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Biochemical and histological changes associated with methanolic leaf extract of *Gongronema latifolium* in acetaminophen-induced hepatic toxicity in wistar albino rats

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Abstract

This study examined the biochemical and histological changes associated with methanolic leaf extract of *Gongronema latifolium* in acetaminophen-induced hepatic toxicity in wistar albino rats. The serum liver enzymes ALT, AST and ALP decreased significantly ($p<0.05$) in the test animals treated with 600mg/kg of the leaf extract. Protein concentration increased significantly ($p<0.05$) in the test animals treated with 600mg/kg of the leaf extract.The effect of the *G. latifolium* leaf extract seems to be dose dependent on the liver enzymes and protein concentration measured. The results showed that acetaminophen-induced hepatic toxicity in the wistar albino rats as observed in the negative control was reversed with the administration of the leaf extract of *Gongronema latifolium* (in groups 3, 4 and 5) in the test animals. The histological analysis of the liver showed that the extract had a normalising effect on the effected liver. These results indicate that the leaf extract of *Gongronema latifolium* exhibits biochemical and histological changes and can be used against some hepatic inflammations.

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Introduction

Gongronema latifolium (whose leaves are bitter) is commonly called "utazi" and "arokeke" in South Eastern and South Western parts of Nigeria respectively. It is a tropical rainforest plant primarily used as spice and vegetable in traditional folk medicine (Ugochukwu *et al.,* 2003).

Phytochemical analysis of leaf extract of *Gongronema latifolium* shows the presence of essential oil, saponins, alkaloids, minerals with calcium, phosphorus, magnesium, copper and potassium (Atangwho *et al.,* 2009). It is a tropical rainforest plant which has been traditionally used in the South Eastern part of Nigeria for the management of diseases such as diabetes and high blood pressure. The presence of phytochemicals (tannins, saponins, alkaloids, flavonoids and hydrocyanide), proximate (crude fat, ash, fat and protein), mineral elements (Cr, Cu, Se, Zn and Fe) and vitamins (A, C, riboflavin, niacin and thiamine) has been reported in the root bark and twig extracts (Egbung *et al.,* 2011). Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc. that is any part of the plant may contain active components (Ibe *et al.,* 2014).

Elevated levels of serum enzymes are inductive of cellular leakage and loss of functional integrity of cell membrane in liver. Since the plant is used variously by traditional/alternative medicine practitioners to manage various ailments, the biochemical and histological changes was therefore studied.

Acetaminophen (Paracetamol) is a widely used over-the-counter analgesic and antipyretic. It is commonly used for the relief of headaches and other minor aches and pains and is a major ingredient in numerous cold and flu remedies (Chinedu *et al*., 2013). The onset of analgesia is approximately 11 minutes after oral administration of paracetamol (Moller *et al.,* 2005), and its half-life is 1–4 hours. Though acetaminophen is used to treat inflammatory pain, it is not generally classified as an NSAID because it exhibits only weak anti-inflammatory activity.

While generally safe for use at recommended doses (1,000 mg per single dose and up to 4,000 mg per day for adults), acute overdose of paracetamol can cause potentially fatal liver damage and, in rare individuals, a normal dose can do the same; the risk is heightened by alcohol consumption. Paracetamol toxicity is the foremost cause of acute liver failure in the Western world, and accounts for most drug overdose in the United States, the United Kingdom, Australia and New Zealand.

Histology studies the microscopic anatomy of cells and tissues of plants and animals. It is commonly performed by examining cells and tissues by sectioning and staining, followed by examination under a light microscope or electron microscope. The ability to visualize or differentially identify microscopic structures is frequently enhanced through the use of histological stains. Histological assessment of the liver, and thus, liver biopsy, is a cornerstone in the evaluation and management of patients with liver disease and has long been considered to be an integral component of the clinician's diagnostic armamentarium (Rockey *et al.,* 2009).

Liver histology may also be very helpful in patients with coexisting disorders such as steatosis and HCV or hemochromatosis or an "overlap" syndrome of PBC with AIH (Zein *et al.,* 2003).

Materials and methods

Plant material

The leaf of *Gongronema latifolium* was harvested at Itaja-Amaegbu Olokoro in Umuahia, Abia State, Nigeria.

The plant was identified at the Department of Plant Science and Biotechnology, Abia State University, Uturu and voucher specimen deposited at the herbarium of the department. The plant material was sun-dried. The dried leaf of *Gongronema latifolium* was milled to a powder. About 250 gm of the powder was extracted with 625 ml of methanol by cold maceration for 48 hours and filtered. The filtrate was evaporated to dryness using a soxhlet extractor and the concentration of the extract determined.

Experimental design

Thirty male albino rats aged 8 weeks and weighing between 120g-130g were used in this study. The animals were randomly placed into five (5) groups of six (6) rats in each group. Group 1 served as the control group and received a placebo of 0.9% normal saline.

Group 2 were treated with acetaminophen (1000mg/kg body weight) only and served as negative control. Groups 3, 4 and 5 received concurrently acetaminophen 1000mg/kg plus 200 mg/kg , 400mg/kg, and 600mg/kg of *G. latifolium* leaf extract respectively. The drug and extract was administered by oral intubation. The treatment lasted for twenty-one days. All animals were allowed free access to food and water *ad libitum* throughout the study. Enas, 2012 reported that 1000mg/kg body weight of rats causes liver damage.

All processes involved in the handling of animals and the experiment was carried out according to standard protocols approved by the animal ethics committee of the College of Medicine and Health Sciences, Abia State University, Uturu

Blood and Liver collection

Forty eight hours after treatment with the leaf extract, the animals were starved overnight, anaesthetized with chloroform and sacrificed. Blood was collected by cardiac puncture and blood samples from each animal collected into dry test tubes. The blood sample was allowed to stand for about 15 minutes to clot and further spun in a centrifuge. Serum was separated from the clot with Pasteur pipette into sterile sample test tubes for the measurement of liver enzymes and protein concentration.

After sacrificing the animals, the liver of a representative of each of the five groups were taken to University of Nigeria Teaching Hospital Enugu for histological analysis.

Biochemical analysis

The serum aspartate transaminase (AST) and alanine transaminase (ALT) were determined as described by Reitman and Frankel, 1956 using Randox Diagnostic kit. Alkaline phosphatase (ALP) was determined as described by Tietz *et al.,* 1983 also using Randox Diagnostic kits. The assays were performed according to the manufacturer's instructions. Serum total protein was determined using Biuret method as described by Henry *et al.*, 1974.

Statistical analysis

Data were expressed as mean ± standard deviation. The statistical evaluations of the data were carried out with the use of standard student T distribution test and mean was compared for significant at ($p \leq 0.05$).

Results

The results of the study are presented in the table and figures below.

Table 1: Protein concentration and liver enzymes concentration

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
Protein (g/dl)	8.43 ± 0.42	3.68 ± 0.24 ^{d}	6.89 ± 0.25 ^{c}	7.29 ± 0.30 ^{b}	8.33 ± 0.26 ^{a}
ALT (U/L)	27.38 ± 3.80	35.38 ± 1.21^{a}	32.97 ± 2.50 ^{a}	30.77 ± 1.62 ^{b}	28.35 ± 1.56 ^{c}
AST (U/L)	34.32 ± 1.97	45.98 ± 3.37 ^{a}	38.42 ± 0.96 ^{b}	36.43 ± 1.34 ^{c}	35.75 ± 1.94 ^{c}
ALP (U/L)	65.17 ± 1.29	84.33 ± 2.03 ^{a}	77.43 ± 1.90 ^{b}	71.70 ± 1.70 ^{c}	65.92 ± 3.49 ^{d}

* Results represent mean ± standard deviation of group serum results obtained (n=6).
Values in the same row with different superscript are statistically significant.

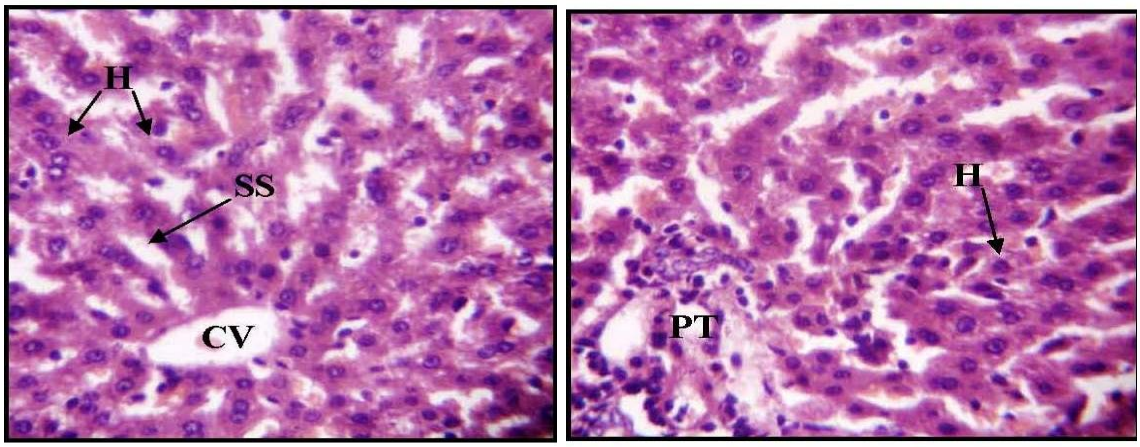

Fig. 1. Light photomicrograph of liver sections from Control rat (group 1).

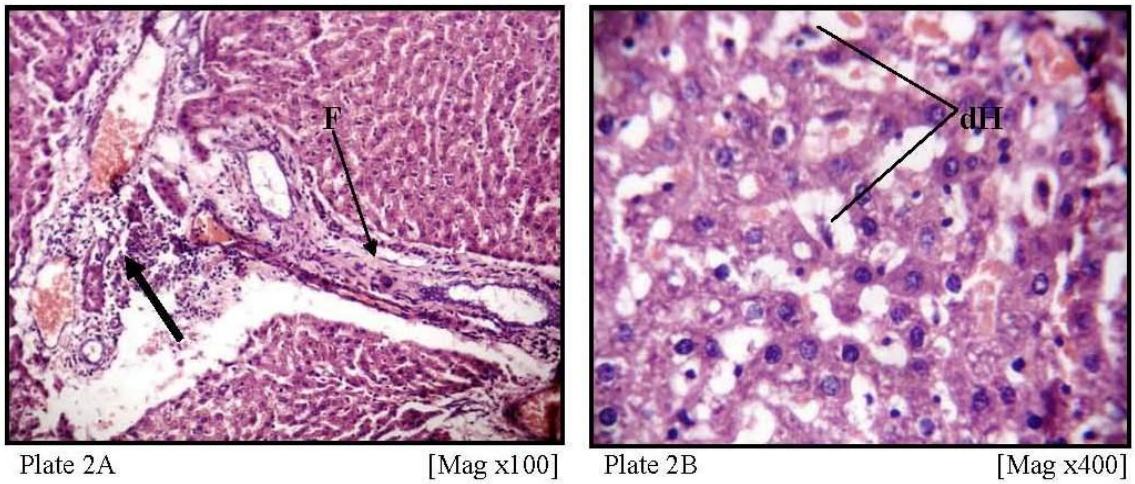

Fig. 2. Light photomicrograph of sections from liver of rat administered with acetaminophen only (group 2).

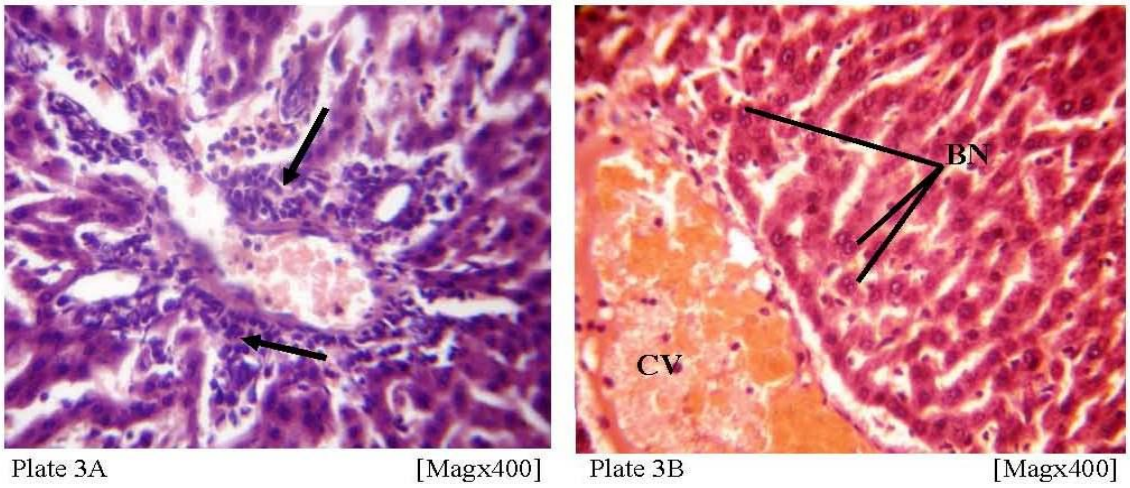

Fig. 3. Light photomicrograph of sections from liver of rat administered with acetaminophen and *G. latifolium* leaf extract [200mg/kg b.wt.] (group 3).

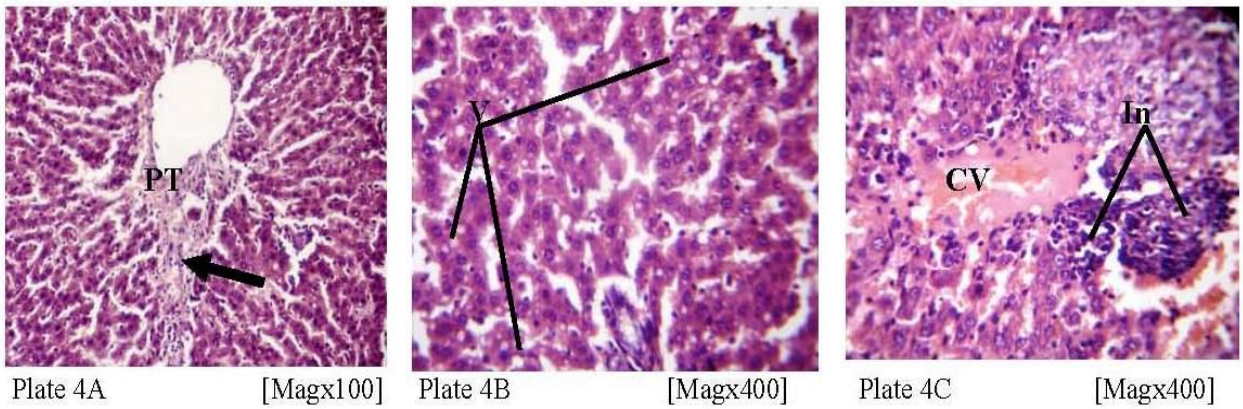

Fig. 4. Light photomicrograph of sections from liver of rat administered with acetaminophen and *G. latifolium* Leaf extract [400mg/kg b.wt.] (group 4).

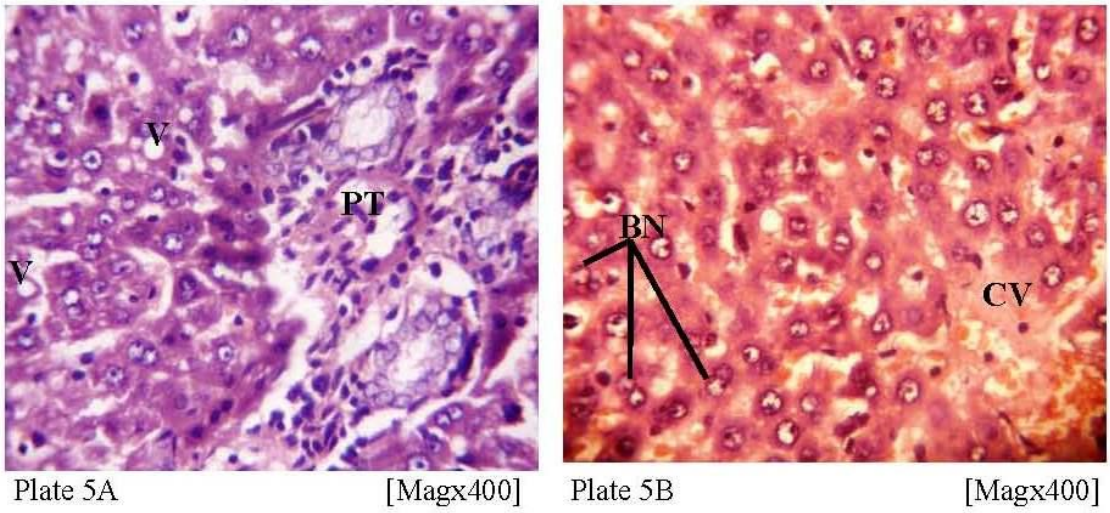

Fig. 5. Light photomicrograph of sections from liver of rat administered with acetaminophen and *G. latifolium* Leaf extract [600mg/kg b.wt.] (group 5).

Discussion

Acetaminophen administration caused increase in liver enzymes in the group 2 animals (negative control). Hepatotoxic drugs cause damage to the liver. Elevated levels of serum enzymes are inductive of cellular leakage and loss of functional integrity of cell membrane in liver. Concurrent administration of acetaminophen and methanolic leaf extract of *Gongronema latifolium* as seen in group 3, 4 and 5 reduced these elevated levels of the liver enzymes. This decrease in serum liver enzymes shows that the leaf extract could have ameliorating properties. Researchers have reported that *Gongronema latifolium* not only possess hypotensive and hypolipidemic activity but also hepatoprotective activity. Protein concentration reduced in the group 2, but increased ($p \leq 0.05$) significantly in all the groups treated with acetaminophen and methanolic leaf extract of *Gongronema latifolium* (groups 3, 4 and 5). Most protein found in the plasma are synthesized by the hepatocytes and secreted into circulation. A reduction in

the protein levels in the serum (group 2) and hepatic tissue may be a result of possible damage to the hepatocytes induced by the ingested toxin. The serum protein level is a marker of the synthetic function of the liver and a valuable guide to assess the severity of the damage (Nair, 2006).

The histological analysis of the liver of rat in the five different groups show: normal histoarchitecture of pericentral and periportal regions of the hepatic tissue (the central vein, hepatocytes interacting with the sinusoidal spaces and portal tract are evident) in group 1 (Fig. 1), distorted histoarchitecture of periportal and pericentral regions of the hepatic tissue (ballooning degeneration of hepatocytes at pericentral region shown; Increased septal and portal fibrosis, dilated vessels, necrosis of periportal hepatocytes and inflammatory cellular infiltration are also observed) in group 2 (Fig. 2), slightly preserved histoarchitecture of periportal and pericentral regions of the hepatic tissue (inflammatory cellular infiltration is shown at periportal regions; binucleates indicating regeneration is also observed, presence of leucocytes and lysed red blood cells are found within the central vein) in group 3 (Fig. 3), slightly preserved histoarchitecture of periportal and pericentral regions (portal fibrosis, vacuolation and lysed red blood cells within the central vein are shown, presence of inflammatory cellular infiltration at pericentral and periportal regions is evident) in group 4 (Fig. 4) and some degree of protection of the periportal and pericentral regions (mild cellular infiltration and hepatic vacuolation are observed at periportal regions; binucleates indicating regeneration and anisonucleosis are also seen) in group 5 (Fig. 5).

Overdose of acetaminophen causes a potentially fatal, hepatic necrosis which can be attributed to the formation of a toxic metabolite N-acetyl-p-benzoquinoneimine (NAPQI) by the action of cyt P_{450}. The reduced necrosis of cells in the group 3, 4 and 5 (treated with *G. latifolium leaf* extract*)* might be due to the presence of chemical constituents which have hepatoproctective properties. Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavonoids, organic acids, lipids, alkaloids and xanthenes (Gupta and Misra, 2006), this may be present in the *Gongronema latifolium* and so responsible for this changes.

From this study, methanolic leaf extract of *Gongronema latifolium* has an appreciable ability to prevent damage to the liver based on the biochemical and histological changes observed.

Conclusion

The results of this study demonstrate that methanolic leaf extract of *Gongronema latifolium* methanolic exhibits biochemical and histological changes against acetaminophen-induced hepatic damage in wistar albino rats. It is possible that, the mechanism of the biochemical and histological changes of *Gongronema latifolium* methanolic leaf extract may be due to the presence of chemical constituents which have hepatoproctective, antioxidant and free radical scavenging properties. Additionally, this is a clear indication of the efficacy of methanolic leaf extract of *Gongronema latifolium* as an antidote to human health. It is pertinent, therefore to note that if adequate attention is given to the findings of this study, the Nigerian population and the world at large will experience laudable progress.

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