hillaire, et al., 2002).

Hypercellularity is characterized by an abnormal proliferation of hepatic cells and slight bleeding from rupture vessels. These features are in line with inflammation.

Finally, the result of the LD_{50} test of the ethanol and aqueous extracts of *Gnetum africanum* root showed no toxicity on the albino rats, even when the concentration of both extracts were increased to 5000mg/kg body weight, could be as a result of entrance of foreign materials. The recovery weight gained and more active observed in the animals showed that the immune system was able to overcome, hence resulting to their recovery. This indicates that the root of *Gnetum africanum* is safe for human and livestock consumption.

V. CONCLUSION

Following the results of this study, the aqueous extracts of *Gnetum africanum* root may passion be safe for consumption without any significant toxic effect on the liver of the rats, especially at lower concentration (250 and 500mg/kg weight). Consequently, care should be taken when consuming *Gnetum africanum* root, as prolonged use at concentration of 500mg/kg body weight could increase the activities of ALT, ALP and total bilirubin in the serum. However increase in ALT, AST, ALP activities and bilirubin concentration in serum of the albino rats influenced by the ethanol of *Gnetum africanum* root may be due to the ability of the ethanol (solvent) to extract the water insoluble active components of the *Gnetum africanum* root which are not present in the aqueous extract.

VI. RECOMMENDATION

From the findings of this study, it is recommended that further studies be done on additional biomarkers such as genetics, proteomics, metabolomics and Micro RNAs of hepatotoxicity in the serum; this can be measured in conjunction with ALT, with respect to specificity of liver injury.

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