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Histopathology Based Comparative Evaluation of Effect of *Piper guineense and Gongronema latifolium* Ethanol Extract on Liver and Kidney of Wistar Rats Exposed with Seventy Percent (70% V/V) Ethanol

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Abstract: The present work studied the effect of ethanol extract *Piper guineense* and *Gongronema latifolium* on the structure and function of the liver and kidney of wistar rats exposed with 70 % ethanol. The animals were divided into three groups; A, B and C. Group A given distilled water and rat feed only, served as the normal control, Group C was subdivided into four C1, C2, C3 and C4. Group B-C were exposed with 70% v/v ethanol for seven days to evoke pathological lesions in cells. Group B was not treated and served as the negative control, while Group C were further treated with 200, 400, 600 and 800 mg/kg, respectively for 21 days and then sacrificed. Tissues were harvested and processed for photomicrographic examinations. Thereafter, Group A kidney showed normal structure, the kidney cortex of Group B showed many histopathological alterations. The renal tubules lost their characteristic appearance and their lining epithelial cells appeared with ghost of glomeruli, tubular cast and necrosis were observed. Glomeruli and the renal blood vessels were degenerated and congested, respectively. The intertubular spaces were infiltrated by inflammatory leucocytic cells. Severe mononuclear leukocyte infiltration of oedemateous surrounding, mild lymyphocytic infiltration around the biliary duct of the portal areas were observed in the liver section of rats of the same Group. The positive control rats had kidney sections with normal tubule and glomeruli. Normal texture of kidney and hepatic cells were observed. Treating exposed animals with *Piper guineense* and *Gongronema latifolium* extract led to significant ($p<0.05$) restoration in the histological structure of the kidney and liver in a dose dependent fashion. The results indicate that *Piper guineense* and *Gongronema latifolium* have ameliorative effect against kidney and liver damage induced in wistar rats.

Key words: *Piper guineense* · *Gongronema latifolium* · Histopathology · Kidney and Liver

INTRODUCTION

Plants have medicinal properties which make them useful for the treatment of some diseases [1]. However, traditional medicine has maintained its popularity in all regions of the developing world and its use is rapidly increasing in industrialized countries [2]. *G. latifolium* belongs Benth Hook, (Asclepiadaceae) is an herbaceous shrub, with yellow flowers and the stem that produces 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 [3]. The Efiks and Quas in Calabar use *G. latifolium* crude leaf extract in the treatment of malaria, diabetes and hypertension and as laxative. Also it is used as a spice and vegetable [4]. The use of crude leaf extract of this shrub in maintaining healthy blood glucose levels have been reported [5]. Scientific studies have established the hypoglycemic, hypolipidaemic and antioxidative effects of aqueous and ethanol extracts of *G. Latifolium* leaf [6]. Research showed that the leaf extract has anti-inflammatory properties while its potential nutritional and food processing [7, 8].

Piper guineense, popularly known as African black pepper is widely consumed in some part of West Africa example Nigeria and Ghana as result of its nutritional and medicinal properties [9]. It belongs to the family Piperaceae [10]. The seeds are put into a variety of uses in traditional herbal medicine, for instance, in some parts

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of Nigeria, the seeds are consumed by women after delivery, to enhance uterine contraction for the expulsion of placenta and other remains from the womb [11], as well as an adjuvant in the treatment of rheumatic pains [12]. The liver has a central role in the maintenance of lipid homeostasis and the kidney also has similar work in maintaining body pH, however the presence of toxicants may alter the concentration of serum lipids which could increase the risk of atherosclerosis. Popularity of herbal remedies is increasing globally and at least one quarter of patients with liver and kidney diseases use ethnobotanicals [13]. More efforts need to be directed to-wards 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 [14]. 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 it was aimed to compare the ameliorating effect of *G. latifolium* and *Piper guineense* ethanol extract in wistar rats exposed with ethanol.

MATERIALS AND METHODS

Animals: Twenty-four Wister male albino rats (weighing150-170g) where obtained from the Animal House of the of Veterinary Medicine, UNN Nsukka, 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.

Plant Materials: *G. latifolium* and *Piper guineense* leaves were purchased from Abakpa market in, Abakaliki metropolis (Nigeria), identification and authenticated by Prof. S. E. Okafor in Botany unit Department of Biology Ebonyi State University Abakaliki, where voucher samples were kept for reference. The leaves were air dried at room temperature, then pulverized into uniform powder using an electric blender 25-28°C, sieved with 2 mm sieve size and packed in airtight bottles and stored until required for extraction.

Preparation and Administration of the Extract: Two hundred gram (200g) was extracted with 600ml of ethanol by maceration for 72 hours the ethanol extract was filtered and the filtrate was allowed to dry at room temperature. This was carefully scraped into a clean sample bottle and stored in a refrigerator at 4°C and used prepare the concentrations used.

Research Design: Twenty four male wistar rats were randomly distributed into three groups A, B and C. Group A served as the normal control, group B Negative control, C were further subdivided into 4, C1 , C2, C3 and C4. Group B-C were exposed with 70% ethanol (7.0g/kg) for seven days. Group C were treated with 200, 400, 600 and 800 mg/kg of the extract respectively by oral gavage using a cannula for 21 days.

Preparation of Tissues for Histopathological Analyses: After 21 days of treatment the animals were starved 24hours and the sacrificed. The liver and kidney samples were quickly excised, fixed in 10% formal saline.

Histopathology: The method adopted were that described by (Akahori *et al.,* 1983) [15].

Statistical Analysis: All data were expressed as mean ±SD one- way analysis of variance (ANVOA) followed by student's't' test. This was done using software (15.0). Differences were considered significant at $P<0.05$.

RESULTS

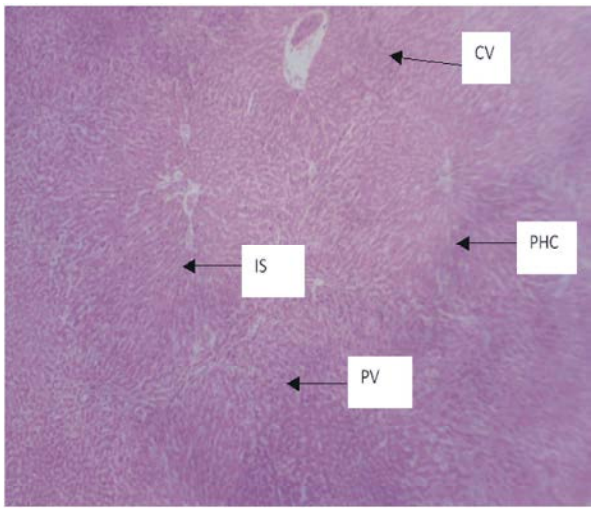

Plate 1: The photomicrograh of Liver Group A(control) x 400mg x40 shows normal liver architecture indicating normal central vein, overall features are consistent with normal liver (H & E stain x400)

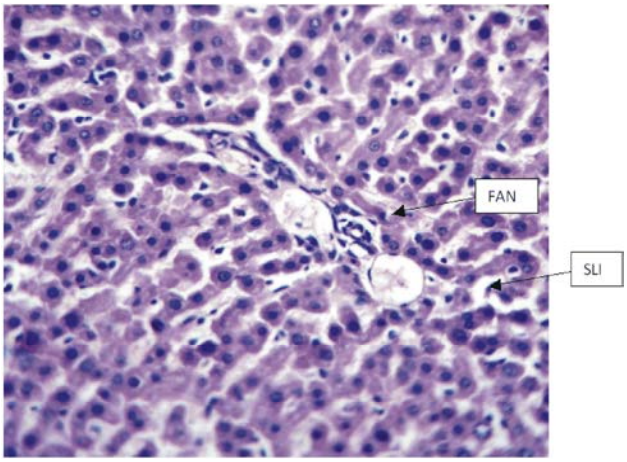

Plate 2: The photomicrograph of Group B Liver (negative control) x400 x40.Rat exposed to 70% EtOH for 7days shows infiltration of the inflammatory cells, the trabecular structure is lightly blurred.The cytoplasm of some cells shows rare empty vacuole-type spaces (H & E stain x400) where FAN = focal area of necrosis, SLI = severe lymphocytic infiltration

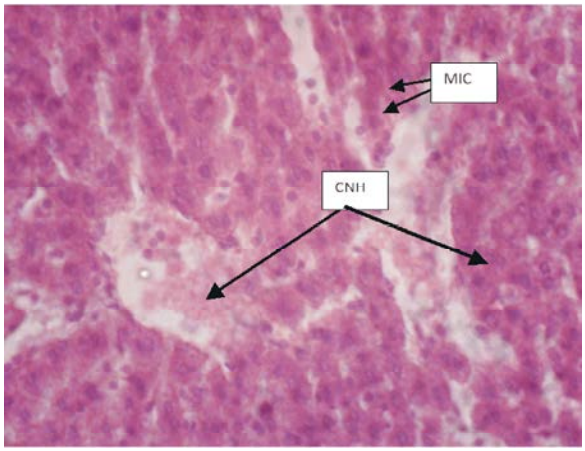

Plate 3: The photomicrograph of rat Liver treated with 200 mg/kg *G.latifolium* x400 mg x40 shows mild inflammatory cells (MIC) and collection of necrotic hypertocytes

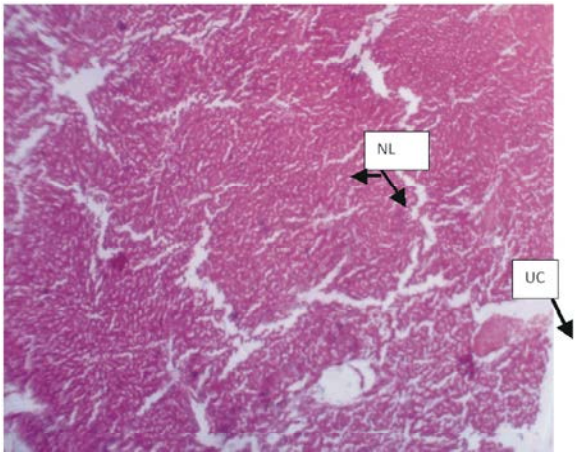

Plate 4: The photomicrograph of rat liver treated with 200 mg/kg of *P. guineense* with normal limits (unremarkable changes)

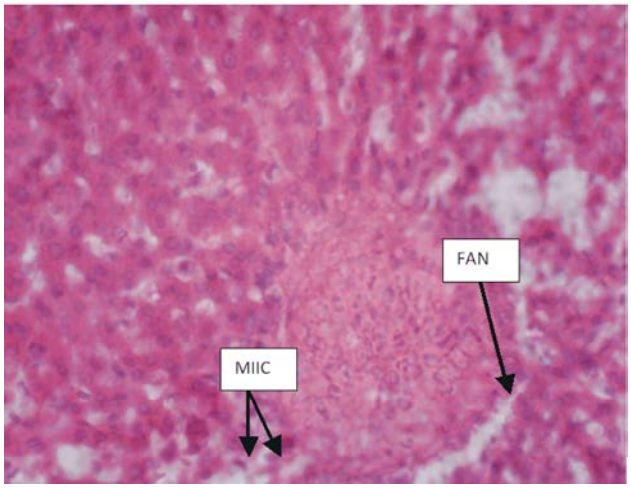

Plate 5: The photomicrograph of rat liver treated with 400mg/kg *G.latifolium* ethanolic extract x400 x40 shows mild infiltrate of the inflammatory cells (MIIC) and focal area of necrosis (FAN) at the hepatocyte

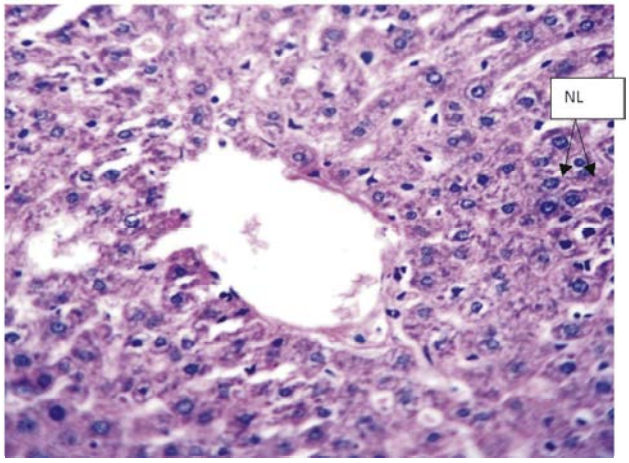

Plate 6: The photomicrograph of rat liver treated with 400 mg/kg *Piper guineense* shows normal limits (unremarkable changes)

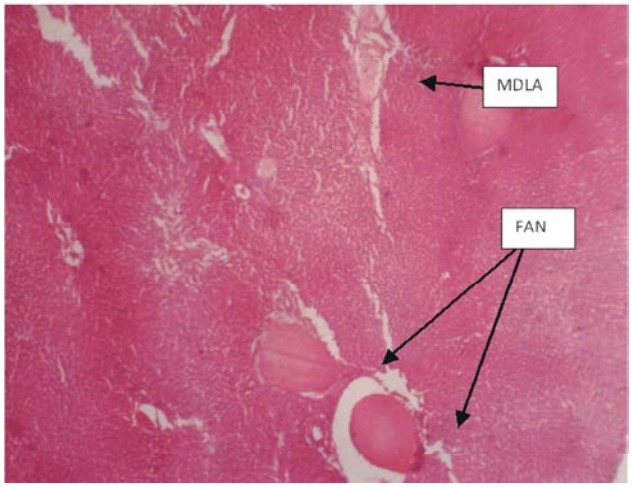

Plate 7: The photomicrograph of rat liver treated with 600mg/kg *G.latifolium* extract x400mg x4 shows the focal area of necrosis (FAN) and mild distortion of liver architecture (MDLA)

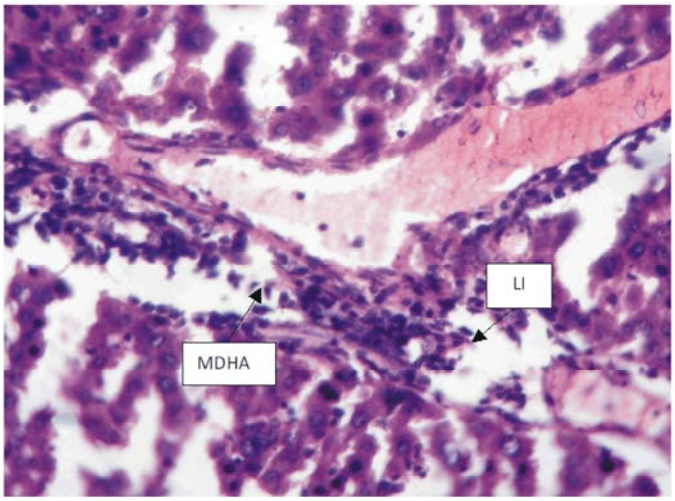

Plate 8: The photomicrograph of rat liver treated with 600mg/kg *P. guineense* shows mild distortion of hepatic architecture (MDHA), modetate to severe infiltration of lymphocytes (LI) within the hepatocytes especially the portal tract (H & E stain x400)

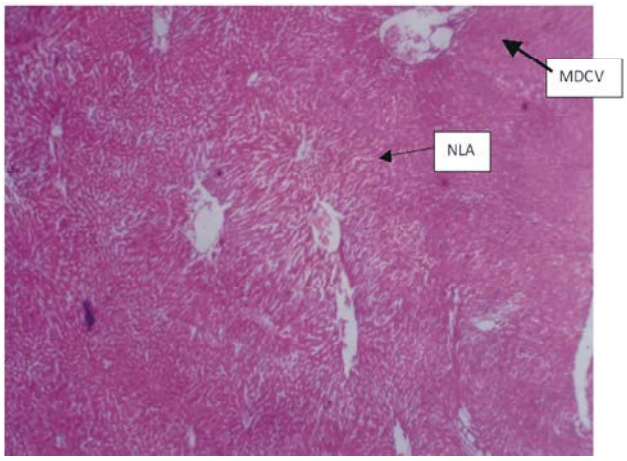

Plate 9: The photomicrograph of rat liver treated with 800 mg/kg of *G.latifolium* x400 x4 shows normal liver archtecture(NLA) and composed of hexagonal lobules with central veins and portal tract

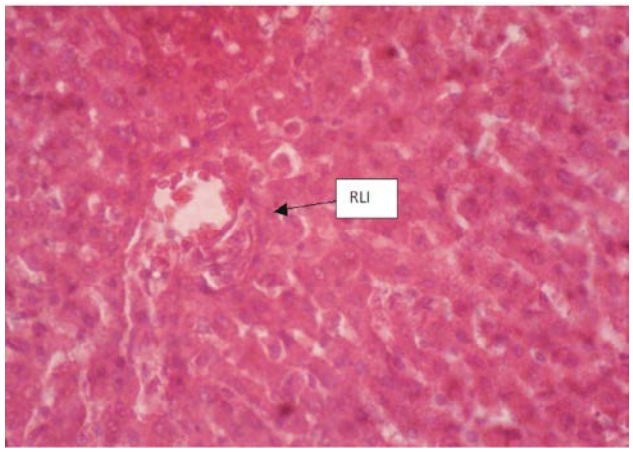

Plate 10: The photomicrograph of rat liver treated with 800 mg/kg of *P. guineense* shows remarkable lymphocytic infiltration of the inflammatory cells (RLI) (H & E stain x 400)

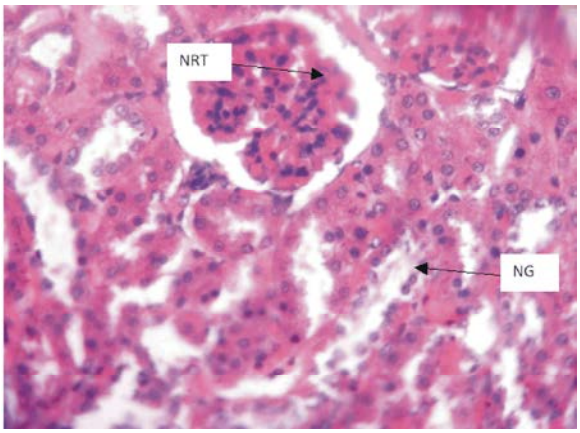

Plate 11: Group A, Photomicrograph of test animal kidney shows normal glomeruli and renal tubule. Features are consistence with normal kidney (H & E stain x 400)

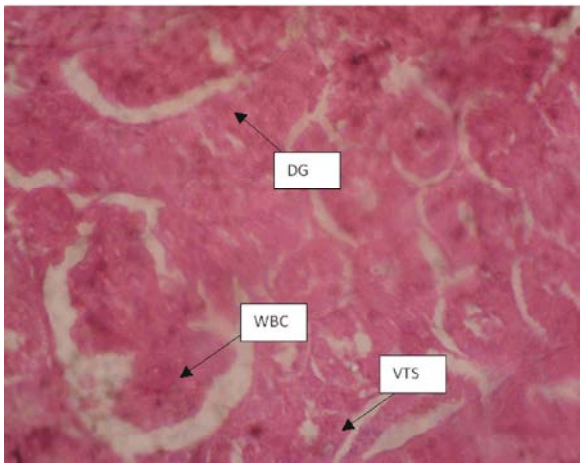

Plate 12: The The photomicrograph of Group B kidney(-ve control) x400 x40.Rat exposed to 70% EtOH for 7days shows distortion of the glomerulus (DG) and widening of the Bowman capsule (WBC). There is a vague and dilation of tubular structure (VTS). Some tubules contain single desquamated cells

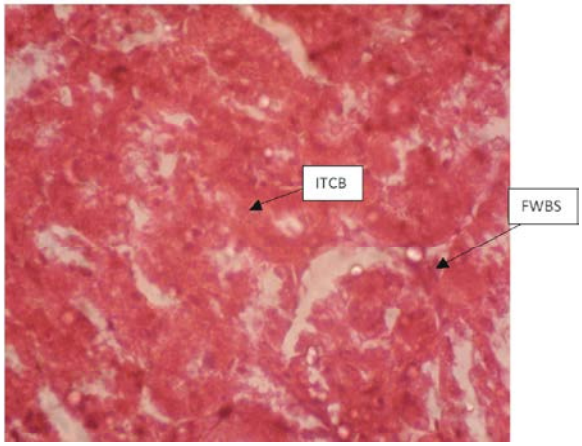

Plate 13: The photomicrograph of rat Kidney treated with 200 mg/kg *G.latifolium* x400mg x40 shows indistent tubules cell border (ITCB) and enlargement of tubules with partial obstruction of the lumen. The epithelial cells that suppose to allign themselves along the tubular membrane are scattered. The glomerulus is contracted giving a focal widening Bowman space (FWBS) and detachment of the tubuli epithelium

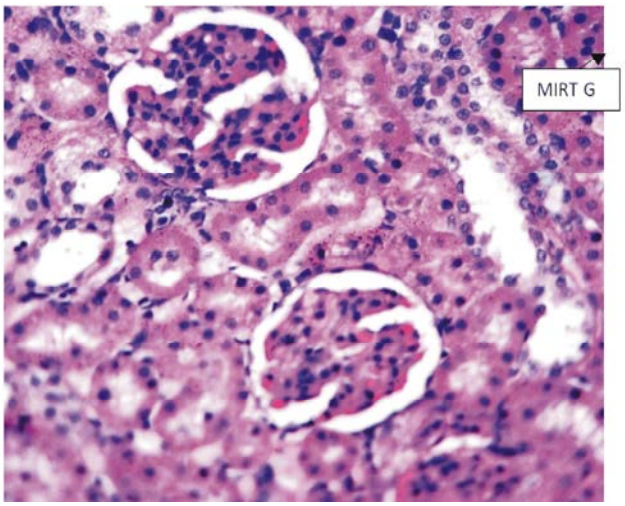

Plate 14: The Photomicrograph of test animal kidney treated with 200mg/kg of *P. guineense* extract shows mild infiltration of the renal tubules and glomeruli (MIRT G) (H & E stain x 400)

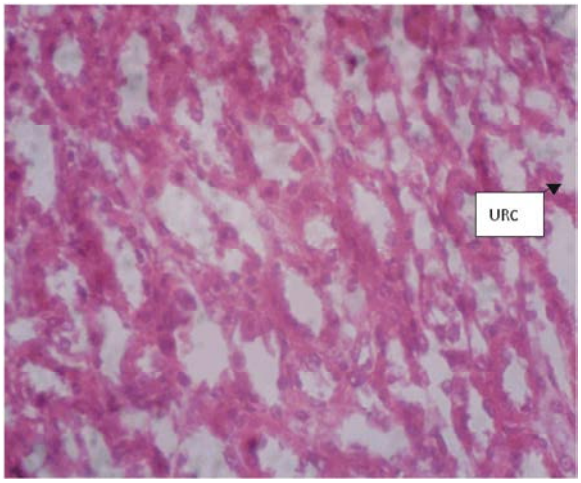

Plate 15: The photomicrograph of rat kidney treated with 400 mg/kg *G.latifolium* extract x400mg x4 shows unremarkable renal changes(URC)

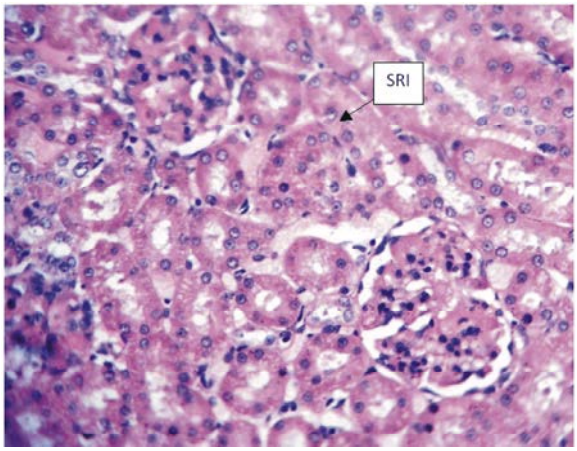

Plate 16: Photomicrograph of test animal kidney treated with 400 mg/kg *of P. guineense* shows scanty renal infiltration (H & E stain x 400)

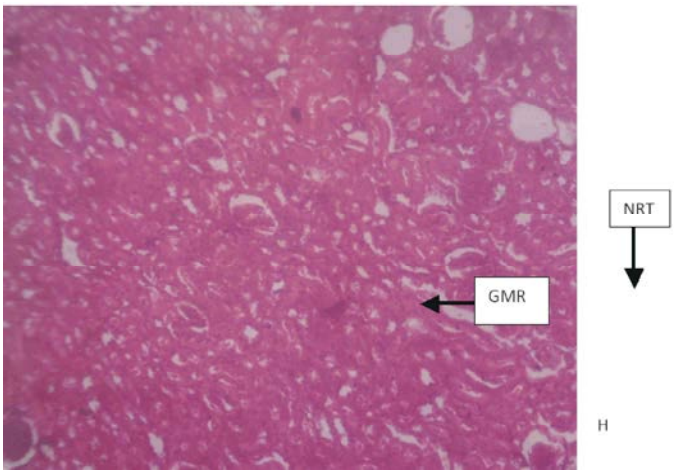

Plate 17: The photomicrograph of rat kidney treated with 600mg/kg *G.latifolium* extract x400mg x4 shows Normal renal tubules (NRT) and glomerular and medulary rays of tubules

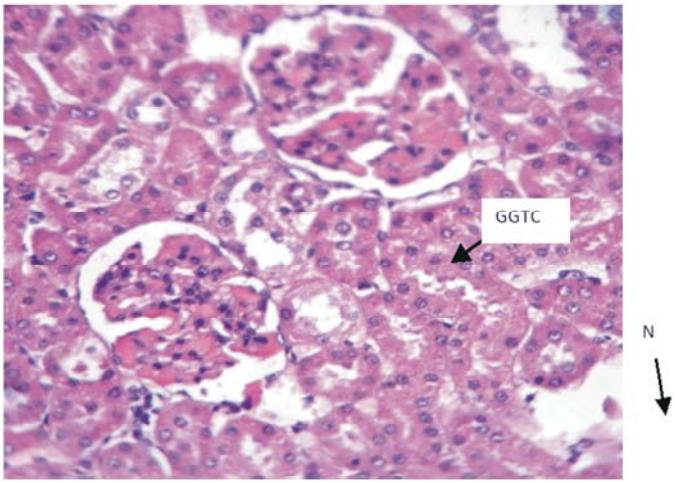

Plate 18: The photomicrograph of rat kidney treated with 600 mg/kg *of P. guineense* showing ghost of glomeruli tubular cast (GGTC) and necrosis (N) (H & E stain x 400)

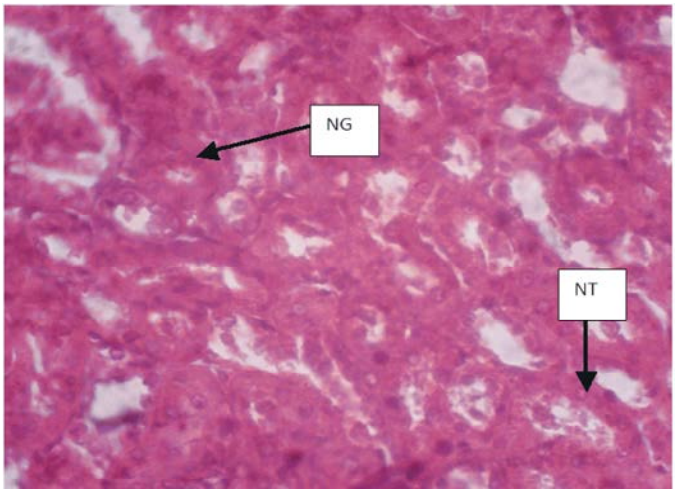

Plate 19: The The photomicrograph of rat kidney treated with 800mg/kg of *G.latifolium* x400 x4 shows normal glomeruli (NG) and normal renal tubules (NT)

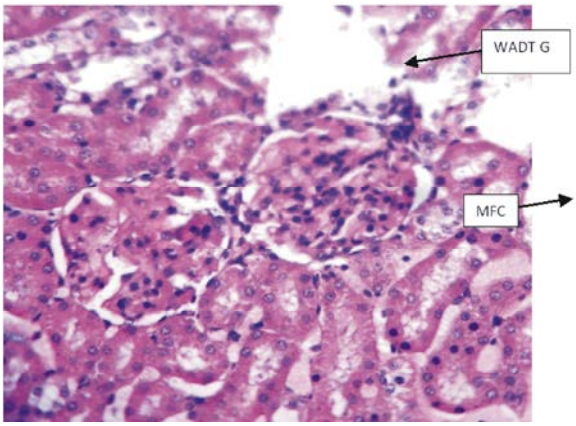

Plate 20: The The photomicrograph of rat liver treated with 800mg/kg of *P. guineense* x400 x4shows ghost of tubular structure and wide area of destroyed tubules and glomeruli (WADTG) with moderate fatty changes (MFC).

Table 1: Summary of Histopathology Results

Extract	Concentration	Tissue	Observation	Comments
	Positive control	Liver	Normal hepatocytes around a central vein	NAD
	Negative control	Liver	Severe lymphocytic infiltration	AD
	200mg/kg	Liver	With normal limitsunremarkable changes	NAD
Piper guineense	400 mg/kg	Liver	With normal limitsunremarkable changes	NAD
	600 mg/kg	Liver	Mild lymphocytic infiltration	AD
	800 mg/kg	Liver	Remarkable lymphocytic infiltration	AD
	Positive control	Liver	Normal hepatocytes around a central vein	NAD
	Negative control	Liver	Severe lymphocytic infiltration	AD
	200mg/kg	Liver	Moderate infiltration	AD
G. latifolium	400 mg/kg	Liver	Scanty portal lymphocytic infiltration	AD
	600 mg/kg	Liver	Normal lymphocytes around venule	NAD
	800 mg/kg	Liver	Normal central vein , portal vein and portal tract	NAD

Key: NAD-No abnormal discovery; AD-Abnormal discovery

Table 2: Summary of Histopathology Results

Extract	Concentration	Tissue	Observation	Comments
	Positive control	Kidney	Normal tubule and glomeruli	Normal texture of kidney observed
	Negative control	Kidney	Dilated tubules lined by thinner epithelium	Widened capsular space, degeneration and necrosis of renal tubular epithelia
	200mg/kg	Kidney	Mild infiltration of the renal tubules and glomeruli	NAD
Piper guineense	400 mg/kg	Kidney	Scanty renal infiltration	AD
	600 mg/kg	Kidney	Ghost ofglomeruli tubular cast and necrosis	AD
	800 mg/kg	Kidney	Ghost of tubular structures and wide area of glomerular	AD
	Positive control	Kidney	Normal tubules and glomeruli	Normal texture of kidney observed
	Negative control	Kidney	Dilated tubules lined by thinner epithelium	AD
	200mg/kg	Kidney	Shrinkage of the glomeruli, widening of the bowmans space, detachment of the tubuli epithelium	AD
G. latifolium	400 mg/kg	Kidney	Unremarkable renal changes	AD
	600 mg/kg	Kidney	Normal renal tubules and glomerular and medulary rays	NAD
	800 mg/kg	Kidney	Normal renal tubules	NAD

Key: NAD-No abnormal discovery; AD-Abnormal discovery

DISCUSSION

The results of the present study demonstrated the synergistic effects of *G. latifolium* and *Piper guineense* ethanol extract on kidney and liver of rats exposed with ethanol. No histological change was observed in the kidney of the positive control rats fed with normal feed. The positive control rats had kidney sections with normal tubule and glomeruli. Normal texture of kidney observed (Plate 1). Exposing of wistar rat with ethanol induced

many histopathological changes in the kidney and liver. The renal tubules, glomeruli as well as the hepatic cells were affected (plate 2). These alterations are in accordance with previously work by some investigators under the effect of different fungicides [16]. Akuodor *et al*. [17], showed that when male and female rats were exposed to mancozeb fungicide, CCL_4, the kidney showed tubular dilation, necrosis and congestion of blood vessels whereas the liver showed centrilobular necrosis, scanty portal lymphocytic infiltration with extramedulary haemopoiesis [18]. Exposing rabbits to other toxicant such as maneb and zineb fungicides have been reported to cause tissue injury, blood congestion and mononuclear inflammatory cell infiltrations in the liver and kidney [19]. These pathophysiological changes as recorded in this work (plate 2) were consequence of decreased glomerular filtration which, in the present work, could be attributed to have developed due to atrophy of glomeruli. It may also be as a result of decrease in renal tubule reabsorption due to degeneration of tubular epithelial cells and degeneration of hepatic cells as observed. Thus, leading to their des-desquamation with the appearance of proteinaceous debris in the lumen tubules [20].

Histological analysis of albino rats liver and kidney treated with ethanol extracts of *Gongronema latifolium* and *Piper guineense* leaves at 200, 400, 600 and 800 mg/kg doses restored nephrotic architectures and hepatic cells significantly towards normal in a dose dependent fashion (Plate 3-20; Tables 1-2). There were mild distortions or inflammatory cells observed in group treated with high dose of *Piper guineense* compared to that of *G. latifolium* and normal control. This is in agreement with those obtained by Anaso and Onochie (1999) [20]. Treatment with different doses of the *G. latifolium* and low doses of *Piper guineense* extract was well tolerated by all the rats, as there were no toxic effects observed by direct visual observation of the rats throughout the experiment. There was no death and apparent behavioral changes recorded during the course of the experiment in all *G. latifolium* treatment groups as compared to the control group whereas toxic changes were observed at high doses of *Piper guineense*. This might suggest the non-toxic effect of the extracts at these concentrations. According to Anders and Jakobson (1985) [21], established that *G. latifolium* was not toxic even at higher doses showing why the plant is edible and use as medicinal plant. Furthermore anders and Jakobson (1985). [22], reported that the adverse effects of *Piper guineense* at high concentration. In addition, rats administered with all the doses of *G. latifolium* extracts did not show any morphological changes in the liver cells. This might be substantiated by (Anderson *et al*., 1991) [23]. This is in agreement with the *in vitro* cytotoxicity study done by (Antai *et al*., 1990) [24], who reported that an ethanol extract of leaves from *Piper guineense* and *G. latifolium*on hepatocytes did not affect cell viability. Furthermore, no significant histopathological changes were observed in the kidneys of the rats treated with all doses (Table 2). The liver and kidney pathology showed that no significant lesions were observed in this study in dose dependent fashion and this may point to the fact that these plants are relatively safe for use nutritionally and medicinally [25]. Also, the results of this study have shown the rationale for the folkloric use of the ethanol extract of *Piper guineense* and *G. latifolium* in the treatment of renal and liver disorders. Hence, this study suggest the use of *Piper guineense* and *G. latifolium* extract in the treatment of diseases associated with exposure of animal models to toxicants such as ethanol.

CONCLUSION

The results of this study indicated that *G latifolium* and *Piper guineense* extract ameliorated ethanol induced nephrotoxicity and hepatotoxicity in wistar rats.

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