INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 3, No. 11, p. 183-188, 2013

RESEARCH PAPER OPEN ACCESS

Ameliorating effect and haematological activities of methanolic leaf extract of *Gongronema latifolium* in acetaminophen-induced hepatic toxicity in wistar albino rats

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Key words: Liver enzymes, acetaminophen, *Gongronema latifolium*, ameliorating and haematological.

doi: http://dx.doi.org/10.12692/ijb/3.11.182-188 Article published on November 17, 2013

Abstract

This study examined the ameliorating effect and Haematological activities of 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. ALT decreased from 35.38 ± 1.35 U/L to 28.34 ± 1.74 U/L, while AST and ALP decreased from 45.98 ± 3.76 U/L to 35.74 ± 1.50 U/L and 84.32 ± 2.26 U/L to 65.92 ± 3.90 U/L respectively. Protein concentration increased significantly ($p<0.05$) in the test animals treated with 600mg/kg of the leaf extract. Protein increased from 3.68 ± 0.24 g/dl to 8.33 ± 0.26 g/dl. The effect of the *G. latifolium* leaf extract seems to be dose dependent on the liver enzymes and protein concentration measured. The haematological parameters Hb, PCV and WBC increased significantly ($p<0.05$) in the test animals treated with 600mg/kg of the leaf extract. Hb increased from 8.93 ± 0.21 g/dl to 10.25 ± 0.47 g/dl, while PCV and WBC increased from 27.73 ± 0.42 % to 32.39 ± 0.52 % and 5.81 ± 0.13 ($\times 10^3/\mu l$) to 7.38 ± 0.38 ($\times 10^3/\mu l$) respectively. 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. These results indicate that the leaf extract of *Gongronema latifolium* has ameliorating effect and Haematological properties and can be used against some hepatic inflammations in human health and could have significant effect on human population.

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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 and Babady, 2002; 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 (Morebise *et al.*, 2002; Eleyinmi and Bressler, 2007; 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 (Ugochukwu *et al.*, 2003). Egbung *et al.,* (2011) reported 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) in the root bark and twig extracts.

When reactive oxygen species generation exceeds the antioxidant capacity of cells, oxidative stress develops, potentially causing tissue damage (Araujo *et al.*, 2006), lipid peroxidation, plasma membrane alterations, and inactivation of enzymes (Anand *et al.*, 2000). 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, we thought it necessary to investigate its effect on the liver and it has ameliorating and haematological properties.

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. 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 (Khashab *et al.*, 2007).

Aim of the study

The aim of this study is to investigate the acclaimed medicinal properties of the leaves of *Gongronema latifolium* by traditional herbal medicine practitioners. It became necessary (as no previous work has been done in this exact form) to investigate the ameliorating effect and haematological activities of leaf extract of *Gongronema latifolium* in acetaminophen-induced hepatic toxicity in wistar albino rats. This will help to justify or not justify the use of *Gongronema latifolium* leaf in folkshore medicine for management of liver disease and to create the public awareness of the possible ameliorating effect and haematological activities of *Gongronema latifolium* leaf extract.

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. Farghaly and Huessien (2010), and 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 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 divided into two. The first part was dispensed in heparinized tubes for haematological analysis. The second part of 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.

Biochemical analysis

The serum aspartate transaminase (AST) and alanine transaminase (ALT) were determined as described by Reitman and Frankel (1957) 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. Haemoglobin was measured using the method described by Ramnik (1994). Packed cell volume and White blood cells were determined by the method described by Cheesbrough (2004).

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 tables below, according to the parameters investigated.

Table 1. Liver Enzymes Concentration (U/L).

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
ALT	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	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	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.

Discussion

Acetaminophen administration (Table 1) caused significant ($p \leq 0.05$) increase in liver enzymes in the group 2 animals (negative control). Hepatotoxic

drugs cause damage to the liver (Kumar *et al.*, 2004; Sturgill and Lambert, 1997). Elevated levels of serum enzymes are inductive of cellular leakage and loss of functional integrity of cell membrane in liver (Moore *et al.*, 1985). 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 (Table 1). This decrease in serum liver enzymes shows the leaf extract could have ameliorating properties. Ugochukwu and Badaby, (2003); Ugochukwu *et al.*, (2003); Nwanjo, (2005); Nwanjo and Alumanah, (2005) in their separate researches have also reported that *Gongronema latifolium* not only possess hypotensive and hypolipidemic activity but also hepatoprotective activity.

Table 2. Protein and Haematological parameters.

Parameter	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}
Hb (g/dl)	10.33 ± 0.41	8.93 ± 0.21 ^{b}	9.18 ± 0.30 ^{b}	9.94 ± 0.29 ^{a}	10.25 ± 0.47 ^{a}
PCV (%)	31.10 ± 0.71	27.73 ± 0.42 ^{c}	31.01 ± 0.59 ^{b}	31.26 ± 0.71 ^{b}	32.39 ± 0.52 ^{a}
WBC ($\times 10^3/\mu l$)	7.42 ± 0.40	5.81 ± 0.13 ^{c}	6.70 ± 0.33 ^{b}	7.55 ± 0.51 ^{a}	7.38 ± 0.38 ^{a}

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

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).

Haematological studies showed a significant increase in white blood cells, packet cell volume and haemoglobin after treatment with 600mg/kg of the leaf extract; this could be explained by a need of oxygen to repair the injury as a result of the toxicity induced by acetaminophen (as seen in group 2). The low level of PCV and Hb in group two when compared with the positive control (group 1) may be attributed to the increase in ALT and AST. Hb synthesis is normally increased by the consumption of plant foods due to their high content of minerals and vitamins (Morebise *et al.*, 2002) that may stimulate synthesis of globin component of Hb as was observed in this study (group 3, 4 and 5). The level of white blood cells (WBC) was used as an index of immune function and there was significant increase ($p<0.05$) in WBC levels in group 3, 4 and 5 (treated with leaf extract), thereby suggesting presence of phytosterols and flavonoids in *G. latifolium* leaf that may have possibly influenced the processes involved in the production of white blood cells. Phytosterols modulate immune function through their effect on T-helper cells and natural killer cells (Bouic *et al.*, 1999).

Conclusion

The results show a clear indication of the efficacy of methanolic leaf extract of *Gongronema latifolium* as an antidote to human health. The results reviewed that methanolic leaf extract of *Gongronema latifolium* methanolic exhibits ameliorating effects and haematological action against acetaminophen-induced hepatic toxicity in wistar albino rats. It is possible that, the mechanism of the ameliorating effects, haematological action and hepatoprotective activity of *Gongronema latifolium* methanolic leaf extract may be due to its antioxidant and free radical scavenging properties which could pose a positive effect on population and human health.

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