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EFFECT OF AQUEOUS EXTRACT OF *GANGRONEMA LATIFOLIUM* (G. LATIFOLIUM) (UTAZI) ON N-ACETYL-PARA-AMINOPHENOL (ACETAMINOPHEN) - INDUCED HEPATIC INJURY ON WISTAR ALBINO RATS

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ABSTRACT

The present study investigated the effect of leaf extract of *Gangronema latifolium* (G. latifolium) on acetaminophen (APAP) - induced liver injury in Wistar albino rats. In this study, sixty (60) male Wistar albino rats were divided into five (5) groups of twelve (12) rats each. Animals in group 1 served as control group and received a placebo of 0.9% saline solution. Group 2 served as APAP control group, administered with 800 mg/kg body weight of APAP only. Groups 3, 4 and 5 served as the experimental groups and received oral dosage of 800 mg/kg body weight of APAP plus 150 mg/kg, 200 mg/kg and 250 mg/kg body weight of G. latifolium respectively. The results showed that the enzymatic activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) in the serum were decreased significantly ($p \leq 0.05$) in the experimental groups dosed with 150 mg/kg, 200mg/kg and 250 mg/kg of G. latifolium respectively. For 150 mg/kg G. latifolium treated group, ALT decreased from 23.3 ± 7.31 to 9.00 ± 1.52 IU/L, while AST and ALP decreased from 17.6 ± 2.66 to 15.00 ± 1.00 IU/L and 92.8 ± 2.34 to 83.8 ± 7.94 IU/L respectively. In conclusion, the results showed that aqueous extract of G. latifolium has a protective effect on rat liver induced with APAP injury.

Key words: Acetaminophen, liver enzymes, G. latifolium.

INTRODUCTION

Acetaminophen (N-acetyl-para-aminophenol (APAP) is non-steroidal antipyretic drug, and its overdose is the most common cause of acute liver failure in the West. In mice, APAP hepatotoxicity can be rapidly induced with a single dose. Because it is both clinically relevant and experimentally convenient, APAP intoxication has become a popular model of liver injury (McGill *et al.*, 2012). Though APAP is beneficial at therapeutic doses, centrilobular hepatic necrosis can result from higher doses, leading to fatality (Hinson *et al*., 2010). As can be expected, APAP poisoning accounts for approximately one-half of all cases of acute liver failure in the United States and Great Britain (Larson *et al.*, 2005), accounting for a very high percentage of inquiries to poison control centers and deaths respectively Litovitz *et al.*, 2002).

G. latifolium is a tropical rain forest plant found throughout Nigeria and other tropical countries such as Guinea-Bissau, Western Cameroon and Sierra Leone. It has been in use in the traditional system of medicine for treatment of various

gastrointestinal disorders such as diarrhea, ulcers and dyspepsia and in the management of diabetes mellitus (Nwinyi *et al.,* 2008). The leaves have been reported to have a hypoglycaemic effect (Ugochukwu *et al.,* 2003) by decreasing activity of glucokinase enzyme and levels of hepatic glycogen, hepatic and blood glucose. It is rich in fats, protein, vitamins, minerals and essential amino acids (Eleyinmi *et al.,* 2007). Additionally, studies on the phytochemical properties of G. latifolium proves that the root contains polyphenols in abundance, alkaloids, glycosides and reducing sugars (Antai *et al*., 2009).

The ethanolic extract of G. latifolium leaves is reported to process antioxidant activity by increasing superoxide dismutase and glutathione peroxidation, and increases the gluthathione/gluthathione disulphide ratio (Ugochukwu *et al*., 2003) and increases white blood cell counts and haemoglobin concentrations in normal condition, while the leaves have a strong modulatory effect against hepatocellular damage induced by carbon tetrachloride (Ugochukwu *et al.,* 2003). The plant also has anti-inflammatory property (Morebise *et al.,* 2002) as well as antimicrobial activities against various microbial pathogens (Ogbedi *et al*., 2017), and could be used to reduce weight loss, and haematotoxicity in diabetic subjects (Owu *et al*., 2012). Evidence abound that extract of G. latifolium leaves proffer protective effect against hepatic toxicity in male albino rats, oxidative stress and diabetes mellitus – induced liver injury as there was significant decrease in the activities of ALT, AST, ALP (Imo *et al*., 2015), and an increase in glutathione peroxidase, superoxide dismutase and catalase in the serum and liver tissue homogenate relative to diabetic control (Akpan and Ekpo, 2015).

Being one of the most easily accessible anti-pyretic over-the-counter drug in the world (Bunchorntavakul and Reddy, 2013), studies have implicated APAP in the inducement of liver and kidney diseases on prolonged exposure and high doses in humans and animals (Wang *et al.,* 2017). The toxicity of this substance on organs such as liver and kidney stems from the intermediate produced during its metabolism by the cytochrome P450 enzyme system known as N-acetyl-p-benzoquinone imine (NAPQI) (Leeming *et al.,* 2015). The over utilization of APAP by the Nigerian population to care for, and manage fevers and pains, and the high dosage consumption is cause for concern as regards its hepatotoxic potential. It is against this backdrop and the long use of G. latifolium as a therapeutic remedy that this study was carried out.

The main aim of this study was to determine the effect of G. latifolium on acetaminophen-induced liver injury, and to determine the effective dose capable of inducing significant hepato protective effect and to further evaluate the possibility of toxicity due to oral exposure to G. latifolium.

MATERIALS AND METHODS

Plant collection

Fresh G. latifolium leaves were obtained from Emelego forest, a tropical rain forest in Odual, Abua/Odual Local Government Area in Rivers State Nigeria, and was identified and authenticated by Dr. Chimezie Ekero of the Department of Plant

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Science and Biotechnology at the University of Port Harcourt, Nigeria.

Plant preparation and extraction

The plants were washed with water to remove dirt and soil particles stuck to the leaves. It was then allowed to sun-dry for ten (10) days after which the leaves were removed and ground into fine powdered form using Blender Lab 2 Speed 1L 120V (Thomas Scientific, Swedesboro, USA). One kilogram (1000 grams) of the powder was extracted using Sexhlot extraction system (Rogo-Sampaic, France). The extract was concentrated under reduced pressure to yield a viscous mass. The aqueous extract was kept in air tight container in a deep freezer to maintain at 40 ˚C until it was used.

Preparation of stock (plant extract) solution

3% of crude extract stock was prepared by dissolving 3 g of crude extract in 100 ml of Tween-80. 5 ml of the extract was taken into the sample bottles and labeled 100 mg/kg, 200 mg/kg, and 400 mg/kg respectively. 20 ml of Tween-80 was added to dilute (100 mg/kg) 5 ml extract, 10 ml of Tween-80 was added to dilute (200 mg/kg) 5ml extract and 5ml of Tween-80 added to dilute (400 mg/kg) 5ml extract.

Animal model

In this study, sixty (60) male Wistar albino rats (weighing between 70 -110 g) were obtained from Ritman University Animal Farm, and experiment performed in adherence to institutional guidelines for the care and handling of laboratory animals. Animals were sheltered in cages and allowed to acclimatize for 5 days prior to drug administration, and were fed *ad libitum*. Animals were divided into five (5) groups of twelve (12) rats each. Group 1 served as control group and received a placebo of 0.9% saline solution. Group 2 served as APAP control group, administered with 800 mg/kg body weight of APAP only. Groups 3, 4 and 5 served as the experimental groups and received oral dosage of 800 mg/kg body weight of APAP plus 150 mg/kg, 200mg/kg and 250mg/kg body weight of G. latifolium respectively.

Sacrifice of Animals

The animals were sacrificed 24 hours after APAP was induced on the tenth day. All animals were weighed before sacrifice. Cotton wool was soaked in chloroform and placed in a Bel-Art™ SP Scienceware™ Lab Companion Vacuum Dessicator (Fisher Scientific Inc., Hampton, UK). Each rat was transferred from the cage to the desiccator in turns. After the rat had been properly anaesthetized, the heart was punctured and blood samples from each animal were collected into heparinized bottled.

Biochemical Analysis

ALP, ALT, AST and GGT were assayed according to manufacturer's instruction using Randox Diagnostic kits (Randox Laboratories Ltd., Crumlin, UK) as described by Reitman and Frankel (1957) and Tietz *et al.*, (1983).

Statistical Analysis

All the results were expressed as mean ± S.E.M. and analyzed using analysis of variance (ANOVA) statistical SPSS package (15.0) version (SPSS Inc, USA). Mean was compared for significance and

p-values $\leq$ 0.05 were considered to be statistically significant.

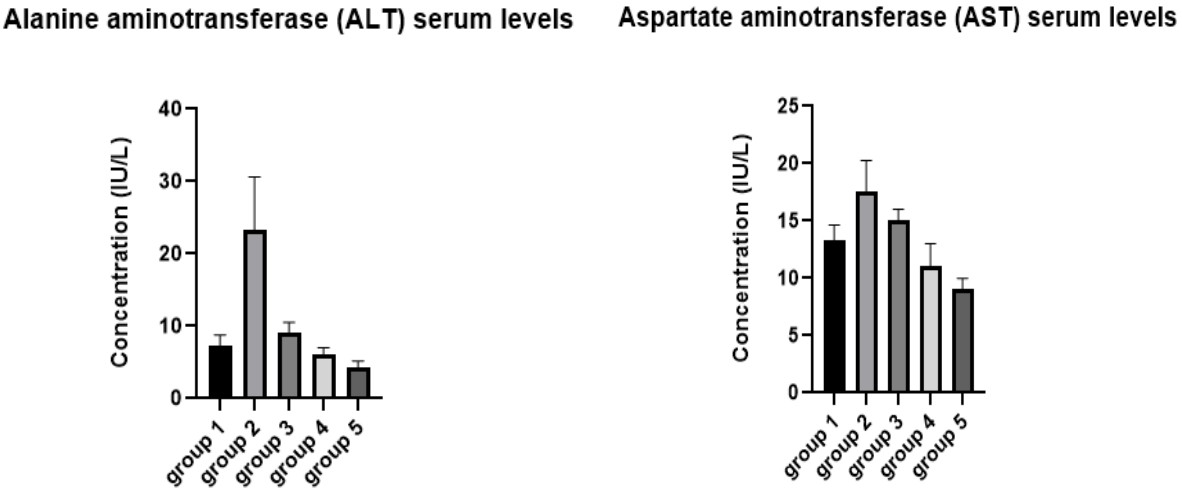
RESULTS

The results of the experiment for the parameters ALT, AST, ALP and GGT are shown in Table 1 below. Notably, ALP and AST in group 1 (control) showed a significant difference when compared with group 2 (APAP control) and G. latifolium treated groups (3, 4, 5). This was also observed for ALP and AST when 250 mg/kg G. latifolium leaves treatment was administered. There was a significant difference when the group 2 was compared with control group and groups 4 and 5 for AST.

Table 1. Mean enzyme concentrations of ALT, AST, ALP, and GGT in male Wistar albino rat liver.

Test Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	GGT (IU/L)
Group 1 (control)	7.33 ±1.45	13.3 ±1.33	81.1 ±8.80	60.2±14.2
Group 2 (APAP control)	23.3 ±7.31[a]	17.6±2.66[a]	92.8±2.34[a]	93.9±6.93[a]
Group 3	9.00 ±1.52[b]	15.0 ±l.00[a]	83.8±7.94[a]	78.8±13.6[b]
Group 4	6.00 ±l.00[b]	11.0+2.00[b]	78.4±3.33[b]	69.1 ±10.0[c]
Group 5	4.30±0.88[c]	9.10±0.88[c]	54.5 ±5.61[d]	45.0±4.61[d]

Results above shows mean ± standard deviation of group serum results obtained (n =12). Values in the same column with different superscript are statistically significant when compared to the control. Values with similar superscripts indicate no statistical difference when compared to the control group.



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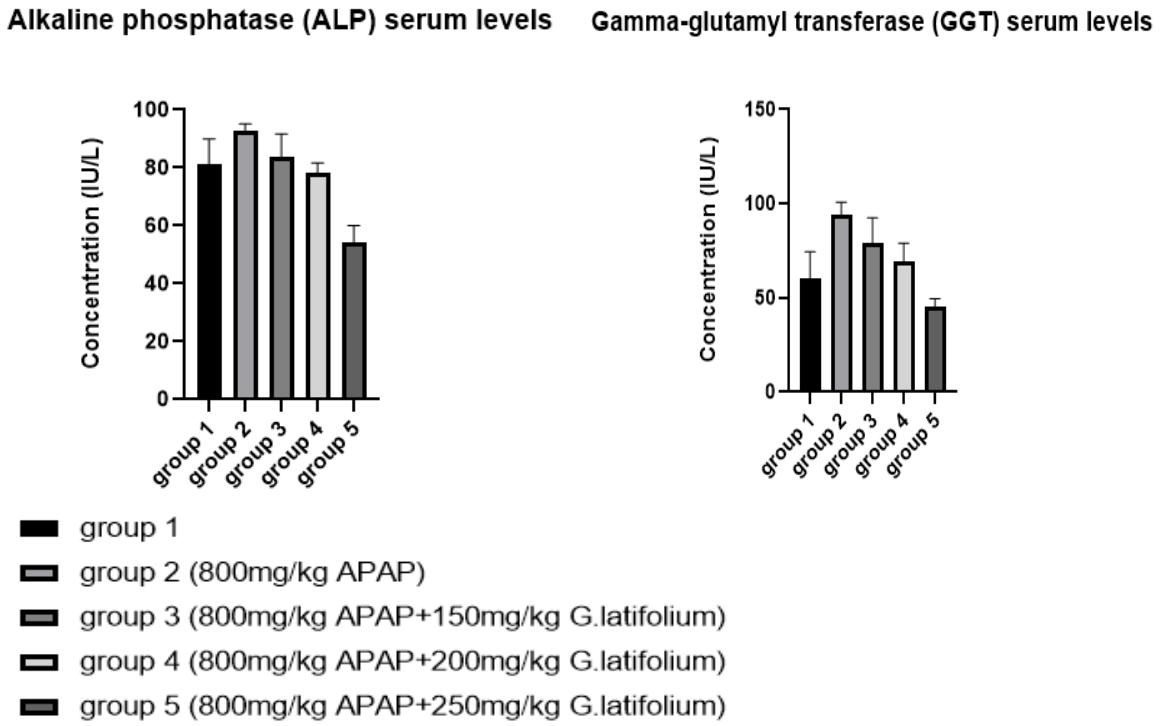

Figure 1 Serum liver enzymes concentration of ALT, AST, ALP, and GGT in 5 groups of male Wistar albino rat's liver following oral administration of APAP and G. latifolium.

DISCUSSION

The commonest enzymes in the diagnosis of hepatocellular damage are the transaminase enzymes which include AST, ALT and ALP (Nnodim *et al.,* 2010). In the present study, oral administration of acetaminophen resulted in significant increase ($p \leq 0.05$) of serum activity of ALT, AST, ALP and GGT enzymes in the rats liver in group 2 (Table 1). Increase in activities of serum enzymes indicates a pathological state resulting to cellular leakage, and if in the liver, causes loss of functional integrity of cell membrane (Edoardo *et al.,* 2005). On the other hand, simultaneous administration of leaf extract of G. latifolium (in APAP-treated rats) (groups 3, 4 and 5) caused a significant decrease in the once elevated activities of the liver enzymes (Figure 1), which is attributed to the fact that the leaf could have efficacy in improving liver toxicity. This is consistent with recent studies which point G. latifolium to not only possessing hypotensive and hypolipidemic activity but also hepatoprotective activity (Ugochukwu and Baddy, 2003; Ugochukwu *et al.,* 2003; Nwanjo, 2005; Nwanjo and Alumanah, 2005). It was deduced that administration of extract of G. latifolium significantly lowered AST, ALT and ALP enzymatic activities when compared with those that received APAP only (group 1) and group 2. This correlates with the work of Etim *et al.,* (2008) and Kumarapp *et al.,* (2011). The impairment in the liver results in increased activities of these liver enzymes. Hence, the mechanism by which G. latifolium lowered liver enzymes may be attributed to their ability to maintain liver cell integrity (Nnodim *et al.,* 2011).

APAP toxicity like many other disease conditions is widely believed to involve the

generation of reactive oxygen species (ROS) and oxidative stress which plays an important role in the liver damage. Free radicals induced lipid peroxidation is believed to be one of the major causes of cell membrane damage leading to a number of pathological conditions (Ayala *et al*., 2014). Also, antioxidants have been linked with the prevention of ROS production and offer protection against acetaminophen toxicity (Du *et al*., 2016). This has led to the evaluation of medicinal plants with free radical scavenging potentials for protective roles against drug induced toxicity. Therefore, G. latifolium has been reported to have antioxidant effect (Ugochukwu *et al.,* 2003). In the APAP control, the activity of ALT liver enzyme increased compared to AST (though not statistically significant), this suggest liver drug toxicity induced by APAP since (AST/ALT ratio approximately equal to one) (Gowda *et al*., 2009; Bayard *et al.,* 2006). Hence, ALT levels is increased in the serum solely due to conditions where cells of the liver have been inflamed or undergo cell death, and is specific for the liver cells but the AST levels can be triggered in other conditions such as myocardial infarction apart from hepatocellular damage (Jensen *et al.*, 2004).

CONCLUSION

This study has demonstrated that aqueous extract of G. latifolium can significantly reduce biomarkers of liver injury. Although, the mechanism through which G. latifolium exerts this therapeutic effect remains unclear, there is need to explore the possible mechanisms by which *G.* latifolium extract lowers liver enzymes and maintains liver cell integrity.

Author Contributions: G.D.C., W.O., conceived and designed the experiments. O.V. contributed to the design of experiments, W.O performed the experiments, and analyzed the data. G.D.C. and W.O. wrote the manuscript with input from O.V.

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Conflicts of Interest: The authors declare no conflict of interest.

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