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Hypoglycemic Effect of *Gongronema latifolia* Extracts in Rats

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Abstract

Phytochemistry and hypoglycemic effect of the water and Ethanolic Extracts of the leaves of *Gongronema latifolium* were screened for. The extracts were Soxhlet extracted and phytochemically screened. Three tolerated doses were estimated from acute toxicity studies and orally administered to three groups of 10 rats per group, daily for 21 days. After treatments, rats of groups 1 to 3 were sacrificed and blood samples collected for glucose analysis and was compared to control group 4. Phytochemistry reveals that, both extracts of the plant contain predominant amount of Saponins and Polyphenols among other phytochemicals. Glucose analysis shows dose related reduction in the blood glucose levels compared to control. The polyphenols content of the plant extracts could cause the hypoglycemic effect observed, suggesting insulin-like activity which justifies the use of the plant leaf in the management of diabetic Mellitus in Nigeria herbal medicine.

Keywords: Phytochemistry, phytopharmacology, hypoglycemia, extracts, *Gongronema latifolium*

1. Introduction:

The plant, *Gongronema latifolium* (Asclepiadeceae) is native to southern Nigeria. It is popularly called "Utasi" by the people of the South-South and South-East Nigeria. The plant is used by herbalist in Nigerian folk medicine for the treatment of diabetes mellitus [Edet, et. al, 2011; Ugochukwu, et. al, 2003; Sakiham, et.al, 2002]. The herbal extract is prepared by grinding a known quantity of the fresh leaves of the plant and dissolving in an appropriate volume of water. The mixture is then filtered and administered orally to patient in a chosen dose, two times daily [Udoh, 2012; Okolie, et. al, 2008].

The leaves of *Gongronema latifolium* have been used as a vegetable in preparation of some Nigerian foods as appetizer. It has a bitter taste which makes most natives to develop appetite for its consumption.

A number of researches have been carried out on the biological activity of the leaf extract of *Gongronema latifolium.* Ugochukwu et al. (2003) reported that the leaf extract of *Gongronema latifolium* is used for the treatment of non insulin dependent diabetic mellitus (NIDDM), investigated in NIDDM induced rats. Morebise et. al. (2002); Sakihama et., al. (2002); and Bloomgarden (2012) reported on the anti-inflammatory properties of the plant drug. *Gongronema latifolium* has also ability to inhibit subcutanous phycomycosis,[Okafor and Nwosu, 1995; Sharma, et. al, 1995]. The study by Edet et. al. (2011), showed that treatment with the extracts of *Gongronema latifolium* is used in Nigeria herbal medicine for management of diabetis mellitus makes it possible to carry out this investigation.

2. Materials And Methods

2.1 Preparation of Extract

The flesh leaves of *Gongronema latifolium* were collected from the swamp forest of Calabar around the months of March and April, 2008. The Plant sample was exposed to dry at room temperature of 28^{o}C for 3 days. The plant was identified by Professor Ani Nkang of Botany Department, Faculty of Science, University of Calabar, Nigeria. The voucher specimen with the number, UNICAL/BOT/2010-00116, was deposited in the herbarium, Department of Pharmacology, University of Calabar, Nigeria.

The dried leaves were ground into powder. The powdered sample, weighing 100 g, was wrapped in thimble and placed in a Soxhlet extractor (M&G Scientific Company, England) and extracted in absolute ethanol (Sigma, USA), or water for 72 h respectively, to obtain either ethanolic or water extract using Rotay Evaporator (Bioscience Co. Ltd., UK).

Extraction in ethanol gave a percentage yield of 30, while water extraction was 60. Both extract-fractions 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 grams of ethanolic extract in 10 ml of 0.85% DMSO-Saline and solution (Normal Saline), to give concentration of 1000 mg/ml respectively.

2.2 Phytochemistry

Phytochemical screening of both water and ethanolic extracts of the leaf of *Gongronema latifolium* were carried out. The quantitative analysis of the phytochemicals was done following the modified methods of Sotomayor (1993), Evans (1993), Trease and Evans (1989) and Sofowora (1993).

2.3 Animals

Young adult rats of average weight of 135±1 gram bought from the animal house, College of Medicine, University of Calabar, were housed in cages of 10 per cage. The rats 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 and ambient temperature of 28±1^{o}C.

2.4 Acute Toxicity Study

The tolerated doses of the extract of the leaf of *Gongronema latifolium* were estimated from the dose response of either ethanolic or water extract of the leaf of *Gongronema latifolium*. Graded doses of either water or ethanolic extract (10, 15, 20, 25, 50, 75, 100, and 150 mg/kg) were investigated on male rats sub lethal response. From the data of the dose response relationship, three tolerated doses of either water or ethanolic extract of the leaf of *Gongronema latifolium*, were obtained and used in the study.

3. Treatment

The young adult albino rats, 80 in number were divided into 8 groups of 10 rats per group and orally administered either ethanolic or water extract of the leaf of *Gongronema latifolium,* daily for 21 days. Group 1 received 2 ml normal Saline (0.85% NaCl) solution as control. Group 2 received 25 mg/kg, group 3 received 75 mg/kg and 4 received 100 mg/kg of ethanolic extract; and groups 5 to 7 received 25, 75 and 100 mg/kg of water extract while group 8 received insulin (8µiu/kg) as standard. 24 h after last treatment, the rats of all groups were anaesthetised in a desiccator saturated with chloroform (Sigma, USA). About 0.5 ml of blood samples were collected from the rats tails by a cut with a new surgical blade. The blood samples were placed separately on a clean test strip of the glucometer and read up.

Blood Glucose Estimation

The rapid stick methods described by Hoffman, (1937) and Kadish et al (1969), were followed. Blood sample from each rat was measured by applying 0.5 ml to the test strip which was then inserted into the Glucometer for reading. The values of the blood glucose levels estimated by rapid stick methods were compared with that of a Beckman Glucose Analyser.

4. Results

The chemical constituents of both extracts of the leaf of *Gongronema latifolium* include alkaloids, saponins, tannins, cardiac glycosides, flavonoids and polyphenols (Table 1). The analysis revealed that the ethanolic extract fraction of the plant contained a very high concentration of Saponins and polyphenols, while water extract fraction contained in excess the polyphenols.

The plant extracts treatment in rats caused a dose and time dependent decrease in the blood glucose levels compared to control. Reduction in the levels of blood glucose was significant ($P< 0.05$) in ethanolic extract (100 mg/kg/d) treatment for 7 and 21 days, and water extract treatment at dose levels of 25, 75 and 100 mg/kg/d for 7 and 21 days. The data derived were analysed using student t-test, *$P< 0.05$ and **$P < 0.01$ respectively (Table 2 and 3).

5. Discussion

In Nigeria herbal medicine, the leaf extracts of the plant, *Gongronema latifolium* have been used to reduce blood sugar levels of diabetic mellitus patients (a claim by herbalist). The water and ethanolic extracts of the plant were

Soxhlet extracted and screened for their biological effects on blood glucose levels in normoglycemic rats. Phytochemistry indicates a higher present of saponins and polyphenols in the ethanolic extract and excess present of polyphenols in the water extract. The phytochemical, polyphenol is being reported to possess antidiabetic activity which might be responsible for the reduction of the blood glucose levels by the plant extarcts [Zhu et, al. 2013; Khunti et al. 2013]. Reduction in the blood glucose concentration by the leaf extracts of *Gongronema latifolium* treatment might be due to their direct action on the insulin receptors to cause reduction in the blood glucose levels of the normoglycemic rats.

The findings show that, pretreatment with the ethanolic or water extract of the leaf of *Gongronema latifolium* caused a decrease in the blood glucose levels in the normoglycemic rats, compared with that of the control. The level of reduction in the blood glucose concentration of the treated-rats with the water extract was significantly higher than that of the ethanolic extract-treated rats. The extracts of the leaf of *Gongronema latifolium* might reduce blood sugar level in a similar manner as insulin.

Insulin stimulates transport of glucose across cell membrane by the glucose transport system 1 (GLUTI) and into the heart and muscle by the glucose transport system 4 (GLUT4), brain by GLUT3 and Liver GLUT2 which is a low-affinity and high capacity glucose transporter [Khunti et, al. 2013].

Therefore, the extract of the leaf of *Gongronema latifolium* might reduce blood sugar level in the similar manner reported for insulin [Gupta et, al. 1992; Wolfe, 2002; Morebise et, al. 2002]. The extracts might bind to insulin-receptor on the plasma membrane or cause the release of insulin from the β-cell of pancreatic islets of Langerhans to initiate a signalling cascade that could promote translocation and fusion of GLUT4 containing vesicles with plasma membrane to facilitate glucose transport into cells and organs. The reduction effect of the extracts on the blood glucose level in the normoglycemic rats probably due to the polyphenols present in the plant. The biological activity of these extracts reveals that the plant possesses insulin-like property and allows the conclusion that the extracts of the leaf of *Gongronema latifolium* plant have hypoglycemic activity and could be used to regulate or reduce blood glucose level which justifies its usage in Nigerian herbal medicinal practice for the treatment of diabetes mellitus.

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TABLE 1: PHYTOCHEMICAL CONSTITUENTS OF THE WATER AND ETHANOLIC EXTRACTS OF THE LEAF OF *Gongronema latifolium*

Chemical Constituents	**Ethanolic Extract**	**Water Extract**
Alkaloids	+	++
Saponins	+++	++++
Tamins	+	+
Cardiac Gylcosides	++	++
Flavonoids	++	++
Polyphenols	+++	++++

KEY
low +
High ++
Higher +++
Excess Present ++++

Table 2: Effect of Pretreatment with the Ethanolic Extract of the leaf of *Gongronema latifolium* ,daily for 7 and 14 days on Blood Glucose levels in rats.

Experimental Group	Blood Glucose Level (µmol/l)		% - Reduction	
	7 Days	21 Days	7 Days	21 Days
Control (0.85% NaCl)	6.8 ± 0.1	6.2 ± 0.01	0	0
25 mg/kg	5.1 ± 0.01	4.8 ± 0.05	25.7	22.5
75 mg/kg	4.8 ± 0.005	4.5 ± 0.1	29.4	27.4
100 mg/kg	4.6 ± 0.001*	3.8 ± 0.001**	32.2	38.7
Standard (Insulin)	3.2 ± 0.05	1.9 ± 0.01	53.0	72.0

*P <0.05 and **P < 0.1 by Student t-test

Table 3: Effect of pretreatment with the Water Extract of the leaf of *Gongronema Latifolium*, daily for 7 and 21 days on Blood Glucose levels in rats.

Experimental Group	Blood Glucose Level (µmol/l)		% - Reduction	
	7 Days	21 Days	7 Days	21 Days
Control (0.85% NaCl)	7.0 ± 0.01	7.1 ± 0.05	0	0
25 mg/kg	4.5 ± 0.1*	3.1 ± 0.01**	37.5	55
75 mg/kg	3.8 ± 0.01*	2.7 ± 0.1**	47.2	61
100 mg/kg	3.0 ± 0.001*	2.2 ± 0.05**	58.3	68.1
Standard (Insulin)	2.8 ± 0.01	1.5 ± 0.05	60.0	77.5

*P <0.05 and **P < 0.1 by Student t-test

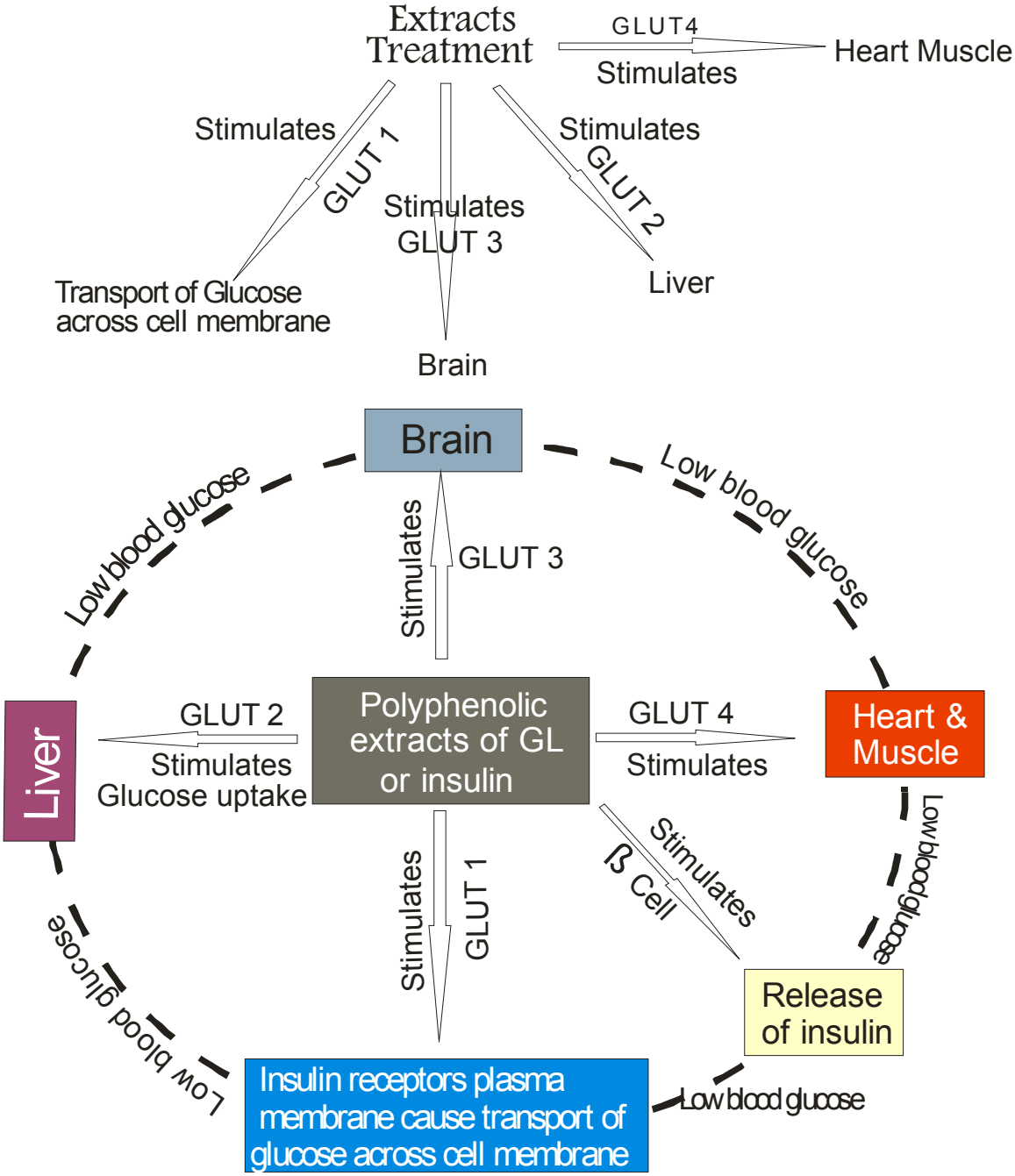

Figure 1: Structural summary of the activity of the leaf extracts of *Gongronema latifolium* on blood glucose level