

# Acute and chronic toxicity studies of the ethanol leaf extract of *Gongronema latifolium*

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Acute and chronic toxicity evaluation of the ethanol leaf extract of *Gongronema latifolium* (GL) was carried out in Swiss mice and Wistar rats respectively in this study. Whereas acute toxicity study was done in two phases via oral and intraperitoneal (i.p) route to determine the lethal dose (LD<sub>50</sub>), chronic toxicity study involved treatment with the extract in two doses {150mg/kg b.w and 300mg/kg b.w) daily for an interval of 30 and 60 days. At the end of treatments, haematological and biochemical parameters were analyzed in the blood and serum. Changes in behaviour and body weight were also noted. The results indicated an LD<sub>50</sub> of 5000mg/kg b.w by oral route but 1500mg/kg b.w intraperitoneally. There were no significant changes (p< 0.05) in body weight, haematological and biochemical parameters in experimental groups in comparison with the control. The results therefore suggest that *Gongronema latifolium* is non toxic at the doses studied.

**Keywords:** *Gongronema latifolium*, acute toxicity, chronic toxicity, biochemical parameters, haematological indices, mice, Wistar rats.

## INTRODUCTION

*Gongronema latifolium* Benth Hook, (Asclepiadaceae) is an herbaceous shrub, with yellow flowers and a stem that yields characteristic milky exudates when cut. It is commonly grown in gardens in Calabar, Cross River State, Nigeria. It is locally called "utasi" by the Efiks, Ibibio and Quas; "utazi" by the Igbos and "arokeke" by the Yorubas in Nigeria (Edet *et al.*, 2009). It is a rain forest plant which has been traditionally used in the South-Eastern part of Nigeria for the management of diseases

## Abbreviations

**GL** = *Gongronema latifolium*, **i.p** = Intraperitoneal, **b.w** = Body weight, **h** = Hour, **LD<sub>50</sub>** = Lethal dose, **RBC** = Red Blood Corpuscles, **WBC** = White Blood Corpuscles, **Hb** = Haemoglobin, **LYM** = Lymphocytes, **n** = Number of animals in each group.

such as diabetes, high blood pressure and others (Ugochukwu *et al.*, 2003).

*Gongronema latifolium* is also used in the West African sub-region for a number of medicinal and nutritional purposes such as spice and vegetable (Dalziel, 1937). The plant is traditionally used in the control of weight in lactating women and promotes fertility in women (Schneider *et al.*, 2003). In the United States, it is used as a constituent of herbal tea for maintenance of healthy glycemic control (Atangwo *et al.*, 2009).

The plant has been scientifically studied and the antihyperglycaemic, antioxidant, antilipidemic and anticholesterolemic activities of the leaves in both normal and Streptozotocin induced diabetic rats have been reported (Ugochukwu and Babady, 2003; Ugochukwu *et al.*, 2003). Given its uses in the traditional settings and the emerging reports on its pharmacological actions, it is therefore necessary to do a detailed toxicity study to validate its usage or potential usage in the short and long term. Consequently, the present study investigated the

toxicological effects of extract from the plant's leaves in Swiss mice and albino Wistar rats.

## MATERIALS AND METHODS

### Plant materials

Fresh GL leaves were collected from a cultivated land at Ibiaku Itam in Itu Local Government Area of Akwa Ibom State, Nigeria. The sample was authenticated by a Botanist in the Department of Botany, University of Calabar, Nigeria and voucher specimen deposited at the Department of Botany herbarium, University of Calabar.

### Preparation of ethanolic extract of GL

The ethanol extract was prepared using the wet method of extraction. One kilogramme of the fresh leaves of the plant were cut into pieces, blended in 1.5 litres of ethanol (96%) with an electric blender and transferred into amber coloured bottle and kept in a cool (4°C) dark compartment for 72 hours. The mixture was filtered using a cheese material and thereafter with Whatman No 1 filter paper. The extract was concentrated using a rotary evaporator at 37-40°C and dried completely in a desiccator containing a self-indicating silica gel.

### Toxicological studies

Acute and chronic toxicological studies of ethanolic leaf extract of *GL* were carried out.

### Acute toxicity study

#### Animals

Swiss albino mice (20-25g) of both sexes obtained from the Department of Pharmacology and Toxicology, University of Uyo, Nigeria were used for the experiment after a 14 day acclimatization. The animals were kept in clean plastic cages under standard conditions. Studies were carried out in accordance with the principles of good laboratory practice and animal handling.

#### Methods

LD<sub>50</sub> determination was conducted using the method of Nofal et al., (2009). The evaluation was done in two phases. In phase one, four groups of six mice each, were treated with 2000, 4000, 6000 and 8000 mg extract/kg b.w orally respectively. The mice were observed for

clinical signs , symptoms of toxicity and death within 24 h. Based on the results of phase one, another four groups of six (6) fresh mice per group were each treated with 1000, 2000, 3000 and 4000 mg extract/kg b.w intraperitoneally (ip) respectively in the second phase. Clinical signs and symptoms of toxic effects and mortality were then observed for 24h. The LD<sub>50</sub> was then calculated according to Nofal et al., (2009) using the formulae;

$$LD_{50} = Dm - \frac{\Sigma(z \times d)}{n}$$

Where:

**Dm** = the largest dose which kills all animals.

**z** = Mean of dead animals between 2 successive groups

**d** = the constant factor between 2 successive doses.

**n** = Number of animals in each group.

$\Sigma$  = the sum of (z × d)

### Chronic toxicity study

#### Animals

Adult Wistar rats (120-150 g) of either sex used for the experiment were obtained from the animal house of the Pharmacology and Toxicology Department, University of Uyo, Nigeria. They were housed in a plastic cages at room temperature and under standard conditions as well as maintained in 12:12h light/dark cycle, they were acclimatized for 14 days at the animal house of Biochemistry Department of the University of Calabar before the experiment. The effect of prolonged administration of *GL* leaves extracts for one and two months in rats were investigated, using oral doses of 150 and 300 mg/kg b.w. deduced from 1/10 and 1/5 of LD<sub>50</sub> respectively.

#### Methods

Animals were divided into 3 groups of 12 rats each. Group A was treated with distilled water and kept as control, while groups B and C were given extract orally in doses of 150mg/Kg b.w and 300 mg/kg b.w respectively daily. Food and water intake as well as body weight were recorded weekly. Exactly after one month of treatment, six rats per group were sacrificed and blood samples collected for analysis, while the remaining six rats were treated for another one month (total of 2 months) before sacrifice and collection of blood samples for analysis. The following parameters were analysed.

#### Haematological parameters

Haematological parameters: Red Blood Corpuscles

**Table 1.** Effect of oral administration of *GL* on body weight of rats

Group	AT ONE MONTH			AT TWO MONTHS			
	Body weight (g)			Body weight (g)			
	Before treatment	After treatment	% weight gain	Before treatment	After treatment	% weight gain	
A	134.35 ± 12.18	147.63 ± 10.69	13.28 ± 2.60	134.35 ± 12.18	177.45 ± 12.18	43.10 ± 2.63	
B	152.33 ± 10.16	191.08 ± 14.55*	38.75 ± 4.56*	152.33 ± 10.16	215.00 ± 10.16	62.68 ± 12.41	
C	145.85 ± 8.12	175.45 ± 8.70	29.60 ± 2.60*	145.85 ± 8.12	192.98 ± 8.12	47.13 ± 17.38	

\*P<0.05 vs control; means ± SE (n = 6)

(RBC), White Blood Corpuscles (WBC), Haemoglobin (Hb) and Lymphocytes (LYM) were analyzed using Serono 9110 automated haematology analyzer (Serono-Baker Diagnostic Inc, Allentown, PA).

### Biochemical parameters

Serum biochemical indices were estimated for liver functions including AST, ALT, ALP, and ACP, total protein and albumin. Kidney function parameters including creatinine and serum electrolytes (Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>) were analyzed using a Boehringer Knoll auto analyzer 4010 System (Boehringer GmbH, Mannheim, Germany) and analytical kits obtained from Randox. Whole animal body weight gain and relative changes in organ weight (liver, heart, kidney, pancreas and brain) were measured as well using an electronic balance.

### Statistical analysis

The results were analyzed by one-way ANOVA, using SPSS statistical package. All data were expressed as Mean ± SE and difference between groups considered significant at p<0.05. Inter group comparisons were done at 2 levels (30 and 60 days).

## RESULTS

### Acute toxicity studies

When administered orally, in mice, the ethanolic extract of *GL* produced no lethality even at doses as high as 8000mg/kg. Thus the oral LD<sub>50</sub> was estimated to be >5000mg/kg b.w. Apart from weakness, *GL* did not produce any major signs of clinical toxicity over the 24hour observation period. Intraperitoneal administration produces no noticeable neurological or behavioural

effects within the six hour observation. However, 100% lethality at 2000mg/kg and 0% lethality at 1000mg/kg were recorded after 24 hours, hence intraperitoneally, the LD<sub>50</sub> was calculated to be 1500mg/kg.

### Chronic toxicity studies

#### Body weight

There were 29.60 ± 2.60 and 38.75 ± 4.56 % increases in body weights of rats which received 150 mg/kg and 300 mg/kg respectively. These increases were significant (p<0.05) when compared to the control for the one month phase. There was also no abnormality in behavioural pattern within the one month duration (Table1).

#### Relative organ weights at the end of one month

As shown in Table 2, there was a significant increase (P<0.05) in the relative weight of the livers of groups B (4.3±0.30) and C (4.2±0.30) compared to that of the control (2.9±0.4). However, there was no significant difference (P>0.05) in the relative weights of other organs.

#### Relative organ weights at the end of two months

Also shown in Table 2, two month administration of the extract caused a significant increase (P<0.05) in the relative weight of the livers of groups B (3.8±0.20) and C (3.9±0.4) compared to that of the control (2.8±0.03). There was no significant difference (P>0.05) in the relative weights of other organs.

#### Food and water intake at the end of one month

An average animal in Groups B and C respectively consumed 23.40 ± 0.29 and 25.34 ± 0.10 grammes of

**Table 2.** Effect of oral administration of GL on relative organ weights

Organs	Relative organ weights (%) at one month			Relative organ weights (%) at two months		
	Groups			Groups		
	A	B	C	A	B	C
<b>Liver</b>	2.9±0.4	4.3±0.3*	4.2±0.30*	2.8±0.03	3.8±0.20*	3.9±0.4*
<b>Kidney</b>	0.57±0.06	0.60±0.06	0.66±0.05	0.48±0.03	0.52±0.02	0.55±0.04
<b>Pancreas</b>	0.30±0.05	0.23±0.03	0.33±0.02	0.30±0.10	0.21±0.09	0.21±0.03
<b>Heart</b>	0.26±0.03	0.27±0.02	0.29±0.03	0.34±0.10	0.26±0.01	0.31±0.04
<b>Brain</b>	0.75±0.04	0.60±0.01	0.75±0.05	0.66±0.03	0.65±0.07	0.71±0.07

\*P<0.05 vs A; a = P<0.05 vs B; means ± SE (n = 6)

**Table 3.** Effect of oral administration of GL on food and water intake

Group	Food intake (g)		Water intake (ml)	
	At one month	At two months	At one month	At two months
<b>A</b>	27.14 ± 0.54	31.25 ± 0.68	29.76 ± 1.08	26.48 ± 0.35
<b>B</b>	23.40 ± 0.29*	27.09 ± 0.06*	27.98 ± 1.23	22.65 ± 0.01*
<b>C</b>	25.34 ± 0.10*a	30.20 ± 0.40a	27.95 ± 1.06	29.47 ± 0.14*,a

\*P<0.05 vs control; a =P<0.05 vs B; means ± SE (n = 6)

feed within the period and these were significantly lower (p<0.05) when compared with the control (27.14 ± 0.54). In the intergroup comparison, group C showed significant increase (p<0.05) when compared to group B. There was no significant difference (p>0.05) in water intake when experimental groups were compared with the control (table 3).

#### Food and water intake at the end of two months

The pattern of effect in one month administration was also replicated at the end of two months with an average animal in Group B consuming 27.09 ± 0.06g of feed and group C being 30.20 ± 0.40g of feed. However only group B food intake showed a significant decrease (p<0.05) compared to the control (31.25 ± 0.68). Water intake in Group B animals (22.65 ± 0.01) was significantly lower (p<0.05) when compared to the control (26.48 ± 0.35), while group C (29.47 ± 0.14) was rather significantly higher (p<0.05) compared to the control (Table 3).

#### Haemogram

The red blood corpuscles of the animals were enumerated after one and two month administration of

extract. No significant changes were observed in RBC and HB with respect to the control after the one and two month's treatments (table 4). However, WBC of rats given 300mg/kg (10.35 ± 0.22) was significantly decreased (p<0.05) when compared with the control (15.85 ± 2.12) after the one month administration. Conversely the LYM of this group (12. 27 ± 0.13) after two month administration decreased significantly relative to the control (7.25 ± 1.48).

#### Biochemical indices

##### Observations at the end of one month of administration

There were varied differences in the activities of the enzymes analyzed both in group B and C when compared with the control. In intergroup comparisons, the ALP (37.62 ± 4.80) of group C was significantly lower than that of group B (91.98 ± 18.92). Also, ACP of group C (3.35 ± 1.87) decreased significantly in comparison with that of group B (9.49 ± 1.49) while AST of group C (173.34 ± 17.83) increased significantly when compared with that of group B (124.87 ± 22.40). Total protein of groups B and C were not significantly different (P>0.05) from the control while albumin of group B (10.25 ± 1.24)

**Table 4.** Effect of oral administration of GL on haematological indices

Indices	Haematogram at one month			Haematogram at two months		
	Groups			Groups		
	A	B	C	A	B	C
WBC ( $\mu\text{L} \times 10^3$ )	15.85 $\pm$ 2.12	10.35 $\pm$ 0.22*	11.90 $\pm$ 1.85	11.50 $\pm$ 2.19	12.43 $\pm$ 2.16	13.70 $\pm$ 1.70
RBC ( $\mu\text{L} \times 10^5$ )	6.32 $\pm$ 0.30	6.44 $\pm$ 0.22	6.93 $\pm$ 0.53	7.47 $\pm$ 0.54	7.57 $\pm$ 0.34	7.70 $\pm$ 0.15
Hb (s/dl)	10.07 $\pm$ 0.46	10.60 $\pm$ 0.13	10.20 $\pm$ 0.40	14.58 $\pm$ 1.16	13.20 $\pm$ 0.78	13.47 $\pm$ 0.43
LYM ( $\times 10^3/\mu\text{L}$ )	9.27 $\pm$ 0.15	6.23 $\pm$ 0.12	8.33 $\pm$ 0.17	7.25 $\pm$ 1.48	12.27 $\pm$ 0.13*	8.05 $\pm$ 1.13*

\*P <0.05 vs A; <sup>a</sup> P<0.05 vs B; means  $\pm$  SE (n = 6)

**Table 5.** Effect of oral administration of GL on serum biochemical indices of rats

Indices	Serum biochemical indices at one month			Serum biochemical indices at two months		
	Groups			Groups		
	A	B	C	A	B	C
ALP (U/L)	41.44 $\pm$ 4.47	91.98 $\pm$ 18.92	37.62 $\pm$ 4.80 <sup>a</sup>	1.63 $\pm$ 0.63	0.40 $\pm$ 0.01	0.20 $\pm$ 0.01
ALT (U/L)	81.00 $\pm$ 0.00	84.50 $\pm$ 3.50	96.50 $\pm$ 13.57	109.50 $\pm$ 14.58	123.00 $\pm$ 0.00	127.50 $\pm$ 4.50
AST(U/L)	168.18 $\pm$ 9.71	124.87 $\pm$ 22.40	173.34 $\pm$ 17.83 <sup>a</sup>	109.50 $\pm$ 14.58	262.50 $\pm$ 21.81*	170.33 $\pm$ 74.48
ACP(U/L)	2.20 $\pm$ 0.86	9.49 $\pm$ 1.49	3.35 $\pm$ 1.87 <sup>a</sup>	565.18 $\pm$ 269.20	103.20 $\pm$ 72.17*	13.40 $\pm$ 2.69*
Total protein (mg/L)	4.44 $\pm$ 2.16	2.68 $\pm$ 1.05	1.21 $\pm$ 0.26	9.21 $\pm$ 0.57	9.17 $\pm$ 0.68	7.46 $\pm$ 0.46 <sup>a</sup>
Albumin (mg/L)	4.38 $\pm$ 0.52	10.25 $\pm$ 1.24*	4.97 $\pm$ 0.46 <sup>a</sup>	48.11 $\pm$ 5.13	43.63 $\pm$ 2.91	45.87 $\pm$ 2.94

a = P<0.05 vs B; \*P<0.05 vs A; means  $\pm$  SE (n =6)

showed a significant increase (p<0.05) in comparison with the control (4.38  $\pm$  0.52). Albumin of group C (4.97  $\pm$  0.46) was significantly decreased when compared with that of group B in the inter group comparison.

#### Observations at the end of two months of administration

The ALP and ALT of groups B and C showed no significant difference (P>0.05) compared with the control. The AST of group B (262.50  $\pm$  21.81) was significantly increased compared with that of the control (109.50  $\pm$  14.58). Whereas, the ACP of group B (103.20  $\pm$  72.17) and C (13.40  $\pm$  2.69) showed a significant decrease (P<0.05) compared with the control (565.18  $\pm$  260.20). The total protein of group C (7.46  $\pm$  0.46) was lower (P<0.05) than the control (9.21  $\pm$  0.57) and group B (9.17  $\pm$  0.68). Albumin of the experimental groups showed no

significant difference (P>0.05) compared with the control group.

#### Kidney function parameters

Table 6 shows the result of changes in selected kidney function parameters - potassium (K<sup>+</sup>), sodium (Na<sup>+</sup>), chloride (Cl<sup>-</sup>) and creatinine after a 30 and 60- day treatment with the extract.

#### Observations at the end of one month of administration

There was no significant difference in the kidney function parameters; K<sup>+</sup>, Na<sup>+</sup>, Cl<sup>-</sup> and creatinine of the experimental groups when compared with the control group. However, within group comparison, showed that

**Table 6.** Effect of oral administration of GL on kidney function parameters in rats

Parameters	Kidney function parameters at one month			Kidney function parameters at two months		
	Groups			Groups		
	A	B	C	A	B	C
<b>Creatinine</b>						
(mg/dl)	0.93±0.44	0.81±0.17	1.07±0.24	2.47±0.20	2.26±0.18	2.32±0.28
Na <sup>+</sup> (mEq/L)	880.28±404.74	2837.72±1535.36	1605.11±1072.62	113.21±13.40	140.41±16.88	179.80±16.32*
K <sup>+</sup> (mEq/L)	9.06±0.86	17.22±1.61	11.62±1.66 <sup>a</sup>	6.99±2.11	7.28±0.24	7.16±0.36
Cl <sup>-</sup> (mEq/L)	116.68±23.28	86.44±4.12	82.61±1.17	148.77±6.74	147.30±16.03	138.74±5.61

\*P<0.05 vs A; a = P<0.05 vs B; means ± SE (n =6)

K<sup>+</sup> in group C (11.62 ± 1.66) was significantly decreased (p<0.05) compared with that of group B (17.22 ± 1.61).

#### Observations at the end of two months of administration

K<sup>+</sup>, Cl<sup>-</sup> and creatinine of the experimental groups showed no significant difference compared with the control even after two months of administration. However, Na<sup>+</sup> concentration in group C (179.80 ± 16.32) increased significantly (P<0.05) compared with the control (113.21 ± 13.40).

#### DISCUSSION

This study indicates a significant increase in percentage weight gain at one month in a dose dependent fashion, but a non significant increase after two months of administration. Consumption of food in the absence of disease normally leads to increase in weight. The unpalatability of the GL due to the presence of bitter substances in the leaves, may have reduced the quantity of food consumed by the rats, hence their weight decrease. In a similar study, another commonly consumed vegetable, *Telfairia occidentalis* increased the weight of animals that consumed it for a long term due to its palatability (Iweala and Obidoa, 2009). The bitter taste of the leaves extracts may prevent its culinary use as a major source of nutrients. Hence, serum protein in this study was not statistically significant (P>0.05) at one month compared with the control even though the plant has been shown to be a good source of protein and other essential amino acids (Eleyimi, 2007). However, at two months trials, the animals became used to the bitter taste of the extract and thereby consumed normal quantity of food, thus the protein was statistically increased (P<0.05).

The non-significant difference in liver enzymes suggests that GL may not be toxic to the liver but hepatoprotective. Liver damage is assessed by the determination of serum levels of its enzymes such as ALT and AST (Dobbs *et al.*, 2003). High levels of AST and ALT indicate liver damage, cardiac infarction and muscle injury. However, ALT is more specific to the liver and is thus a better parameter for detecting liver injury. Serum ALP on the other hand, is related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis of the enzyme, in the presence of increasing biliary pressure (Moss and Butterworth, 1974). Generally an increase in these liver enzymes indicates injury or toxicity to the organ (Ghadi, 2000). Several African medicinal plants have been shown to have hepatoprotective effects (George and Chaturvedi, 2008; Chaturvedi *et al.*, 2007; Roy *et al.*, 2006 and Chattopadhyay, 2003). Hepatoprotection is possibly due to flavonoids which exert a membrane-stabilizing action that protect the liver cells from injury (Hahn *et al.*, 1968). A good liver function is inextricably linked to a good detoxification and antioxidant system, which is indispensable in ensuring optimum physiological function and prevention of diseases. Hb's were not significantly different from the control both at one and two months of administration which is contrary to the work of Iweala and Obidoa, (2009) where Hb levels were significantly increased in all animals fed GL leaves extracts. This probably could have resulted from the difference in administration route. 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. Hb and RBC levels were increased though not significantly. Thus, no undesirable effects were detected in the experimental animals thereby suggesting why the traditional Nigerian folklore medicine employs aqueous extracts of some leafy vegetables in the treatment of anaemia (Alada, 2000).

Most phytochemical constituents of plant food affect the immune system (Kubena and McMurray, 1996). An example is the antioxidant phytochemicals known to protect lymphocytes and reduce their destructive abilities (Duthie *et al.*, 1996). This was observed in this study as lymphocytes increased significantly in the experimental group B after long term administration, suggesting the presence of antioxidants in the plant. Phytosterols modulate immune function through their effect on T-helper cells and natural killer cells (Bouic *et al.*, 1999; Middleton and Nsikan, 1993). In this study, the level of white blood cells (WBC) was used as an index of immune function and there was significant increase in WBC levels in group B at one month, thereby suggesting presence of phytosterols and flavonoids in GL that may have possibly influenced the processes involved in the production of white blood cells. The WBC of rats at two months was insignificantly different from control because their body system might have become acclimatized to the extract effect.

## CONCLUSIONS

These toxicological results suggest that *Gongronema latifolium* may be non haematotoxic, non hepatotoxic and non nephrototoxic at the doses studied, hence, may be recommended for further clinical studies.

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