

**Investigation of Effects of Aqueous Extracts of *G. latifolium* on Selected Biochemical Indices in Ethanol Intoxicated Albino Rats**

Ali Fredrick U<sup>1</sup>, Orinya O.F<sup>2</sup>, Ominyi MC<sup>1.</sup>, Ebenyi L.N<sup>1</sup>. Ogbanshi M.E<sup>3</sup>, Ezenwali M. O.<sup>4</sup>and Eze U.S<sup>5</sup>

<sup>1</sup>Biotechnology Department Ebonyi State University Abakaliki ,Nigeria

<sup>2</sup>Medical Biochemistry Department Ebonyi State University Abakaliki ,Nigeria

<sup>3</sup>Biochemistry Department Ebonyi State University Abakaliki ,Nigeria

<sup>4</sup>Biochemistry Department Enugu State University of science and Technology ,Nigeria  
Department of Biotechnology Ebonyi State University Abakaliki, Nigeria.

<sup>5</sup>Biology Department Ebonyi State College of Education Ikwo ,Nigeria

Email:[alifredrik13@gmail.com](mailto:alifredrik13@gmail.com). Or [fredrickali.@ebsu-edu.net](mailto:fredrickali.@ebsu-edu.net) +2348063893022,

---

**ABSTRACT**

Herbal medicinal products play an important role in the management of liver diseases for the lack of satisfactory liver protective drugs in allopathic medical practices. The present study was designed to evaluate the hepatoprotective and antioxidant effect of aqueous extract of *G. latifolium* on ethanol intoxicated wistar rats. In order to assess the hepatoprotective effect of this extract in experimental animals, twenty-four Wistar albino rats (weighing 150-213 g) were divided into six groups of four animals each. Toxicity was induced by administering 70% ethanol (7.0 g/kg b.w) by oral gavage for 7 days to group B - C. Serum marker enzymes were assayed after 7<sup>th</sup> day before commencement of treatment, there after graded doses of the extract (200, 400, 600 and 800 mg/kg b.w.) were administered to C group for 21 days. Serum triglyceride (TG), cholesterol, HDL levels, alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities, and weight of the rats were monitored. At the end of which, reduced glutathione (GSH) levels, and glutathione-S-transferase (GST) activities were determined in the liver, kidney and intestine. At the end of the experiment, chronic administration of ethanol resulted in enhanced lipid peroxidation (LPO) with depletion in the levels of GSH as well as reduction in the activities of GST. TG levels, cholesterol, HDL, ALT and AST activities were elevated. Administration of the plant extract after ethanol exposure inhibited hepatic LPO and ameliorated GST activities as well as restoring GSH, AST, ALT, and ALP levels significantly. Vitamin B, C and K values increased significantly (P > 0.05) with the increase in concentration of the extracts compared to group B. The present studies suggest that the aqueous extract may be beneficial in ameliorating ethanol-induced oxidative damage in the liver of wistar rats

**Keywords:** Gongronemalatifolium; rats; extract; antioxidant; serum lipid; aqueous extract, kidney, intestine.

---

## INTRODUCTION

Chronic alcohol consumption increases the capacity of cytochrome P450 2E1 (cyp2 E1) to oxidize ethanol up to 10-fold which consequently increases the peroxidative burden Adaramoye *et al.*, 2005 and Adaramoye,(2010)[1],[2] reactive oxygen species (ROS) generated during ethanol oxidation via cyp2E1 contributes to ethanol-induced liver injury[3]. Although the pathogenesis of alcohol-induced liver diseases remains the subject of debate, one factor that has been suggested as playing a central role in many pathways of alcohol - induced damage, and which has been the focus of much research is the excessive generation of these free radicals, which can result in a state called oxidative stress[4]. Numerous studies have indicated that excessive ethanol intake induces the mass production of free radicals in the body, which are considered to be associated with alcoholic liver diseases Aguilar-Santamaria *et al.*, 2009 and Hsiao *et al.*, (2003)[5],[6]. The most important characteristic of toxic free radicals either in vivo or invitro is peroxidation of lipids resulting in tissue damage and death of affected cells Iga *et al.*, 1977 and ensen *et al.*,(2004)[7],[8]. Several reports have implicated free radical-induced lipid peroxidation in the pathogenesis of alcohol-induced liver toxicity. Antioxidants play an important role in the protection of cells and tissues against free radical-mediated tissue injury[9],[10]. Although significant progress has been made in understanding the pathogenesis of alcoholic liver diseases, current therapies for these diseases are not effective. At present, except for abstinence from alcohol intake, there is no effective modality of either prevention or treatment. Antioxidants of plant origin have been reported to either inhibit or prevent the development of fundamental cellular disturbances resulting from excessive alcohol consumption [11].

*G. latifolium* belongs Benth Hook, (Asclepiadaceae) is an herbaceous shrub, with yellow flowers and the stem that yields characteristic milky exudates when cut. It is commonly grown in gardens in Abakaliki, Ebonyi State, Nigeria. It is locally called “utasi” by the Efiks,

Ibibios, and Quas, “utazi” by the Igbos and “arokeke” by the Yorubas in Nigeria Mourelle *et al.*, (1987)[12]. The Efiks and Quas in Calabar use *G. latifolium* crude leaf extract in the treatment of malaria, diabetes, and hypertension and as laxative Price (1980) [13]. Also it is used as a spice and vegetable Norman, 1998 and Okafor(1989)[14],[15]. The use of crude leaf extract of this shrub in maintaining healthy blood glucose levels have been reported Amos *et al.*, 1997; Murray *et al.*, (1990)[16],[17]. Scientific studies have established the hypoglycemic, hypolipidaemic and antioxidative effects of aqueous and ethanol extracts of *G. latifolium* leaf Braide *et al.*,(2003)[18]. Research showed that the leaf extract has anti-inflammatory properties while its potential nutritional and food processing Morebise *et al.*, 2002 and Akpantah *et al.*, (2005)[19],[20] investigated preservation values of food.

Some phytochemical such as *B*- sistostem lupenyl esters, pregnane ester, glycosides, essential oils and saponin are associated with parts of this herb. It is plausible that one or more of these phytochemical that are found in *G. latifoliumis* likely to influence cellular proteins with enzymatic activity. This study was designed to evaluate the chronic alcohol-induced liver oxidative damage and the efficacy of pre-treatment of aqueous extract of *G. latifolium* leaf on chronic ethanol-induced liver damage.

## MATERIALS AND METHODS

### (a) Animals

Twenty-four Wister male albino rats (weighing 150-170g) were obtained from the Animal House of the of Veterinary Medicine, University of Nigeria Nsuka (UNN), Enugu State the animals were allowed access to feed (obtained from Safari feed mill 5 Zik Avenue Abakaliki, Nigeria) and water *ad libitum* for a period of seven days, for their acclimatization prior to the commencement of the experiment. The animals were kept in well ventilated cages at room temperature, and under controlled light dark cycles (12/12h). The guidelines of the

ethical care and treatment of animals followed the regulations of the ethical committee Ebonyi State University, Abakaliki Nigeria.

### **(b) Chemicals**

Randox alanine aminotransferase (ALT), aspartate (AST), and triglyceride (TG) assay kits were purchased from Gerald chemicals, Enugu (Nigeria), glutathione and bovine serum albumin (BSA) were purchased from Sigma chemical (St. Louis, MO, U.S.A). All other chemicals were of the highest purity commercially available.

### **(c) Plant material**

*G. latifolium* leaves were purchased from Abakpa market in, Abakaliki metropolis (Nigeria), identification and authenticated by Prof Emeka Okafor of Botany Department, Ebonyi State University Abakaliki, where voucher samples are kept for reference. One kilogram (1kg) of the leaves from air dried leaves was pulverized into uniform powder using an electric blender and packed in airtight bottles and stored until required for extraction.

## **PREPARATION AND ADMINISTRATION OF THE EXTRACT**

Pulverized leaves (600g) was extracted with 1500ml of distilled water by maceration for 72 hours the aqueous extract was filtered and the filtrate was concentrate in a rotary evaporator, to field a yellowish brown extract. This was carefully scrape into a clean sample bottle and stored in a refrigerator at 4°C for further use. Aqueous extract of *G. latifolium* were used for the experiment. Twenty four male Wister rats were randomly distributed into six groups of four animals each. Group A served as the positive control and received water and feed. Group B and C received 70% ethanol (7.0g/kg) and feed only, for seven days. Group B was not treated and served as negative control. Furthermore, group C were treated with group 200mg/kg, 400 mg/kg, 600mg/kg, 800mg/kg of the extract for 21 days respectively. Administration of ethanol and the extract was by oral gavage using a

cannula and animals were observed daily for psychomotor changes and other signs of toxicity including death throughout the period of study.

#### **(d) Preparation of tissues for biochemical analyses**

Following the daily treatment of the animal for 21 days, the animals were sacrificed 24 hours after the last dose. Liver samples were quickly excised and weighed. They were then homogenized in 4 volumes of 56 mM Tris-HCl buffer (pH 7.4) containing 1.15% KCl, and then centrifuged at 10,000g for 15 minutes. The supernatant was collected and stored until needed for assays. Small pieces of liver renal and intestine sections were harvested and fixed in 10% formal saline and used for the study.

#### **(e) Biochemical Assays**

Protein concentrations of the various samples were determined by means of the Biuret method as described by Piyachaturawat *et al.*, (1995)[21]. The levels of reduced glutathione (GSH) in the supernatant fraction of the liver homogenate were estimated using the method described by Gornall *et al.*, (2003)[22]. Glutathione -S- transferase (GST) activity was determined according to Beutner *et al.*, (2010)[23]. Serum ALT, AST and ALP activities, cholesterol, HDL, and TG, and vitamin C levels were quantified spectrophotometrically using Randox commercial assay kit. While vitamin B and K were evaluated using immunoassays and prothrombin time respectively.

### **STATISTICAL ANALYSIS**

All data were expressed as mean  $\pm$  SD. One-way analysis of variance (ANOVA) was used for the analysis of the biochemical indices. This was done using software (15.0). Differences were considered significant at  $P > 0.05$ .

### **RESULTS**

#### **PHYSICAL OBSERVATION**

It was observed that during the period of ethanol administration, the animals were weak, not agile. Increased water and reduced food intake whereas their agility increased during the 21 days of extract administration.

Table 1 shows effects of *G. latifolium* on the level of some selected biochemical parameters.

Parameters/ Treatment	AST(IU/L)	ALT(IU/L)	ALP(IU/L)	$\beta$ - carotene(ug/ml)	Ascorbic acid(mg/dl)	Vitamin k(nmol/l)
<b>Group A</b>	40.69 $\pm$ 3.81 <sub>b</sub>	4.37 $\pm$ 0.41 <sub>d</sub>	71.08 $\pm$ 1.84 <sub>d</sub>	0.74 $\pm$ 0.03 <sub>c</sub>	1.29 $\pm$ 0.04 <sub>c</sub>	<b>1.27<math>\pm</math>0.15<sub>d</sub></b>
<b>Group B</b>	52.78 $\pm$ 0.87 <sub>a</sub>	24.62 $\pm$ 0.63 <sub>a</sub>	89.20 $\pm$ 0.08 <sub>a</sub>	0.23 $\pm$ 0.02 <sub>e</sub>	0.08 $\pm$ 0.08 <sub>f</sub>	<b>0.27<math>\pm</math>0.03<sub>f</sub></b>
<b>Group C</b>						
<b>200mg/Kg</b>	29.29 $\pm$ 0.63 <sub>c</sub>	24.11 $\pm$ 0.93 <sub>a</sub>	76.29 $\pm$ 0.89 <sub>b</sub>	0.26 $\pm$ 0.19 <sub>e</sub>	0.73 $\pm$ 0.09 <sub>e</sub>	<b>0.57<math>\pm</math>0.05<sub>e</sub></b>
<b>400mg/Kg</b>	17.09 $\pm$ 0.16 <sub>d</sub>	24.89 $\pm$ 1.11 <sub>a</sub>	74.60 $\pm$ 0.29 <sub>c</sub>	0.69 $\pm$ 0.02 <sub>d</sub>	1.60 $\pm$ 0.07 <sub>d</sub>	<b>2.00<math>\pm</math>0.09<sub>c</sub></b>
<b>600mg/Kg</b>	15.94 $\pm$ 0.43 <sub>d</sub>	23.83 $\pm$ 0.10 <sub>b</sub>	59.40 $\pm$ 0.41 <sub>e</sub>	0.97 $\pm$ 0.04 <sub>b</sub>	3.98 $\pm$ 0.06 <sub>b</sub>	<b>2.59<math>\pm</math>0.20<sub>b</sub></b>
<b>800mg/Kg</b>	<b>9.40<math>\pm</math>0.49<sub>e</sub></b>	<b>21.85<math>\pm</math>0.08<sub>c</sub></b>	<b>45.20<math>\pm</math>0.39<sub>f</sub></b>	<b>1.28<math>\pm</math>0.02<sub>a</sub></b>	<b>7.40<math>\pm</math>0.13<sub>a</sub></b>	<b>3.16<math>\pm</math>0.34<sub>a</sub></b>

At ( $p < 0.05$ ), means with the different letters (subscript) are significantly different in each parameter.

Table 2: Table shows effect of *G. latifolium aqueous* extract on the level of triglyceride, HDL and cholesterol

Treatment	Triglyceride	HighDensity lipoprotein	Cholesterol
<b>Positive control</b>	1.5599 $\pm$ 1.2a	6.9780 $\pm$ 2.33a	3.1758 $\pm$ 0.22a
<b>Negative control</b>	0.6397 $\pm$ 4.1b	8.0595 $\pm$ 3.33b	17.4296 $\pm$ 3.1b
<b>200mg/kg</b>	0.2002 $\pm$ 2.3c	6.6995 $\pm$ 2.43c	4.7132 $\pm$ 5.0c
<b>400mg/kg</b>	1.3139 $\pm$ 5.14d	5.5636 $\pm$ 6.33d	5.100 $\pm$ 2.11d
<b>600mg/kg</b>	0.3100 $\pm$ 3.55e	3.8636 $\pm$ 2.12e	6.3390 $\pm$ 4.3e
<b>800mg/kg</b>	0.1476 $\pm$ 6.32e	6.7990 $\pm$ 3.11d	5.2297 $\pm$ 3.4e

At ( $p < 0.05$ ), means with the different letters (subscript) are significantly different in each parameter.

Table 3: Table shows effects of aqueous extract of *G. latifolium* on the level of reduced GSH( $\mu\text{g/g}$  tissue) in ethanol intoxicated albino rats.

Group	Hepatic GSH level	Renal GSH level	Intestinal GSH level
<b>Group A</b>	14.12 $\pm$ 0.91a	7.92 $\pm$ 1.78a	7.48 $\pm$ 1.57a
<b>Group B</b>	4.23 $\pm$ 0.74b	3.42 $\pm$ 0.65b	3.44 $\pm$ 1.01b
<b>Treated</b>	13.79 $\pm$ 0.67a	6.85 $\pm$ 0.44a	8.10 $\pm$ 0.77a

Values are expressed as mean  $\pm$ S.D for four animals per group  $p < 0.05$  against control.

Table 4: Table shows effects of aqueous extract of *G. latifolium* on the level of Malondialdehyde(MDA) concentration( $\text{molg}^{-1}$  tissue) in ethanol intoxicated albino rats.

Group	Hepatic	Renal	Intestinal
<b>Group A</b>	51.36 $\pm$ 4.09a	11.08 $\pm$ 1.96a	19.36 $\pm$ 2.2a
<b>Group B</b>	109.37 $\pm$ 0.4b	16.25 $\pm$ 1.3b	36.47 $\pm$ 2.34b
<b>Treated</b>	52.17 $\pm$ 1.6a	10.53 $\pm$ 0.34a	20.7 $\pm$ 0.87a

Values are expressed as mean  $\pm$ S.D for four animals per group

## DISCUSSION

The liver has a central role in the maintenance of lipid homeostasis and the presence of toxicants may alter the concentration of serum lipids which could increase the risk of atherosclerosis, given that increased LDL cholesterol and decreased HDL cholesterol are implicating risk factors of atherosclerosis and related cardiovascular diseases Habig *et al.*, 2001 and Price *et al.*, (1987)[24],[25]. Popularity of herbal remedies is increasing globally and at least one quarter of patients with liver diseases use ethnobotanicals

Adedeji *et al.*, 2006 and Arai *et al.*, (2011) [26],[27]. More efforts need to be directed towards methodological scientific evaluation for their safety and efficacy by subjecting to vigorous pre-clinical studies followed by clinical trials to unravel the mysteries hidden in

the plants. This approach will help exploring the real therapeutic value of these natural pharmacotherapeutic agents and standardized the dosage regimen on evidence-based findings to become more than a fashionable trend Banerjee *et al.*, (2002)[28]. Many herbals are on the market to support health, relieve symptoms and cure diseases. However, most of these products lack scientific pharmacological validation. Hence, in this study

Estimation of the activity of ALT, AST and ALP are good marker of assessment liver function. These enzymes are normally located in the cytosol of hepatocytes. When liver cells are damaged, these enzymes are released in the plasma /serum and increased their activity in plasma and serum is a useful marker of the extent and type hepatocellular damage. In this study, exposure of the animals to ethanol increased the levels of liver marker enzymes negatively compared to positive control (Table1). Hence, ethanol administration to experimental animals induced acute pathological changes in the liver Amrani *et al.*, (2009)[29]. Biochemical mechanism for ethanol toxicity based on mitochondrial damage was previously described Lipkin (1995)[30]. Alcohol impairs nutrient absorption by damaging cells lining the stomach and intestines, and disabling transport of some nutrients into the blood Murray *et al.*, (2002)[31]. Alcohol also inhibits the breakdown of nutrients into usable substances, by decreasing the secretion of digestive enzymes from the pancreas [32].

Research have shown that individuals with chronic alcohol abuse frequently exhibit lowered plasma levels of pyridoxal 5'-phosphate, the coenzyme form of vitamin B6. The liver is the primary source of this coenzyme in plasma and also the principal organ that oxidizes ethanol Bode and Bode (2009)[33]. The effect of ethanol on hepatic pyridoxal phosphate metabolism has been reversed in this study by the action of plant extract on ethanol exposed Wister rats in tissues and reversing of liver marker enzymes .Thus the derangement of pyridoxal phosphate metabolism produced by ethanol is dependent upon

its oxidation. These is in line with previous findings which indicated that acetaldehyde is the responsible agent which acts by accelerating the degradation of intracellular pyridoxal phosphate Albano *et al.*, (1994)[34]. Folate, is essential for the development and growth of new cells, the absorption of this vitamin is inhibited by ingestion of ethanol. Ethanol inhibits absorption of folate and other vitamins by killing the cells lining gastrointestinal tract that mediate the absorption of these nutrients.

Alcohol reduces the ability of the body to absorb the vitamin thiamin, also called vitamin B-1 that helps break down carbohydrates, proteins and fat in the food you eat. It also aids in production of hemoglobin, the protein that binds oxygen in red blood cells. A severe deficiency in thiamin leads to a life-threatening brain disorder called Wernicke-Korsakoff Syndrome, characterized by memory loss, confusion and trouble maintaining balance. If severe, it can even lead to permanent brain damage Pritchard and Butler(1989)[35]. Hence, in this research the altered vitamin were restored significantly [  $p > 0.05$  ] by the plant extract in a dose dependent fashion.

Research indicates that alcohol affects protein nutrition by causing impaired digestion of proteins to amino acids, impaired processing of amino acids by the small intestine and liver, impaired synthesis of proteins from amino acids, and impaired protein secretion by the liver [36].

Vitamins A, C, D, E, K, and the B vitamins, deficient in chronic alcoholics , these vitamins are all involved in wound healing and cell maintenance Mathew *et al.*, (2012)[37]. In particular, because vitamin K is necessary for blood clotting, deficiencies of that vitamin can cause delayed clotting and result in excess bleeding. However, plants extracts have profoundly restored vitamins significantly ( $p > 0.05$ ) in a dose dependent fashion (Table1-4). Furthermore deficiencies of other vitamins involved in brain function can cause severe neurological damage [38].

Ethanol significantly altered serum lipid profile in this study. Report have shown that moderate consumption of alcohol results (30 g ethanol/day) increases the concentration of HDL-c in approximately 4 mg/dL, and apoA-I in 8.82 mg/dL Olatunde(2000)[39], with a reduction to the risk of cardiac disease estimated at 24.7% [41 ]. Furthermore, ethanol promotes less degradation of HDL-c King and King (1954)[40] and a greater liver metabolism of LDL-c Anusuya and Sellamuthu(2013)[41]. Administration of aqueous extract of *G. latifolium*

Significantly ( $p>0.05$ ) restored the lipid profile of the test animals .However, this action may be attributed to the bioactive components of the plant extract .Report by Ali *et al.*, (2013)[42] have indicated ameliorating effects of plant extract treated with animals exposed to toxicants. There is a progressive increase of diseases attributed to greater alcohol consumption Afiukwa *et al.*, (2013)[43], but research have shown efficiency of plants extracts in reversing the effects of ethanol Ali *et al.*, (2013)[44]. Biochemical analysis of our results showed significant increase ( $P<0.05$ ) of the hepatic, renal and intestinal reduced glutathione (GSH) and malondialdehyde (MDA) concentrations compared with negative control respectively. Similarly, the hepatic, renal and intestinal GST activities were significantly increased ( $p>0.05$ ) respectively. The levels of reduced glutathione (GSH) and malondialdehyde (MDA) in the liver, kidney and intestine of ethanol-treated animals were significantly ( $p>0.05$ ), altered relative to the positive control group .Furthermore, treatment of test animals with the extract ameliorated the reduced glutathione and malondialdehyde significantly compared to negative control (Table 3-4).

Previous studies have demonstrated that *G.latifolium* Ali and Ibiam(2014)[45] exhibited hepato- and renal protective effect. The restoration of hepatic of hepatic, renal, and intestinal GSH and MDA in rats are probably due to membrane stabilizing effect. The reduced lipid peroxidation in rat liver homogenates were reported to be due to total

phenolic or flavonoids contents Ali *et al.*, (2015)[46]. Therefore the protective effect of *G.latifolium* extract against ethanol hepatotoxicity may be attributed to the presence of phytochemicals[47].

Many compounds known to be beneficial against ethanol-mediated liver injury exert their protective action by toxin-mediated lipid peroxidation either via decreased production of carbon tetrachloride derived free radicals or through the antioxidant activity of the protective agents themselves. These constituents were reported to exhibit strong antioxidant properties and cytoprotective actions in rats[48],[49].

The present work clearly demonstrate that oral administration of aqueous extract of *G. latifolium* has restored the biochemical parameters which were altered after ethanol-induced intoxication. The anti-hepatotoxic, renal and intestinal activity of *G. latifolium* extract may be due to its direct radical scavenging activity Moses *et al.*, 2014 and Ogbanshi *et al.*, (2015)[50],[51]. In conclusion, *G. latifolium* was effective in reversing altered biochemical parameters antihepatotoxic and can be considered as good natural sources of hepatoprotective constituents.

## CONCLUSION

In conclusion, the study has revealed that aqueous leaf extract of *G. latifolium* ameliorate hepato-, renal and intestinal toxicity due to ethanol exposure in wistar rats ,hence it suggest it potential source of ethno medicine.

## ACKNOWLEDGEMENTS

The authors are thankful to Mr Ogbu Keneth, of Biotechnology Research and Development centre Ebonyi State University, Abakaliki Nigeria who assisted in the laboratory work and

manager Brain Phosphorylation research laboratory 9 Ogui Road Enugu State, Nigeria for data analysis.

#### REFERENCES

1. Adaramoye, O. A., Nwaneri, V. O., Anyanwu, K. C., Farombi, E. O. and Emerole, G. O. (2005). Possible anti-atherogenic effect of kolaviron (a *Garcinia kola* seed extract) in hypercholesterolaemic rats. *Clinical and Experimental and Physiology* **32**(1-2): 40-46.
2. Adaramoye, A.O. (2010). Protective effect of kolaviron, a biflavonoid from *Garcinia kola* seeds in brain of Wistar albino rats exposed to gamma radiation. *Biological and Pharmaceutical Bulletin* **33**(2): 260-266.
3. Bruckner, J.V., Luthra R and Kyle GM (1984). Influence of time of exposure to carbon tetrachloride on toxic Liver injury. *Ann. Rev. Chronopharmacol.* 1: 373-376.
4. Bruckner, J.V, Mackenzie WF and Muralidhara S (1986). Oral toxicity of carbon tetrachloride. *Acute, Fundam. Appl. Toxicol.*, 6: 16-34.
5. Aguilar-Santamaria, L., Ramirez, G., Nicosio, P., Alegria-Reyes, C. and Herrera-Arellano, D. (2009). Antidiabetic activities of *Tecoma stans* (L.) Juss. Ex-Kunth. *Journal Ethnopharmacology* **10**:1010-1016.
6. Hsiao G, Lin YH, Chou DS, Lin WC and Sheu JR (2003). The Protective effects of PMC against chronic carbon tetrachloride - induced hepatotoxicity in vivo. *J. Pharmacol.*, 4: 9-10.
7. Iga T, Sugiyama Y, Yokota M, Tomono Y, Ansazu S and Hanano M (1977). Pharmacokinetic aspects of sulfobromophthalein transport in chronically carbon tetrachloride intoxicated rats. *Biochem. Pharmacol.*, 26: 1867-1873.
8. Jensen JE, Stainberg SE, Freese P and Marino E (2004). Liver function tests. *J. Digest. Disord.*, 6: 1-3.

9. Chung HS, Chong LC, Lee SK, Shamon LA, Breemen RBV, Mehta RG, Farnsworth NR, Pezzuto JN and Kinghorn, A.D (1999). Flavonoids constituents of chlorinzan diffused with potential cancer chemopreventive activity. *J. Agric. Food Chem.*, 47: 35-41.
10. Lambo JO (1979). The Healing powers of herbs with special reference to obstetrics and gynaecology. In: Hepatotoxicity of carbon tetrachcoride: Protective effect of *Gongronema latifolium* Pak. *J. Pharm. Sci.*, Vol.21, No.3, July 2008, pp.268-274 .
11. Lillie RD (1965). Nuclei, nucleic acid, general oversight stains. In: *Histopathology Technique and Practical Histochemistry*, 3rd edition, McGraw Hill Book Company, pp.142-179.
12. Mourelle M, Ameziua JL and Hong E (1987). Effects of riprostit and colchicines on carbon tetrachloride acute liver damage in rats. Relationship with Plasma Membrane Lipids. *Prostaglandins*, 33: 869-877.
13. Price JE (1980). Carbon tetrachloride, Carborundum. *New Age Ecylopedia*, 4: 93-94.
14. Norman JJ (1998). *Common Laboratory Test in Liver Diseases*. Columbia-Edu. Columbia.
15. Okafor JC (1989). Tropical plants in health care delivery (Guest Lecture Delivered on Y6 Pharmacology Society of Nigeria at the University of Nigeria Nsuka).
16. Amos, A. F., McCarty, D. J. and Zimmet, P. (1997). The Rising Global Burden of Diabetes and its Complications: Estimates and Projections to the Year 2010. *Journal Animal Veternary Advances* 14: 51-85.
17. Murray PR, Drew WL, Kobayashi GG and Thompson JH (1990). *Medical Microbiology*. Wolf Pub. Ltd., USA., pp.696-697.
18. Braide, K. V., Agabe, C. A., Essien, G. E. and Udoh, F. V. (2003). Effect of *Garcinia kola* seed alkaloid extracts on levels of Gonadal Hormone and Pituitary Gonadotrophins in Rat serum. *Nigeria Journal Physiology Science* 18(1 - 2):59- 64.

19. Morebise O, Fatunso MA, Makinde JM, Olajide OA, Awe EO (2002). Anti-inflammatory property of leaves of *Gongronema latifolium*. *Phytother. Res.* 1: 75-77.
20. Akpantah, A. O., Oremosu, A. A., Moronhna, C.C., Ekanem, J. B. and Okanlawon, A.O. (2005). Effect of *Garcinia kola* seed extracts on ovulation, oestrous cycle, and foetal Development in Cyclic Sprague Dawley Rats. *Nigeria Journal Physiology Science* 20(1-2): 58 - 62.
21. Piyachaturawat P, Kingkaeohoi S and Toskulkao C (1995). Potentiation of carbon tetrachloride hepatotoxicity by piperine. *Drug Chem. Toxicol.*, 18: 333-344.
22. Gornall, A. G., Bardawill, C. J and David, M. M (2003). Determination of serum protein by means of biuret reaction. *Journal of Biological chemistry* 177:751-766
23. Beuther, H. Henning, T and .Linz, H (2010). Estimation of liver homogenate. *Journal of Pharmaceutical Science* 45:67-78
24. Habig, W. H. Pabst, M. J. Jakoby W. B (2001). Glutathione s-transferase; the first enzymatic step in mercapturic acid formation. *Journal of Biological chemistry* 249:7130-7139.
25. Price, K.R., Johnson, L.I. and Feriwick, H. (1987). The chemical and biological significance of saponins in foods and feeding stuffs. *CRC Critical Reviews in Food Science Nutrition.*, 26: 127-135.
26. Adedeji, O. S., Farimu, G. O., Amen, S. A. and Olayemi, J. B. (2006). The Effects of dietary bitter kola (*Garcinia kola*). Inclusion on body weight, haematology and survival rate of pullets chicks. *Journal Animal Veterinary Advances* 5(3): 184 - 187.
27. Arai, T., Yamashita, S., Hirano, K., Sakai, N., Kotani, K., Fujioka, S., Nozaki, S., Keno, Y., Yamane, M. and Shinohara, E. (2011). Increased plasma cholesteryl ester transfer protein in obese subjects. A possible mechanism for the reduction of serum HDL cholesterol levels in obesity. *Arterioscler Thrombosis* 14: 1129-1136.

28. Banerjee, S. K., Patra, B. C., Bandoypahyay, P. and Teway, A. (2002). Changes of blood parameters in Carp *Catla catta*. *Journal Aquatic Biology* 17(11):79 -84.
29. Amrani, F., Rhallab, A., Alaoui, T., Badaoui. K., Chakir, S. (2009). Hypoglycemic effect of *Thymelaeahirsute* in normal and streptozotocin-induced diabetic rats. *Journal Medicinal Plant Resource* 3(9): 625-629..
30. Lipkin R (1995). Secondary plant metabolites. *Sci. News*, 14: 8-9.
31. Murray RK, Granner DK, Mayes PA and Rodwell VM (2000). *Harper's Biochemistry*, 25th Ed. McGraw Hill, USA, pp.868-869.
32. Lius SL, Espoti SO, Yao T, Diehl AM and Zern MA (1995). Vitamin E therapy of acute CCl<sub>4</sub>- induced hepatic injury in mice as associated with inhibition of nuclear factor- $\kappa$ B binding. *J. Hepatol.*, 22: 1474- 1481.
33. Bode, C. and Bode, J.C. (2009). Alcohol's role in gastrointestinal tract disorder. *ACADEMIC Journal Article* 21:13-25
34. Albano, E., French, S. and Ingelman-Sundberg, M. (1994). Cytochrome p450 2E1, hydroxyl ethyl radicals, and immune reaction associated alcoholic liver injury. *Alcoholism: Clinical and Experimental Research*. 18:1057-1068
35. Pritchard DJ and Butler WH (1989). Apoptosis; The mechanism of cell death in dimethyl-nitrosamine- induced hepatotoxicity. *J. Pathol.*, 156: 253-260.
36. Adesanya, O. A., Oluyemi, K. A., Olusori, D. A., Omotuyi, I. O., Okwuonu, C. U. Ukwenya, O. V. and Adesanya, A. A. (2011). Micromorphometric and Stereological effects of ethanolic extracts of *Garcinia cambogia* seeds on the testes and epididymides of Adult Wistar Rats. *Internet Journal of Alternative Medicine* 5(1):1-9.
37. Mathew LA, Dhanyaraj D, Prathibhakumari P.V, Prasad G. (2012). Hepatoprotective and antioxidant potential of *Sphaeranthus indicus* [Linn] on liver damage in Wistar rats. *Int.J Pharm. Pharm science* 4[3]:222-225.

38. Balkan, J., Dogru-Abbasoglu, S., Kanbagli, O., Cevkbağ, U., Aykac-Toker, G. and Uysal, M. (2010). Taurine has a protective effect against thioacetamide induced liver cirrhosis by decreasing oxidative stress. *Human Express Toxicology* **20**: 251-254
39. Olatunde F (2000). Mechanisms for the hepatoprotective action of Kolaviron. Studies on hepatic enzymes, microsomal lipids, and lipid peroxidation in carbon tetrachloride treated rats. *Pharmacol. Res.*, 42: 75-80.
40. King PR and King EJ (1954). Estimation of alkaline phosphatase. *Journal Clin. Path.*, 7: 322-323.
41. Anusuya N, Sellamuthu M, (2013). Antioxidant and free radical scavenging potential of different solvent extracts of *Indigofera tinctoria* L. leaves. *Int. J. PharmPharm. Science*(1):142-147.
42. Ali, F. U Afiukwa, C. A., Oko, A.O (2013). Nutritional and antinutritional characterization of two wild yam species from Abakaliki, Southeast Nigeria. *Research. Journal of pharmaceutical ,biological and chemical sciences* volume 4:840-848.
43. Afiukwa, C A, Ali, F. U. and Oko, A. O. (2013). Proximate and mineral element compositions of five edible wild grown mushroom species in abakaliki, southeast Nigeria. *Research Journal of Pharmaceutical ,Biological and Chemical Sciences* volume 4: 1056-1064.
44. Ali, F. U, Oko, A O, Afiukwaca, Ahamefule, ka, Ezeonu, O. A, and Nwoba, S. T. (2013). Evaluation of effects of supplementation of sun-dried leaves of *Mucunapoggei* on the growth and carcass characteristics of broiler birds. *American journal of life sciences*1(3 ):82-87.
45. Ali, F. U, and Ibiam, U. A. (2014). Phytochemical studies and Gc-MS analysis of *Gongronema latifolium* and *Piper guineense*. *International journal of innovative reseach and development*. volume 3:108-115.
46. Ali, F. U, Ominyi, M. C. and Ogbanshi M.E. (2015). Comparative evaluation of effect of *Gongronema latifolium* and *Piper guineense* ethanol extract against scavenging enzymes and marker of oxidative stress in ethanol induced liver injury in wistar rats. *International journal of innovative reseach and development*. volume 4(1):61-71.

47. Ali, F. U., Ominyi M.C., Ogbanshi M.E and Eze U.S (2014). Phytochemical analysis of *Spondias mombin*. *International Journal of Innovative Research and Development*. volume **3**:101-107.
48. Ali Fredrick U, Ominyi MC, Nwankwo OVU, Ibiam U.A and Ogbanshi M.E(2015). Comparative effects of ethanolic extract of *Gongronema latifolium* and *Piper guineense* on blood electrolytes in ethanol exposed wistar rats. *Journal biochemistry and analytical biochemistry open access***4(2)**:171-175.
49. Ali , Fredrick U,U.A Ibiam ,Ominyi ,M. C ,Nwankwo O.V.U Ogbanshi M.E and Eze U.S (2015). Evaluation of effect of ethanol extract of *Gongronemalatifolium* and *Piper guineense* on lipid profile in ethanol intoxicated wistar rats. *Biochemistry and physiology: Open access*.vol4:3:171.
50. Moses E.Ogbanshi,Kingsley N.Agbafor,Benjamin S. Nwanze, Celestine,O.Ogbu,Anayo J.Uraku Stanley c. Onuoha and Ali Fredrick U.(2014). Biochemical changes in albino rats exposed to rice husk dust at Abakaliki rice mill industrial area. *International journal of biochemistry research and review*.**4(6)**:644-652.
51. Ogbanshi, M. E., Agbafor, K. N. Ominyi,C.M., Ugwu Okechukwu, P. C., Nwali , B. U. and F.U. Ali.changes in reproductive functions of adult male rats administered water and salt samples from Okposi and Uburu Nigerian Salt Lakes .*American- Eurasian Journal of Toxicological Sciences* **7 (2)**: 55-62, 2015.