

# EFFECT OF AQUEOUS EXTRACT OF *GONGRONEMA LATIFOLIUM* ON SOME INDICES OF LIVER FUNCTION IN RATS

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## ABSTRACT

The effect of various concentration of aqueous extract of *Gongronema latifolium* leaves on some indices of liver function; serum bilirubin (conjugated and unconjugated), alanine and aspartate aminotransferase and alkaline phosphatase were investigated in albino Wistar strain rats. The classes of chemical components of the aqueous extract of the plant were determined. Glycosides, alkaloids, carbohydrates, saponins tannins, and flavonoids, were found to be present while cyanogenetic glycosides was not detected. Acute toxicity test in rats gave an LD<sub>50</sub> of 1450.45 mg/kg. The activities of aspartate aminotransferases increased for all concentration ( $p < 0.05$ ). The increase in both alanine aminotransferase and alkaline phosphatase activities were not significant ( $p > 0.05$ ) for all the concentrations administered. Also the increase of the mean value of conjugated and unconjugated bilirubin levels for all the concentrations administered were not statistically significant ( $p > 0.05$ ). Administration of 1.5mg/ml cyanide/Kg body weight, a hepatotoxic agent showed an increase in serum enzyme levels, alanine and aspartate aminotransferases and alkaline phosphatase relative to the control groups. The results therefore, strongly suggest that *Gongronema latifolium* leaf extract may not have hepatotoxic effect when used as nutritional or therapeutic agent.

**KEYWORDS** *Gongronema latifolium*, bilirubin, liver enzymes

## INTRODUCTION

The plant *Gongronema latifolium*, Benth (*Asclapiadaceae*) is a common forest climber with hollow stems and broadly ovate, elliptic leaves which is widely chordate at the base. It is perennial edible plant with soft and pliable stem. It has profound bitter principles in every part of the plant. It is known in Nigeria as "Utazi" amongst the Ibos and Arooke in Yoruba.

*Gongronema latifolium* is widely used for both nutritional and therapeutic purposes. Okafor (1975) reported that the leaf rich in iron is commonly used as vegetable and spice "pepper Soup" and vegetable preparation. The Ibos used it for the preparation of "Ngwo-ngwo ji apu" Popularly known as African Salad.

Therapeutically, the stems, which is soft and pliable, are used in Sierra Leone as chewing sticks and cut up and boiled with lime juice, a liquor which is taken as purge for colic and stomach pains and symptoms connected with worm infestation (Dalziel 1987). It is given to a newborn baby in the Joru area of Sierra Leone to make baby grow rapidly. It is also used as an aromatic bitter and stimulant in treatment of dyspepsia (Sofowora, 1980). Gamaniel and Akah (1996) found in their study that the administration of the stem aqueous extract of *G. latifolium* produces potent relaxation of the gastrointestinal tract and inhibition of small intestinal transit. The leaves are bitter and bitter principle from plant has been associated with the improvement in the symptom of diabetes mellitus (Gupta and Seth, 1962). The natives in some communities alleged that the plant possesses hypoglycaemic effects and that fresh extract of the plants is used as hypoglycaemic agent by traditional healers in the treatment of diabetes.

Despite these uses of the plant there have been a dearth of information on the possible toxicological potentials of the plant.

The liver is the central organ that coordinates and modulates most biochemical activities in the body. Plasma enzymes are classified, as functional and non-functional enzymes. Functional enzymes are those, though produced in

the liver, are present in the blood in an equivalent or even higher concentration than in the tissue. The non-functional enzymes though present in the plasma of normal individuals perform no known physiological function. Their presence in the plasma at levels elevated above normal values suggest an increased rate of tissue destruction. Therefore, measurement of these classes of plasma enzymes and bilirubin levels can provide a valuable evidence for the toxicity of the tissue (Rodwell *et al*, 1991).

It is therefore intended in this study to examine the effect of aqueous extract of *G. latifolium* leaf on some indices of liver function in rats.

## MATERIALS AND METHODS

### MATERIALS

Fresh and apparently uninfected leaves of *G. latifolium* were collected from plants growing within Owerri, Imo State. Botanical identity was kindly confirmed by Dr. C. Okeke (Head of Department of Plant Biology and Biotechnology, Imo State University, Owerri), where voucher samples are kept for reference. They were properly washed, before extraction. Other materials include liver function test kits (Sigma Chemicals, England) Sodium hydroxide, sodium bicarbonate, potassium ferricyanide, acetic acid, concentrated sulphuric acid and bovine serum albumin from BDH analytical grade. Chloroform, ferric chloride and 4-amino antipyrine were from Merck chemicals, Denmark.

### ANIMALS

Experimental animals (rats) were obtained from the Animal House of College of Medicine and Health Sciences, Imo State University, Owerri. Twenty four albino Wistar strain rats of both sexes weighing 150 - 200g were employed. The rats were randomly assigned into 4 groups of six rats each and housed in stainless steel cages and a twelve hour light dark cycle was maintained. They were allowed free access to water and feed (product of pfizer Nigeria Ltd.) *ad libitum* throughout the period of the experiment.

### PREPARATION OF THE EXTRACT

Fresh leaves of *G. latifolium* were collected and cleaned. The leaves were dried in carbolite moisture extraction drying oven (Grant Instruments, Cambridge, England) at 45°C -50°C for 3hr. Grinding was done using Thomas Contact Mill (Pye Unicam, Cambridge, England). The ground materials were sieved through a 1mm sieve. One hundred gram (100g) of fine powder was boiled in about 500 ml of water for 3hr (wet extraction). The extract was cooled and filtered. The filtrate was evaporated by hot air oven (Grant Instruments, Cambridge, England) treatment at 45°C -50°C to a volume of 50 ml. The yield of the extract was 2g/ml.

### PHYTOCHEMICAL STUDIES

Preliminary phytochemical tests were performed as described by Harbone (1973) and Trease and Evans (1996). The presence or absence of saponins, flavonoids, glycosides, tannins, alkaloids, etc. was tested.

### ACUTE TOXICITY TESTS

The acute toxicity (LD<sub>50</sub>) test of the extract was carried out to define the range of the lethal dose and the safe range for the extract. Thirty six (36) wistar rats of both sexes weighing 150-200g were randomly divided into 6 groups of 6 each. Groups were treated with extract (100, 200, 500, 1000, 2000 mg/kg) by the i.p. route. Deaths within a period of 24 hours were recorded and the median lethal dose LD<sub>50</sub> of the extract was determined according to the method of Miller and Tainter (1944). The LD<sub>50</sub> of the aqueous extract was calculated to be 1450.45 mg/kg bodyweight and doses up to 500 mg/kg bodyweight were observed to be safe (with no recorded deaths). All the doses used in this study were carefully chosen to exclude the lethal range.

### ADMINISTRATION OF THE EXTRACT

The rats were divided into four groups according to the dose of the extracts to be administered. Groups 1 and 11 were administered high dose (100mg/kg) and low dose (200mg/kg) body weight respectively. Group 111 animals received 1.8mg/ml cyanide per kilogram body weight. Administration was through intra peritoneal (I.P) route. The control (group 1V) was maintained on feed diet and water without any administration of the extract. Injection was done on the test group twice daily.

### EXPERIMENTAL AND ANALYTICAL PROCEDURE

Twelve four hours after the last injection and after the last feed given, the rats in all the groups were weighed and sacrificed under chloroform anesthesia. Using a sterile syringe and needle, 8ml of blood was collected by cardiac puncture.

The blood collected was transferred into a centrifuge tube and allowed for 30 min to clot. The blood was then centrifuged using Wisperfuge Model 1384 centrifuge (Tamson, Holland) for 5 min to facilitate separation.

The supernatant after centrifugation was used for bilirubin and enzymes assays. Total and conjugated bilirubins were determined by coupling with diazotized sulphanilic acid in the presence of urea as described by Malloy and Evelyn (1932). The activities of plasma liver aminotransferases were determined by measuring the amount of pyruvate released after conversion with aniline citrate solution (Reitman and Frankel, 1951). The activity of alkaline phosphatase was determined using disodium phenol phosphate as substrate in a phosphate buffer of pH 10.0 (Bassey *et al*, 1947).

### STATISTICAL ANALYSIS

Data collected were summarized as mean  $\pm$  SD. Differences between individual groups were assessed by Student's t-test. A P-value of less or equal to 0.5 was considered significant.

### RESULTS

The result of the phytochemical analysis is presented in Table 1. Phytochemical analysis showed the presence of glycosides, alkaloids, saponins, tannin, and flavonoids while cyanogenic glycosides and indoles were not detected.

Table 11 shows the result of administration of aqueous leaf extract of *Gongronema latifolium* on the concentrations of plasma total, conjugated and unconjugated bilirubin for the treated and control rats. The increase in the mean values of total, conjugated and unconjugated bilirubin concentrations in both groups 1 and 11 were not significant when compared to the control group or to one another ( $P>0.05$ ).

TABLE 1. Phytochemical Studies

COMPONENTS	<i>Gongronema latifolium</i>
Glycosides	+++
Alkaloids	++
Saponins	+++
Tannin	+
Flavonoids	+
Cyanogenic glycosides	ND
Indoles	ND

+ = relative abundance of compound  
ND = not detected

TABLE 11. Concentration of serum bilirubin (mg/dl)

	HD	LD	CYND	CD
Total bilirubin (mg/100ml)	1.22 $\pm$ 0.32	1.06 $\pm$ 0.22	1.34 $\pm$ 0.26***	0.98 $\pm$ 0.16
Conjugated bilirulim (mg/100ml)	0.70 $\pm$ 0.41	0.58 $\pm$ 0.16	0.68 $\pm$ 0.30***	0.53 $\pm$ 0.08
Unconjugated bilirulim (mg/100ml)	0.52 $\pm$ 0.16	0.48 $\pm$ 0.68	0.66 $\pm$ 0.18***	0.45 $\pm$ 0.12

HD = High dose LD = Low dose CD = Control dose CYND = Cyanide dose.

\*Significantly different from control group ( $P<0.05$ )

\*\* Significantly different from group administered 100mg extract/kg body weight ( $P<0.05$ ).

\*\*\* Significantly different from other groups ( $P<0.05$ ).

Each group n = 6 albino Wistar strain rats.

Total n = 24 albino Wistar strain rats

TABLE 111. Activities of Serum Alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase. (iu / L).

	HD	LD	CYND	CD
Alanine aminotransferase (iu/L)	27.6±3.04	25.2±2.44	38.33±2.61***	23.8±1.89
Aspartate aminotransferase (iu/L)	48.8±3.62**	34.8±1.56*	56.65±3.35***	15.0±2.32
Alkaline phosphatase (iu/L)	110.18±4.86	107.6±6.2	187.42±9.86***	94.6±3.46

HD = High dose LD = Low dose CD = Control dose CYND = Cyanide dose.

\* Significantly different from control group (P<0.05)

\*\* Significantly different from group administered 100mg extract/kg body weight (P<0.05).

\*\*\* Significantly different from other groups (P<0.05).

Each group n = 6 albino Wistar strain rats.

Total n = 24 albino Wistar strain rats

Table 111 shows result of administration of aqueous leaf extract of *Gongronema latifolium* on the activities of the serum liver enzymes, alanine and aspartate aminotransferases and alkaline phosphatase. Statistical analysis showed that the aspartate aminotransferase activity significantly increased in both low dose and high dose group when compared with the control group (P<0.05). The increase in high dose group over the low dose group was also significant. In other words, as the concentration of the extract increases, the activity of the aspartate aminotransferase increases proportionally.

There is a general increase in the activities of both alanine aminotransferase and alkaline phosphatase in the presence of *G. latifolium* leaves extract when compared with the control. The increase in the case aspartate aminotransferase was not statistically significant (P>0.05) when compared to control or to one another. Administration of 1.8mg/ml cyanide/Kg body weight, a hepatotoxic substance (Ezeanyika *et al*, 2001), showed an increase in serum enzyme levels, alanine and aspartate aminotransferases and alkaline phosphatase relative to the control groups (P>0.05).

## DISCUSSIONS AND CONCLUSIONS

Phytochemical analysis revealed the presence of glycosides, alkaloids, saponins, tannin, and flavonoids while cyanogenic glycosides and indoles were not detected. The acute toxicity test of the extract in thirty six albino Wistar strain rats gave LD<sub>50</sub> of 1450.45 mg/kg bodyweight. This value falls outside the range of toxicity (Loomis, 1978) All the doses used in this study were carefully chosen to exclude the lethal range.

The investigation on the aqueous leaf extract of *G. latifolium* on some biochemical parameters of the liver showed that the extract elicited an increase in the activities of the enzymes studied but only the increase in aspartate aminotransferase activity was significant both in low dose and high dose when compared to the control group or to one another and was comparable to that produced by 1.8mg/ml cyanide/Kg body weight, a hepatotoxic substance.

The increase of alanine aminotransferase and alkaline phosphatase activities were not significant and were also lower than that produced by 1.8mg/ml cyanide/Kg body weight, a hepatotoxic substance. Biochemical alterations in bilirubin were also investigated. Though it was found that the increase was not significant for total, conjugated and unconjugated bilirubin. Serum bilirubin level may rise following liver damage. An increase may be in conjugated and unconjugated bilirubin level. Conjugated bilirubin levels often rise with hepatocellular dysfunction, although not as high as with obstructive jaundice.

If there is indeed hepatotoxic effect, there is going to be an increase in the serum enzymes indigenous to the liver (alkaline phosphatase, alanine and aspartate aminotransferases) and unconjugated bilirubin as was found in this work. However significant increases occurred only for the aspartate aminotransferase in both low dose and high dose. In liver disease associated with some degree of destruction of hepatocytes, serum levels of these enzymes will be elevated even before clinical symptoms of disease like jaundice appear.

Aspartate and alanine aminotransferase levels may reach values as high as 100 times the upper limit of normal, although 30-50 fold elevations are frequently encountered, it must be pointed out that the elevation found in this work was not as much as this except for aspartate aminotransferase for high dose. Also the elevation in alkaline phosphatase activity observed in this study was not as much as to suggest any form of toxic hepatitis. The increase in both conjugated and unconjugated bilirubin was not as higher as to suggest any form of hepatocellular dysfunction.

Alterations in the acid and alkaline phosphatases and lactate dehydrogenase of albino rats treated with *Azadirachta indica* leaves have been shown to be dose dependent (Katsuri *et al*, 1995). The findings agree with this though the increase was not significant for alkaline phosphatase, alanine aminotransferase activities and conjugated and unconjugated bilirubin concentration. It is known that most natives use the *G. latifolium* leaves after squeezing and removing out the extract for nutritional purpose and the macerated form of the leaf extract administered especially in dilute form for therapeutic purposes. Such preparation could be regarded as non-hepatotoxic.

Also, this significant increase is only for aspartate aminotransferase and not for bilirubin and all the other enzymes. Work is currently on to determine the source of the increase of serum aspartate aminotransferase

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