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Anti-hepatotoxic Effect Of *Gongronema latifolium* In Paracetamol - Induced Hepatotoxicity In Rats

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ABSTRACT

The anti-hepatotoxicity effect of aqueous extract of *Gongronema latifolium* (GLE) on Paracetamol-induced hepatotoxicity was studied in 16 albino Wistar rats. Biochemical examination included assay for the following liver enzymes: alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Liver tissue injury was examined by histological staining of the liver with necrosis of the tissue. Administration of the toxicant (induced) in group B shows that there was significant ($p<0.05$) and rapid increase in the AST, ALT and ALP level (55.80 ± 0.96, 23.07 ± 0.77 and 47.29 ± 1.15 respectively), when compared with the control. Treatment with 100mg/kg GLE (group C) in experimental condition showed that there was significant ($p<0.05$) decrease in the AST, ALT and ALP levels (38.51 ± 1.81, 19.7 ± 0.46 and 37.2 ± 1.56 respectively) when compared with group B (induced only). In addition, treatment with high dose of 200mg/kg GLE (group D), also under experimental condition, shows that there was further decrease in the AST, ALT and ALP level (28.6 ± 0.74, 14.98 ± 0.27 and 32.00 ± 1.38 respectively) when compared with group B and C. Histological examination in group B showed that there were poorly perfused hepatic tissues with cytoplasmic ground glass appearance, moderate infiltration of inflammatory cells, extravasation of red blood cells and clearing of hepatocytes. Treatment with 100mg/kg GLE showed moderate restoration of the damage tissue with mild extravasation of red blood cells, clearing of hepatocytes and mild cytoplasmic ground glass appearance in group C. Treatment with 200mg/kg GLE in group D showed regeneration of hepatic tissue with good perfusion, showing that GLE has anti-hepatotoxicity effect against the liver damage induced by paracetamol.

Keywords: Paracetamol, hepatotoxicity, *Gongronema latifolium*, anti-hepatotoxic effect.

INTRODUCTION

Gongronema latifolium (*G. latifolium*) is a perennial climber crop, native of the humid tropic of south east Nigeria (Okafor, 1989). *G. latifolium*, of family Asclepiadaceae, is a climber with woody hollow glabrous stem below and greenish yellow flowers. *G. latifolium* is locally known as "utazi" by the Igbo, Efik and Ibibio speaking communities in Nigeria. Utazi is primarily used as staples, vegetables or spice. It is used as spice for food like African salad, nkwobi, isi-ewu and pepper soup (Morebise et al. 2002; Ugochukwu and Babady, 2002; Ugochukwu et al. 2003). It has a bitter taste and its phytochemical composition indicates that it contains Saponin (Morebise et al. 2002). *G. latifolium* is also known to have anti-microbial functions (Oshodi et al. 2004; Eja et al. 2010), hepatic protective functions (Etim et al. 2013), renal protective functions (Onuoha and Chinaka, 2013) and pharmacological studies speculate that utazi has both analgesic and anti-sickling properties.

Acetaminophen is a medication used to relieve fever and pain, typically used for mild to moderate pain. It is sold in combination some Opiods. It can also be used for more severe pain such as cancer pain and pain after surgery through oral, rectal or intravenous routes. It produces an Alanine Derivative by hydrolysis which is directly converted into hydroxycamine, N-acetyl-p-Benzoquinoneimine (NAPQI). It is this intermediate of acetaminophen metabolism ,in the presence of cytochrome-p450, that causes hepatic damage (Davidson and Eastham, 1996) in both human and experimental animals (Mudge et al. 1978).

Liver damage can be caused by an acute overdose of Paracetamol. In 2011, the US food and drug Administration (FDA) launched a public education program to help consumers

avoid overdose, warning "Acetaminophen can cause serious liver damage if more than directed is used". In a 2011 safety warning, the FDA immediately required manufacturers to update labels of all prescription combination acetaminophen products, to warn of the potential risk for severe liver injury and required that such combinations contain no more than 325mg of acetaminophen. The overdose risk may be heightened by frequent consumption of alcohol. Paracetamol toxicity in the western world accounts for a large percentage of drug overdoses in the United States, United Kingdom, Australia and New Zealand.

The present study was aimed at evaluating the effect of *G. latifolium* on paracetamol-induced hepatotoxicity in wistar rats. The study intends to reveal the structural and functional defect caused by paracetamol-induced toxicity in man and how *G. latifolium* can be effective in such condition, this is particularly necessary since paracetamol belongs to the class of drugs that can be easily purchased over the counter (OTC) without prescription and hence prone to abuse by most individuals.

MATERIALS AND METHOD

Plant Material

Fresh *Gongronema latifolium* leaf was obtained from a farm land in Awgu LGA Enugu; authenticated, dried and weighed. Maceration was the method used for extraction. The extract obtained was concentrated to 10% of the original volume at 80^0c using water bath evaporator. It was then preserved in sample bottles kept at 4^0c. The extract was dissolved in water before use.

Experimental Animal

16 albino wistar rats were obtained from an experimental animal house at University of Nigeria Nsukka. The rats were weighed and housed in the animal house department of Anatomy, Faculty of Basic Medical Sciences, Enugu State University of Science and Technology, Nigeria. It was allowed to acclimatize for 14 days with access to commercial feed and water in a clean wire cage with 12h/12h light and darkness.

Experimental Design

This study was designed to investigate the in vivo effects of *Gongronema latifolium* aqueous extract on histopathological changes of hepatic tissue and biochemical biomarkers, indicating hepatocellular injury induced by paracetamol in the adult albino wistar rats by oral ingestion of 800mg/kg of paracetamol in each group.

1. **Group A:** (Negative Control) Animals received normal diet and water.
2. **Group B:** (Negative Control) Animals received single daily dose of Paracetamol for two weeks
3. **Group C:** Animals received 100mg/kg body weight of GLE +Induced.
4. **Group D:** Animals received 200mg/kg body weight of GLE +Induced.

The treatment lasted for 14 days, on the 15^{th} day the rats was sedated with chloroform, blood samples was collected from the heart with the use of sterile syringe for each rat and transfer into a non-heparin and anti-coagulant sample bottles for further biochemical analysis. The liver was surgically removed and suspended in 10% buffered formaldehyde for further histological analysis.

Determination of Liver Assay

Biochemical analysis was carried out to determine the activities of the liver enzymes such as AST, ALT and ALP using diagnostic kits. alanine transaminase and aspartate transaminase were determined based on colorimeter measurement of hydrazone formed with 2, 4 dinitrophenyl hydrazine (Reitman and Frankel, 1957), Alkaline Phosphatase by the phenolphthalein monophosphate method (Babson, 1965).

Histopathological Studies

10% buffered formaldehyde was freshly prepared and the liver of control and treated were fixed in 10% buffered formaldehyde for 72 hours. It was subsequently dehydrated in alcohol ranging from 50%-absolute alcohol for 30mins each, cleared in xylene 3 times, 30 mins each, and embedded in molten paraffin wax till it solidified. It was mounted on woody blocks, sectioned with a microtome to about

5micrometer and mounted on glass slides. Finally, it was stained with Haematoxlyin and Eosin.

Statistical Analysis

The data obtained from the study was statistically analyzed using SPSS version 20.0 and the results obtained were reported as Mean ± standard deviation (± SD), differences were considered significant at p‹0.05.

RESULTS

The empirical result values were expressed as Mean

Table 1: Liver enzymes activities of rats in the different groups.

Groups	AST	ALT	ALP
A: Negative Control	20.93±0.25	11.07±0.68	30.45±1.34
B: Positive Control	55.80±0.96	23.07±0.77	47.29±1.15
C: 100mg/kg GLE + induced	38.51±1.81	19.70±0.46	37.26±1.56
D: 200mg/kg GLE + induced	28.63±0.74	14.98±0.27	32.00±1.36

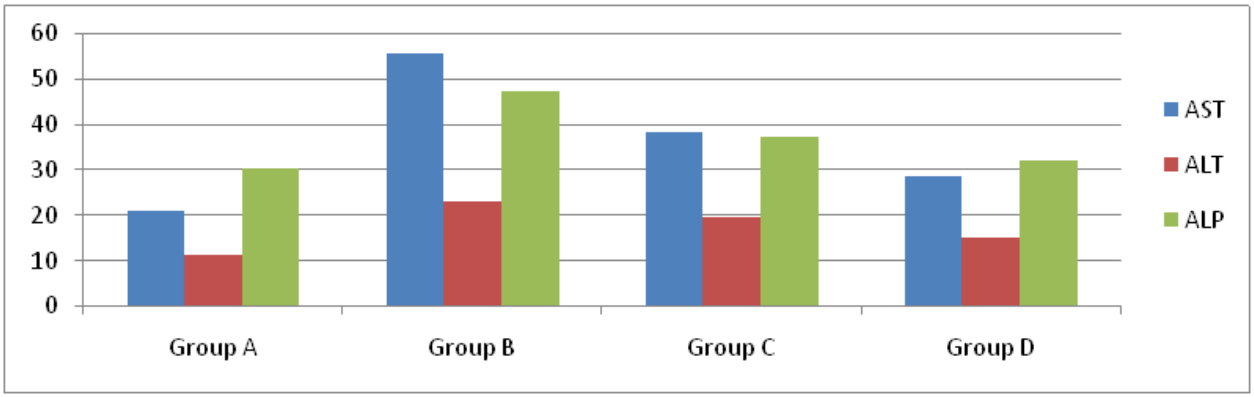

Fig.1: AST, ALT and ALP levels of animals in the different groups.

Values are Mean ± SD (n=2);

p<0.05= (statistical significant difference between group A and group B).

p<0.05= (statistical significant difference between group B and group C, D)

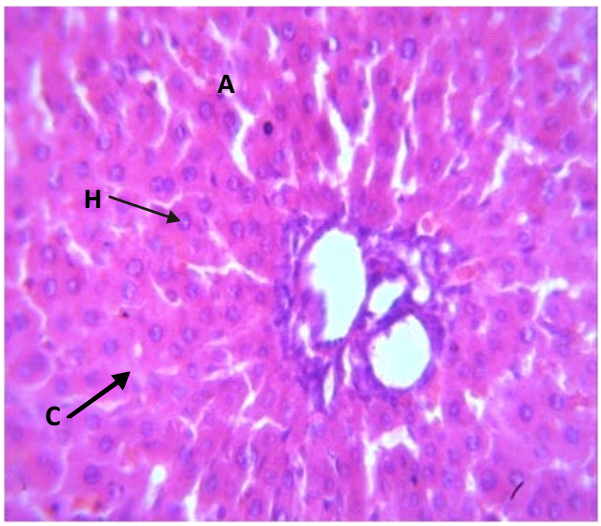

Fig 2: Micrograph of control section liver (x400) (H/E)
It shows normal hepatic architecture with, Cytoplasm (C) and hepatocyte (H) that appears normal

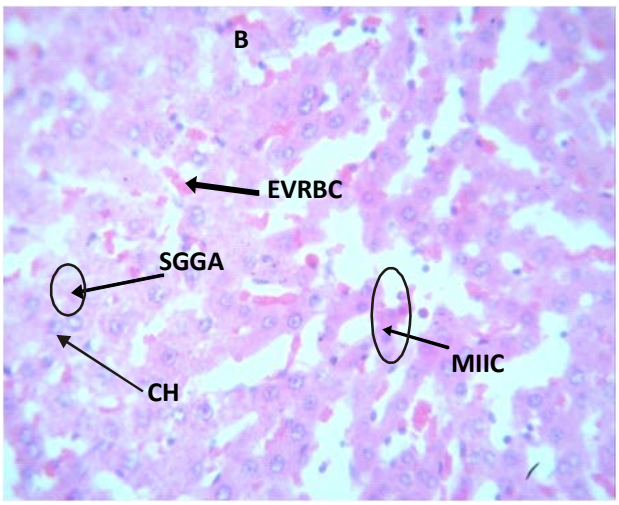

Fig 3: Micrograph of group B section of liver induced with800mg of Paracetamol for 14 days (x400) (H/E).
It shows poorly perfused hepatic tissue with severe cytoplasmic ground glass appearance (SCGGA) moderate infiltration of inflammatory cell (MIIC), extravasation of red blood cell (ERC) and clearing of the hepatocyte (CH).

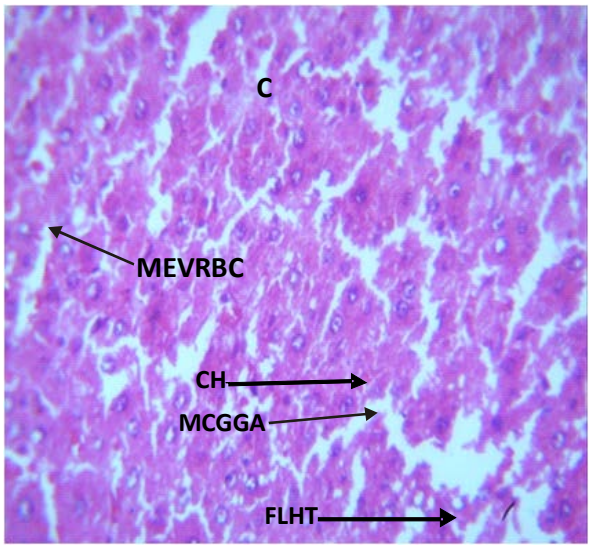

Fig 4: Micrograph of group C section of liver induced with 8oomg of Paracetamol and 100mg/kg of GLE for 14 days (x400) (H/E)
It shows moderate restoration of the damaged tissue with mild cytoplasmic ground glass appearance (MCGGA) mild extravasation of red blood cell (MERC) and clearing of the hepatocyte (CH) and focal loss of hepatic tissue (FLHT).

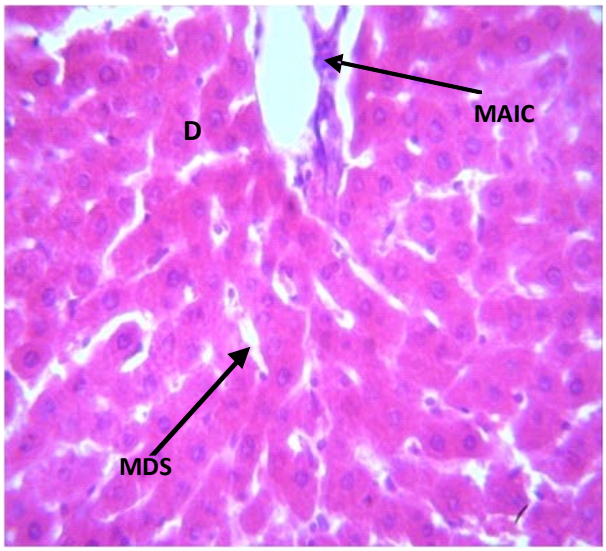

Fig 5: Micrograph of group D section of liver induced with 8oomg of paracetamol and treated with 200mg/kg of GLE for 14 days (x400) (H/E)
It shows well regenerated hepatic tissue with good perfusion and hepatic tissue that appear normal

Despite the efforts put in place to maintain good health, man is still being confronted by a number of disease conditions which are due to exposure to physio-pathological agents (Lambo, 1979). Though the body is built in such a way that helps it tackle any invading foreign substance, sometimes the system is unable or incapable of doing so and needs to be protected, enhanced and activated (Murray *et al.* 1990). The ability to activate the body defense mechanisms or protect the body system has been found to be present in some natural food (Okafor, 1989; Morebise et al. 2002).

This study was aimed at evaluating the effect of *G. latifolium* on the liver toxicity in wiatar rats induced by paracetamol.

G. latifolium has phytochemicals that contain some bioactive compounds like saponin, tannin and flavonoid etc. which have medicinal importance. On the other hand, paracetamol is a commonly used analgesic and anti-pyretic agent but is now recognized as a common cause of the potential devastating clinical syndrome of acute liver failure in many western countries (Schiodt et al. 1997). Acetaminophen, which is also commonly known as paracetamol has been reported to induce a wide spectrum of toxicity especially when taken in large single doses either alone or in combination with an equally large amount of alcohol.

Phyotochemical compositions of *G. latifolium* indicate that it contains saponin (Morebise et al. 2002). *G. latifolium* is known to have an anti-microbial function (Oshodi et al. 2004; Eja et al. 2010), probable anti-hyperglycemic function (Udosen et al. 2012), anti-inflammatory function (Morebise et al. 2002), hepatic protective function (Etim et al. 2008), and anti-renal toxicity (Onuoha and Chinaka, 2013)

The biochemical influence of extract from *G. latifolium* leaf on albino wistar rats with respect to hepatotoxicity with paracetamol, were clearly expressed in group C and D, compared with the positive control (group B).

Result from table above suggest that in group B, there was significant increase ($p<0.05$) in the AST, ALT and ALP level respectively compared with the control. This was as a result of 800mg/kg of paracetamol at a single dose administered to the rats which caused necrosis of liver cells, thereby releasing AST, ALT and ALP into blood stream.

There was moderate reduction of AST, ALT and ALP level in group C compared with

the induced control. This was due to the effect produced by low dose of *G. latifolium* extract which was administered to the rats. Furthermore in group D, there was a mild reduction in the AST, ALT and ALP level in the blood compared with group C and a remarkable reduction of AST, ALT and ALP level compared with induced control. This effect was caused by the higher dose of *G. latifolium* and it suggests that there is an anti-hepatotoxicity effect exhibited by *G. latifolium* leaf.

The histological influence of *G. latifolium* on a toxic liver suggests that there was regeneration and repair of the damaged liver cells in the liver tissue in albino wistar rat induced with paracetamol. The charts show the difference in the degree of effect of *G. latifolium* on AST, ALT and ALP level in blood.

The findings from this present study demonstrate the anti-hepatotoxicity effect of *G. latifolium* leaf aqueous extract against experimental hepatic damage induced by paracetamol. The increase in ALT in group B suggests that there was damage to liver cells which caused its release into the blood. The increase in AST, ALT and ALP are generally produced by cellular necrosis (Brucker et al.1986).

The liver plays a central role in metabolism, transformation and clearing of chemicals in the body and it is susceptible to toxicity from this agents. Certain agents when taken overdose or frequently within therapeutic range may injure organs in the body like the liver and kidney. Paracetamol overdose causes acetaminophen-induced hepatotoxicity and nephrotoxicity (Jackson and Marrow, 2001).

The result from this study shows the therapeutic effect of *G. latifolium* on the liver which can be used as an alternative to other liver pharmacological drugs, based on the anti-hepatotoxicity effect of *G. latifolium* which was reported in this work. The consumption of *G. latifolium* as an anti-hepatotoxicity agent would be preferable by the society because it is cheaper, accessible, common and convenient when compared with other hepatic drugs which are expensive and inconvenient with numerous side effects.

However, further research needs to be done on *G. latifolium* in order to know the particular bioactive element that has anti-hepatotoxicity effect. There is also need for longer duration study.

CONCLUSION

G. latifolium has shown to have good anti-hepatotoxicity effect against the liver damage induced by paracetamol. Further research will be necessary to identify the actual bioactive element and to extrapolate this to realize a commercial quantity and benefit.

REFERENCES

Babson L, Sharon JG, Charles MC, George EP. (1966). Phenolphthalein monophosphate as a substrate for serum alkaline phosphate. Clinical Chemistry. 12. 482-90.

Davidson DG.; Eastham WN. (1996). Acute liver necrosis following over dose of paracetamol.*Br. Med. Journal.* 5512; 497-499.

Eja ME, Arikpo G, Enyi-Idoh KH, Ikpeme EM. (2011). An Evaluation of the Antimicrobial Synergy of Garlic (Allium sativum) and Utazi (*Gongronema latifolium*) on Escherichia coli and Staphylococcus aureus.Malaysian Journal of Microbiology. 7. 45-49.

Etim OE, Akpan EJ, Usoh IF. (2008). Hepatotoxicity of carbon tetrachloride; protective effect of gongronemalatifolium, *Pakistan journal of pharmacological sciences*, 3(21): 268-274.

Jackson-Roberts IL, Morrow JD. (2001). Analgesic-antipyretic and anti-inflammatory agents and drugs employed in the treatment of gout. The Pharmacological Basis of Therapeutics.10th edition. New York: McGraw-Hill Medical Publishing Division. 687-732.

Lambo JO. (1979). The Healing powers of herbs with special reference to obstetrics and gynaecology. In: Sofowora A. (ed). Conference on African Medicinal Plants. University of Ife Press, Ile-Ife, pp.23-30.

Morebise O, Fafunso MA. (1998). Antimicrobial and phytotoxic activities of saponin extracts from two Nigerian edible medicinal plants. Biochemistry.8(2):69–77.

Morebise O, Fafunso MA, Makinde JM, Olajide OA, Awe EO. (2002). Anti-inflammatory property of the leaves of *Gongronema latifolium* . Phytother Res. 16(s1):S75–S77.

MudgeGH.;Gemborys MW.; Duggin GG. (1978).

Covalent bonding of metabolites of acetaminophen to kidney protein and depletion of renal glutathione. J. pharmaco. Exp. Ther. 206; 218-226.

Murray PR, Drew WL, Kobayashi GG, Thompson JH (1990). Medical Microbiology.Wolf Pub. Ltd., USA., pp.696-697.

Okafor JC, Fernandez EC. (1987). Compound farms of Southeast Nigeria. A predominant agro- forestry home garden system with crops and small livestock. Agroforestry systems. 5(2): 153- 168.

Okafor JC. (1989). Tropical plants in health care delivery (Guest lecture delivered on pharmacology society of Nigeria at the university of Nigeria Nsuka).

Omoruyi SI, Enogieru AB, Momodu OI. (2015). Evaluation of hepatoprotective effect of *Uvaria afzelii* against paracetamol induced liver toxicity in wistar rats. *Int. J. Pharm. Sci Res*. 6(7): 2734-2740.

Onuoha SC, Chinaka NC. (2013). Carbon tetrachloride induced renal toxicity and effect of aqueous extract of *Gongronema latifolium* in Wistar albino rats, *drug discovery,* 4(11) pp 15-16.

Oshodi AA, Amoo IA, Eleyinmi AF. (2004). Antimicrobial activity of aqueous extracts of *Vernonia amygdalina*, *Garcinia kola* and *Gongronema latifolium* and their blends on some beer spoilage organisms. Technical Quarterly of the Master Brewers' Association of the Americas. 41(4):398–402.

Schiodt FV, Rochling FJ, Casey DL, Lee WM. (1997). Acetaminophen toxicity in an urban county hospital. N Engl J Med 337:1112-*1117*.

Reitman S, Frankel S. (1957). A colorimetric method for determination of serum glutamate oxaloacetate and glutamic pyruvate transaminase. *Am. J. Clin. Pathol*. 28: 56-58.

Udosen AM, Ugare GU, Ndifon W, Bassey IAE, Oyo-Ita AE, Egba RN, Asuquo M. (2012). Epidemiology of death in the emergency department of a tertiary health centre south-south of Nigeria. African Health Sciences 12:(4): 530-537.

Ugochukwu NH, Babady NE. (2002). Antioxidant effects of *Gongronema latifolium* in hepatocytes of rat models of non-insulin dependent diabetes mellitus. Fitoterapia.73(7-8):612–618.

Ugochukwu NH, Babady NE. (2003). Anti-hyperglycemic effect of aqueous and ethanolic extracts of *Gongronema latifolium* leaves on glucose and glycogen metabolism in livers of normal and streptozotocin-induced diabetic rats. Life Sci. 73(15):1925–1938.

Ugochukwu NH, Babady NE, Cobourne M, Gasset SR. (2003). The effect of *Gongronema latifolium* leaf extract on serum lipid profile and oxidative stress of hepatocytes of diabetic rats. J Biosci. 28:1–5.