

Influence of *Gongronema Latifolium* Leaf-Extracts Treatment on Some Hepatic Enzymes Activity In Rats

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Abstract

Influence of extracts of the leaf, *Gongronema latifolium*, on serum levels of aminotransaminases, α - amylase and urea was investigated in rats. The plant is native to Southern Nigeria. The plant extracts were Soxhlet extracted and evaporated to solid using Rotary evaporator. Four tolerated doses 25, 50, 75 and 100 mg/kg were estimated from the results of acute toxicity study. About 50 adult healthy rats weighing between 120 and 150 grams, were divided into five groups. Group 1 received DMSO- Saline as control, groups 2 - 5 or 6 - 9 received either ethanolic or water extracts daily for 7 days. On the 8th day, rats of all groups were subjected to chloroform anaesthesia and blood samples collected through cardiac puncture and used for biochemical metric. The results showed that the extract- treatments induced enhanced activity of aspartate amino-, alanin amino-transferases (AST & ALT), α -amylase and insignificant effect on the serum level of urea. The induced enhanced enzymes activities were gradually inhibited by graded doses of the extracts.

The conclusion suggests that chronic oral administration of these plant extracts, could cause liver cells damage.

Keywords: *Gongronema latifolium*, hepatic enzymes, and rats

1. Introduction

The medicinal plant, *Gongronema latifolium* (Asclepiadeceae) has been used in Nigerian herbal practice to treat diabetic Mellitus and malaria infections. The plant is grown in most parts of the South South and South Eastern of Nigeria. The natives cultivate the plant in their farm yards as a vegetable. It has been used extensively in herbal preparations for treatments in the country's traditional healthcare (Ahamefule et al 2006). Affinity of herbal preparation for treating diseases depends on the physiochemical property of the preparation (Udoh, 2007; Udoh et al 2011) and the biochemical function of the drug-receptor complex formed in the process. Toxicological concept reveals that no substance is safe despite benefits (Udoh et. al 2012). Based on this concept, oral administration of the extracts of the leaf of *Gongronema latifolium* could influence liver enzymes activities, pathologically, depending on the dose administered.

A toxicological concept that the severity of toxicity of substance depends on the dose and the rate of administration (Sakihama et. al 2002). Therefore, repeated oral administration of the ethanolic and water extracts of the leaf of *Gongronema latifolium* could adversely interfere in the biochemical functions of the liver (Sharma et al 1995). This has become a problem in the public health of the country since it has been reported that, ingestion of chemical substances could either stimulate or inhibit the activities of liver enzyme markers in the hepatic metabolic pathway which might lead to Cirrhosis of the hepatic cells (Sharma et al, 1995).

The influence of the leaf extracts of *Gongronema latifolium* on the liver enzymes could also induce or inhibit drug metabolising enzymes or inhibit drug metabolising enzymes such as Cytochromes P450, Mixed Function Oxidase and Monoamine Oxidase (Farombi, 2003). Most herbal preparations have not been evaluated scientifically. Once therapeutic benefits are known, the preparations are employed in the traditional healthcare delivery system without proper scientific evaluation.

In folk medicine, most side effects of herbal medicine are considered as therapeutic benefits (Morebise et al, 2002). Incooperation of the leaf of *Gongronema latifolium* in rats' feed caused hypoglycemic effect in rats (Sedgwick et. al, 1991). The hypoglycemic effect of the preparation caused Nigerian herbalist to employ the leaf of the plant in herbal medication to treat diabetic mellitus in human patients.

In the study of the plant's mechanism of the hypoglycemic action in rat, Ugochukwu et. al (2003) reported that the leaf extracts of *Gongronema latifolium* could be used for the treatment of non-insulin dependent diabetic mellitus (NIDDM). Akah, et al (2011), reported on the anti-inflammatory properties of the leaf extract.

The leaf extract of *Gongronema latifolium* has also ability to inhibit subcutaneous phycomycosis (Sabinus et. al, 2013).

Having considered the above facts, it became necessary to investigate the toxic effects of *Gongronema*

latifolium leaf ingestion on some biochemical indices on rats.

2. Materials and Methods

a. Plant Extract Preparation

The fresh leaves of the plant of *Gongronema latifolium* were fetched from the farm of Calabar inhabitants in Nigeria, around the months of March and April, 2008. The plant was identified by professor Ani Nkang; of the Department of Botany University of Calabar, Nigeria. The plant sample was deposited in the Herbarium Unit of the Department of Pharmacology, University of Calabar, Nigeria; with the specimen number, UNICAL/PHM/2008/0157.

The fresh leaves of *Gongronema latifolium* collected were exposed to dry under a room temperature of 28°C for 3 days. The dry leaves were ground into paste. A weighed quantity of paste plant sample 100g, was wrapped in a thimble and placed in a Soxhlet extractor of 500 ml capacity and extracted in ethanol or water for 72 h respectively, to obtain either ethanolic or water extract separately. The solid form of the ethanolic or water extract of the plant was recovered by evaporation, using Rotary evaporator (Searl Instruments Ltd, England). The extraction in ethanol gave a percentage extract yield of 30 and in water 60. Both extracts were stored in refrigerator (Thermocool Nig. Ltd) for use during the experiment.

Stock solution of either ethanolic or water extract was prepared by dissolving 10 g of ethanolic extract of *Gongronema latifolium* in 10 ml of 50 % DMSO - Saline and water extract of *Gongronema latifolium* in 10 ml of 0.85 % NaCl Solution (Normal Saline) to give a stock solution of 1 g/ml respectively.

2.2 Animal

Young adult rats, weighing between 120 and 150 grams were bought from the Animal House Unit, Department of Pharmacology, University of Calabar. The rats were housed in cages of 10 per cage. They were allowed free access to feed (Agro feeds Ltd, Calabar) and water ad Libitum.

The rats were allowed to acclimatise in the laboratory for 7 days under 14 hours light and 10 hours dark per day at ambient temperature of 28± 1°C.

2.3 Determination of Doses

The four tolerated doses of either water and ethanolic extract were estimated from the acute toxicity test following the method described by Udoh (2007). Effects of graded doses (10, 15, 20, 25, 50, 75, 100 and 150 mg/kg) of either water or ethanol extract of the leaf of *Gongronema latifolium* were investigated on male rats physical response as overt toxicity.

2.4 Serum Sample Preparation

Serum samples were prepared from the coagulated blood and the sera from the rats were stored in fridge at -5°C for the biochemical metric.

2.5 Enzyme Assay

Ethanolic and water extracts of *Gongronema latifolium* (25 mg/kg) induced a significant increase ($p < 0.05$) in the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Table 1) and a significant dose related increase ($p < 0.01$) in the activity of the serum α -amylase (AMS), Table 2. The data obtained from these studies were statistically analysed by the ANOVA and Student t-test.

2.6 Urea Estimation

Ethanolic extract of *Gongronema latifolium* (25 - 100mg/kg) administered daily to rats did not significantly ($p > 0.1$) alter the blood level of urea, Table 2.

Notwithstandingly, water extract of the plant (25- 100 mg/kg) administered to rats daily for 7 days induced gradual increase in the urea blood level in a dose related manner. The increase in the blood levels of urea was not significantly different ($P > 0.1$), compared to control by Student t-test.

3. Statistical Analysis

Data generated from the study were expressed as means (\pm s.e.m). Difference between control and the test groups was statistically analysed using paired Student t- test and ANOVA (one way) followed by a post test. Differences with $P < 0.01$ and 0.05 were considered significant.

4. Results

4.1 Effect of Extracts on Aminotransaminases

The ethanolic and water extracts of the leaf *Gongronema latifolium* (25 -100 mg/kg/d), administered orally to rats for 7 days induced enhanced activity of aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT) in rats. The induced increase in the liver enzymes activity was dose related for both extracts (Table 1) and the maximal effects obtained for ALT activity was $33.3 \pm 1 \mu\text{L}^{-1}$ at a dose of 25 mg/kg/d and AST was $27.3 \pm 3 \mu\text{L}^{-1}$ at a dose of 50 mg/kg/d, compared with control values, respectively.

Further increase in the doses of extracts administered produced a dose related reductions in AST and ALT activity compared to maximal activity (Table 1). The degree of reduction was significant, $P < 0.05$, by student t-test, compared to maximal activity.

4.2 Repeated Treatment

The ethanolic and water extracts of the leaf of *Gongronema latifolium* (25 -100 mg/kg/d) given orally to rats for 7 days, induced significantly ($P < 0.05$) the activity of the enzyme, α -amylase (AMS) in rats. The increase in the activity of AMS was a dose related (Table 2).

Ironically, repeated oral treatment with the extracts caused insignificant ($P > 0.1$) influence on the serum level of Urea (Table 2).

5. Discussion

Liver enzymes play an essential role in the biochemistry of human body. The enzymes catalyse and regulate most biochemical reactions in the human body, from the replication of DNA by DNA polymerases as well as metabolism of xenobiotics (Okolie et. al, 2008; Agbo and Obi, 2007; Udoh et al 2011). The xenobiotics, ethanolic and water extracts of the leaf of *Gongronema latifolium*, administered orally to rats are enzymatically metabolised in the liver. The metabolites in turn could induce the activity of the enzymes including that of the α -amylase (AMS) in the pancreas to reduce blood glucose level and the DNA polymerase to enhance DNA replication. Also, catalyse addition of nucleotides to the ribosomal subunits for protein synthesis. This phenomenon could result in hepatic cell proliferation or hepatic cell hyperplasia.

The study shows that 25 mg/kg of both extracts administration to rat induced aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT) activities. The induced increase in the activities of AST and ALT was later reduced by the subsequent or further increase in the dosage regimens of the extracts (50, 75 and 100 mg/kg/d). The reduction in the enzymes activity by high doses of both ethanolic and water extracts of the leaf of *Gongronema latifolium*, indicates toxic effects of the high doses of the extracts.

In another study, extracts treatments induced increase serum levels of α -amylase (AMS) in a dose related manner. The enzyme, AMS is the plant constituent responsible for the glucose degradation or insulin-like activity of the plant extracts (Nwajo and Alumanah, 2006; Akah et al, 2011). In human, α -amylase is the major enzyme that breaks down long-chain carbohydrates (Bowman and Rand, 1980).

Therefore, increased α -amylase (AMS) activity by the extracts of the leaf of *Gongronema latifolium* could be responsible for the fall in blood glucose level of the normoglycemics, compared to control rats as reported by Aka et al (2011). One would therefore agree with the fact that the leaf of *Gongronema latifolium* possesses hypoglycemic action, justifying its usage in Nigeria phytomedicine to treat diabetic Mellitus (Agbo, 2012; Naidu, 1992).

However, abuse in the use of this plant as a vegetable or herbal medication in phytomedicine could result in hepatotoxicity.

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References

1. Agbo, CU and Obi, IU (2007). Variability in propagation potentials of stem cuttings of different physiological ages of *Gongronema latifolium* Benth. World Journal of Agricultural Science. 3(5): 576- 581.
2. Agbo, CU (2012). Emergence and early growth of *Gongronema latifolium* in relation to sowing depth and date. Journal of Applied Bioscience. 54: 3916- 3924.
3. Ahamefule FO, Obua BE, Ibeawuchi JA, Udosen NR (2006). The Nutritive Value of Some Plants Browsed by Cattle in Umudike, South Eastern Nigeria. Pakistan Journal of Nutrition (5): 404 -409.
4. Akah, PA; Uzodinma, SU; Okolo, CE (2011). Antidiabetic Activity of aqueous and methanol extract and fractions of *Gongronema latifolium* (Asclepidaceae) leaves in Alloxan diabetic

- rats. Journal of Applied Pharmaceutical Science. 01 (09): 99- 102.
5. Bowman, WC and Rand, MJ (1980). The Endocrine system and drug affecting endocrine function. In W C Bowman and M J Rand (Eds). Textbook of Pharmacology (2nd ed) pp. 19.1- 19. 64. Oxford: Blackwell Scientific Publication.
 6. Farombi, EO (2003). African indigenous plants with Chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents. African Journal of Biotechnology. 2(12): 662- 671.
 7. Morebise, O; Fafunso, MA; Makinde, JM; Olajide, OA; Awe, EO (2002). Anti-inflammatory property of the leaves of *Gongronema latifolium*. Phytother Res. 16(51) S75- 77
 8. Naidu, R (1992). Dietary advice for diabetics in Africa. International Diabetics Digest, 3(4): 114- 118.
 9. Nwajo, HU and Alumanah, EO (2006). Effect of Aqueous extract of *Gongronema latifolium* on some indices of liver function in rats. Global Journal of Medical Sciences 5 (1): 17- 20.
 10. Okolie, UV; Okeke, CE; Oli, JM and Ehiemere, IO(2008). Hypoglycemic indices of *Vernonia amygdalina* on postprandiol blood glucose concentration of healthy humans. African Journal of Biotechnology 7(24): 4581- 4585.
 11. Sabinus, OO; Eze and Benneth, CN (2013) . Effect of Tannin Extract from *Gongronema latifolium* leaves on Lipoxxygenase Cucumeropsis manii seeds. Vol. 2013(1): 1- 7. Article ID 864095
 12. Sakihama, Y; Cohen, MF; Stephen, C and Yamasaki, GH (2002). Plant Phenolic Antioxidant and Prooxidant Activities. Phenolics- induced oxidative damage mediated by metals in plants toxicology. 177: 67- 80.
 13. Sedgwick, GW; Fenton, TF; Thompson, JR (1991): Effect of Protein precipitating agent on the recovery of Plasma free amino acids. Can J Anim. Sci. 71: 953- 957.
 14. Sharma, A; Mathur, R; Shukia, S (1995). Hepatoprotective Action of Proprietary Herbal Preparation Against Carbon Tetrechloride Intoxication. Indian Drugs, 32: 120- 124.
 15. Udoh, FV (2007). The Effects Of *Gnetum Africanum* Leaf Extracts on Some Biochemical Indices and Endocrime Functions in Albino Rats. A doctorate degree thesis submitted to Graduate School, University of Calabar, Calabar, Nigeria. Page 165- 168.
 16. Udoh, FV; Eyoung, EU; Udoh, PB; Ebong, PE (2011). Dynamic Activity of the Ethanolic and Water Extracts of the Leaf of *Gnetum africanum*, Repeated Treatment on Uterine Muscle Morphology of Rats. Journal of Reproduction and Contraception. Vol 22(3). Page 169- 175.
 17. Udoh, FV (2012). Effect of Ethanolic Extract from the Leaf of *Gnetum africanum* on Endocrine Function in Female Rats. Journal of Natural Science Reasearch. Vol. 2(8). Page 67- 73.
 18. Ugochukwu, NH; Babady, NE; Cobourne, M and Gasset, SR (2003). The Effect of *Gongronema latifolium* Extracts on serum Lipids profile and oxidative stress in hepatocytes of diabetic rats. Journal of Bioscience, 28(1), 1- 5.

Table 1: Effects of Ethanolic and Water Extracts of *Gongronema latifolium* Treatment on The Serum Levels of Aspartate (AST) and Alanin (ALT) Aminotransaminases in Rats.

Experimental Group (mg/kg)	Aspartate Amino Treatment (AST)		Alanin Transaminases (ALT)	
	AST (μL^{-1}) Ethanol	AST (μL^{-1}) water	ALT(μL^{-1}) Ethanol	ALT(μL^{-1}) Water
Control	38.0 \pm 8.19	36.0 \pm 5	15.67 \pm 4.33	21.33 \pm 2.2
25	81.33 \pm 5.36*	46.67 \pm 4.82	33.33* \pm 1.21	25.33 \pm 2.16
50	41.67 \pm 4.85**	22.0 \pm 1.57	22.0* \pm 0.36	27.33* \pm 2.93
75	38.33 \pm 4.13	32.67 \pm 1.89	19.0* \pm 0.73	23.23 \pm 3.06
100	30.0* \pm 2.0	1.67** \pm 0.05	23.0** \pm 0.0	24.66* \pm 2.72

*P < 0.01, **P < 0.05 by Student t –test.

Table 2: Effects of Ethanolic and Water Extracts of the Leaf *Gongronema latifolium* Treatment on The Serum Level of α – amylase in Rats

Experimental Group (mg/kg)	Serum Levels of α - amylase		Serum Levels of Urea (mmol/l)	
	Ethanol	Water Extract	mmol L ⁻¹ Ethanolic Extract	mmol L ⁻¹ Water Extract
Control (DMSO - Saline)	230.88 \pm 50	282 \pm 41	5.13 \pm 2.35	5.58 \pm 0.15
25	253.53 \pm 65*	207.9* \pm 16.31	5.67 \pm 1.22	4.6 \pm 0.4
50	311.4** \pm 34.94	304.1** \pm 76.8	4.8 \pm 0.35	8.7** \pm 2.17
75	319.97 \pm 77.71	325** \pm 57.11	4.46 \pm 1.0	7.7* \pm 1.13
100	506.6** \pm 0.1	329.7** \pm 14	3.6* \pm 0.21	8.22** \pm 1.1

*P < 0. 01; **P < 0.05, by Student t -test

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