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Hepatotoxicity Effect of *Gongronemal latifollium* Aqueous Leave Extract on Some Biomarker Liver Enzyme of Albino Wister Rats

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Abstract

Gongronemal latifolium plant has been widely utilized in the treatment of many illnesses and nutritional purposes, however the side effects associated with the consumption of this plant has been greatly neglected due to its therapeutic potentials. Therefore, this study investigated the hepatotoxicity effect of Gongronemal latifolium aqueous leaf extract on the liver enzymes using animal modeling. A total of twenty albino Wister rats were used and grouped into five different groups of 4 animals each and treated with various doses of the plant extract such that group: A serves as control and groups: B-E serves as test groups with different doses such as 100mg- 500mg/kg.bw of the aqueous leaf extract and the treatment lasted for 21days after which the animals where sacrificed and blood samples were collected through cardiac puncture. The hepatotoxicity effect of the plant was evaluated through examination of the various levels of the liver enzymes in the extract treated groups and compared with the control using the colorimetric method. The results indicated that the activities of the Aspartate Trans amylase (AST), Alanine trans amylase enzyme and Alkaline phosphate shows significant increases for all the concentration at P< 0.005 when compared with the control group. Furthermore, there was a slight decrease in the Alanine Trans amylase level observed at 100mg of the extract. In conclusion the result of this study reveals that Gongronemal latifollium, leaf extract has no significant hepatotoxicity effect on the liver enzyme and its use in human nutrition and therapeutic agent is advised at a lower does.

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Keywords: *Gongronemal latifollium*, Liver enzyme, Alanine trans amylase, Alkaline phosphate, Aspartate trans amylase

INTRODUCTION

Gongronema latifollium is a tropical rain forest plant and it is widely common in tropical African countries. It belong to the plant family called Aslepodaceal, it a slender climber often 3-4m long in length.The leaves are relatively simple, heart shaped, it is an edible plant with a soft and pliable stem. It is widely known in Nigeria as Utazi among Igbos and Arkoko in Yoruba language (Ekundayo, 20015).This plant has been greatly utilized in both nutritional and therapeutic purposes, the leaves are commonly use has a vegetable and spices.

Medically the stem part of the plant is widely used in Ghana as chewing stick and when boiled with lime juice, it can be used to purge for colic and treatment of stomach pain due to symptoms connected to worm infection. It is also utilized to improve the growth of new born baby in west African countries (Akuodor, *et al*., 2016, Gamaniel, *et al.,* 1996) indicated in their studies that the administration of the stem aqueous extract of *Gongronema latifollium* produced a potent relaxation of the gastro intestinal tract and inhibition of the small intestine transit. The leave extract of the plant can be added as a spices or combined in the diet of nursing mother with an intension to increase uterine contraction (Nwosu, *et al*., 2006). The aqueous leaf was found to be useful in treatment of ulcer, malaria and fungi infection and also when combined with bitter leave it also widely used to control blood glucose level in diabetes patient. (Gupta,*et a*l., (2005).

The liver is an out grown organ of the digestive system, a very large and vital organ endowed with metabolic active cells which independently secrete execrate, storage, detoxify, protect and synthetic function among others (Doumas, *et al*., (2018). Furthermore, in reference to the nutritional and therapeutic effect of this plant, there is need to investigate the pharmacopotential side effect of the plant. This study investigated the hepatotoxicity effect of *Gongronema latifollium* leaves extract on the liver of albino Wister rat to determine it effect and increase the level of knowledge about the safety profile of *Gongronema latifofllium* as well as it relatively safe dose.

MATERIALS AND METHOD

Plant Collection and Processing

Fresh leaves of the plant were purchased from Relief market in Owerri in Imo – state of Nigeria. Botanically identified and authenticated by a botanist in the department of Biochemistry of Federal Polytechnic Nekede Owerri, Imo -state Nigeria. The leaves were washed with clean water, dried at room temperature and grinded into powdered form using mortar and pestle.

Experimental Animals

Albino rats weighing 152- 250gm were purchased from the Pharmaceutical technology department of Federal Polytechnic, Nekede Owerri Imo state Nigeria.

Preparation of the Aqueous Leave Extract

250 grams of the powdered leaves of *Gongronema latifollium* was dissolved in three liters of distilled water and stirred vigorously at interval of 1hour for four hours and allowed to stand on bench for an hour without disturbance, the solution was then sieved with a laboratory sieve of 0.5 micron in size and allowed to stand for another 1hours to allow the heavy particle to settled down and the supernatant was decanted and filtered using what-man filter paper

and the residue was transferred to an open tray and oven dried at 100^0C for 2days, scraped with a spectacular and grinded into fine powder using laboratory pestle and mortal (Edet,, *et al.*, (2011). The percentage yield was calculated as follows

$$\text{Yield \%} = \frac{\text{Weight of extract in powder}}{\text{Weight of powder leaves dissolved}} \times 100$$

Experimental Design

20 albino Wister rats were used for this study, the animals were selected randomly and grouped into 5 groups of four animals each and treated as follows

Table 1: Groups, numbers of Rats and dosage of the extract administration.

Group	No. of Animals	Treatment with plant extract
A	4	Distilled water
B	4	100mg
C	4	200mg
D	4	400mg
E	5	500mg

Determination of oral acute toxicity of the plant extract.

Acute oral toxicity study was carried out using a standard limit test dose of 1000 mg according to the organization, co-operation and development guideline for testing of chemicals using rat or mice of 2011 (Doumas, *et al.*, (2018). The results indicated that there were no visible sign of acute oral toxicity and no death was recorded at the limit dose test of 1000mg/kg.bw within the 10 days of administration and observation period of the animals.

Treatment of the experimental animals

The animals were housed in a cage under standard housing condition at normal temperature of 25^0C to 29^0C. The animals were fed with a standard raw food and water for 7days after which the administration of the leaf extract was carried out at different does for 21days.

Collection of Blood samples

After 21days all the animals were sacrificed under anaesthesazion using chloroform. The blood samples were obtained by cardiac puncture into EDTA bottles then centrifuge at 5000rm for 15min after which the serum was collected into a non- heparinized bottle, kept in a refrigerator at 4^0C until it was required for analysis.

Determination of Biochemical Analysis of the liver

i. Alkaline phosphate (ALP) test was carried out using colorimetric method described by Wemer, *et al.*, 1999
ii. Alanine trans amylase (ALT) test was carried out by U/V method that based on the oxidation of redox by (Demas, *et al.,* 2018)
iii. Aspartate trans amylase test was carried out using modified colorimetric method described by(Doumas*, et al.,*(2018)

Statistical analysis

The results were expressed as mean and standard deviation of mean + SD using one way analysis of variance (ANOVA) to compare the mean result of different sample group at $P< 0.01$ significant level.

RESULTS

The hepatotoxicity effects of *Gongronema latifolium* leaves extract shown in Tables 2-4

Table 2: Effect of *G. latifollium* leaves extract on the Aspartate Trans amylase enzyme of Albino rats.

Groups	Treatment in concentration mg/kg.bw	AST (u/L)
A	Distilled water	$9.03^{c} \pm 0.05$
B	100	$9.07^{c} \pm 0.05$
C	200	$10.20^{b} \pm 0.10$
D	400	$15.10^{a} \pm 0, 10$
E	500	$15.20^{a} \pm 0.10$

The superscript a, b, c indicated significant difference of mean value at $P \leq 0.01$

Table 3: effect of *G. latifollium* leaf extract on the Alanine Trans Amylase liver enzyme of albino rats.

Group	Treatment in Concentration (mg)	ALT (u/L)
A	Distilled water	$6.02^{c} \pm 0.05$
B	100	$6.13^{c} \pm 0.05$
C	200	$7.50^{b} \pm 0.10$
D	400	$9.23^{a} \pm 0.15$
E	500	$13.40^{a} \pm 0.10$

The superscript a, b, c indicated significant difference of mean value at $P \leq 0.01$

Table 4: Effect of *G. latifollium* leaf extract on the Alkaline phosphate of liver function of albino rats.

Groups	Treatment in concentration mg	ALP (u/L)
A	Distilled water	$50.07^{e} \pm 0.05$
B	100	$52.27^{d} \pm 0.05$
C	200	$57.30^{c} \pm 0.10$
D	400	$74.33^{a} \pm 0, 28$
E	500	$24.0^{f} \pm 0.10$

The superscript a, b, c indicated significant difference of mean value at $P \leq 0.01$

DISCUSSION

The results of administration of aqueous leave extract of the plant at the various concentrations on the liver enzymes such as Aspartate Trans amylase (AST), Alanine transamylase (ALT) and Alkaline phosphate (ALP) reveals various changes related to the concentration of the leaves extract. This result is in line with the result of the study carried out by (Abinu, *et al*., (2007) that evaluate the hepatocellular effect of different part of *Citrus aurantifolia(* Lime tree) and the result indicated various changes in the hepatic functions of the studded animals. Therefore, in table 1 no significant serum decrease in the level of AST across the group treated with the extract when compared with the control group, this result is in contrast with the report of (Lukong, *et al*.,(2008) who reported the effect of administration of aqueous extract of G. *latifollium* leaves on the kidney and liver enzyme of rodent and it was reported that is only one group showed sign of significant change of decrease in AST level which is an indication that the extract is relatively safe to the human health due to lack of significant side effect on the liver of the animals when compared with the control. There was a significant increase in the level of ALT in group C, D and while that group E was significantly decrease, when compared with the control group this result suggested that aqueous leaves extract of the plant may have caused some level of hepatic injury in the test animals when group B result on ALT is considered, in which there is a significant decrease in serum ALT level compared to group A animals. Therefore this observation suggested that the

aqueous leave extract of the plant do not significantly contain potent pharmacological agents that are antagonistic to the liver function, rather this observation indicate that it may contain a potential ingredient that are deleterious to other organ of the body. However, there was no strong evidence in group B, ALT which slightly suggest remedy to any side effect of the extract to the liver. This result is in accordance with the observation of (Nwanjo, *et al.* (2003) who reported that the aqueous extract of *Gongronema latifollium* administration that the lower dose of 1.8mg/kg.bw. showed a decreases in ALT activities when compared with the control group. This result suggested that *G. latifolium* leave extract may not have hepatotoxicity effect when used as nutritional or therapeutic agent at lower dosage. Furthermore, it was observed that there was an increase in the serum ALP level in the test group B, C, D. and E this may be attributed to a toxicity effect in any other organ other than liver as it was noted that increase in ALP is a marker for bone disease and other organs such as kidney (Edet, *et al.*, (2011), (Friedman, et al., 1996). Also observed a similarly result in a related study conducted by (Kochman, *et al*,(2008) in which he stated that Alkaline phosphate (ALP) is distributed almost in every tissue in the body.(Kaplan, 2012) reported that ALP are present in many human tissue including bone, intestine, Kidney, Liver, Placenta and white blood cell and any damage or injury to any of this organs will caused high realize of ALP into the blood stream. More so, (Damera, *et al*., (2012), reported elevated serum activity of ALP has been associated with chronic kidney disease. The increase in ALP level in this study could mean that the leaves extract of the plant may be injurious to the bone, heart, and other organs but not liver. This result is in agreement with the report of Edet, *et al.*, (2011 on effect of *G. latifollium* leave extract on some liver enzyme of albino and stated that a test dose of 400mg/kg.bw increased ALP level in the test group relatively to control hence this indicated that the aqueous extract of *G. latifollium* leave is not likely to cause liver pathology and can provide alleviation and protection to the animal liver in a dose lower than 400mg/kg.bw, therefore usage of the plant extract at concentration higher than 500mg/kg.bw will result into detrimental effect on the other internal organ due to increase in ALT following it administration at 500mg/kg.bw.

CONCLUSION

Based on the outcome of this present study, it would be beneficial to conduct additional studies on the long term effect of *Gongronema latifolium* leaf extract on liver enzymes, investigating the impact of prolonged exposure to different dose of the extract could provide valuable insight into its safety profile over extended period. Furthermore, exploring the potential interaction of the plant extract with other medication or supplements commonly used in conjunction with it could offer a comprehensive understanding of its overall effect on liver health.

RECOMMENDATION

There is need for exploration of the mechanism of action of the plant extract by delving into the underlying mechanisms of how *G. latifolium* affects liver enzyme could enhance our knowledge of its physiological impact, investigating the specific pathways or biochemical processes involved in the modulation of liver enzyme activity by the plant extract could pave the way for targeted therapeutic intervention or the development of novel treatment for liver related condition.

There is need for clinical trials and human studies to evaluate the efficacy and safety of *Gongronema latifolium* leaf extract in real –world setting is essential. Assessing its effect on liver function in human subject with varying health condition, such as liver disease or metabolic disorders, could provide valuable clinical data to support its use as a therapeutic agent or dietary supplement

Collaborative with experts in fields such as pharmacology, Biochemistry and traditional medicine could enrich the research on *Gongronema latifolium* and its potential hepatoprotective properties. Engaging in interdisciplinary studies and knowledge sharing initiatives could foster a holistic approach to understanding the plant therapeutic benefit and mechanisms of action.

Considering the widespread traditional use of *Gongronema latifolium* in various communities, it is crucial to disseminate evidence based information on its effect on liver health. Educating healthcare provider, policymakers, and the general public about the findings of this research studies can promote informed decision –making regarding the safe and effective use of the plant extract for health and wellness purposes.

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