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Effect of Ethanolic Leaf Extract of Gongronema Latifolium on Blood Glucose and Cholesterol Levels in Alloxan-Induced Diabetic Rats

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Abstract
This study was carried out to evaluate the effects of ethanolic leaf extract of *Gongronema latifolium* (Family: *Asclepiadaceae*) on blood glucose and cholesterol levels in alloxan-induced diabetic albino wistar rats. 30 male albino wistar rats were randomized into 4 groups (A-D). Groups A and B had 10 animals each while Groups C and D had 5 each. They were fed with standard feed and water *ad libitum*. Diabetes was induced in Groups A and B animals with 150mg of alloxan/kg body weight of animal. Group A rats were orally administered 400mg of the extract/kg body weight of animal twice daily. Group C rats also received 400mg of extract/kg body weight of animal for the same duration. Groups B and D animals served as diabetic and normal control respectively and were given distilled water in place of the extract. Treatment lasted for 10 consecutive days and blood samples for analysis were collected from the tail tips of the animals every two days. Results obtained showed that there were significant ($p < 0.05$) reductions in blood glucose and cholesterol levels in Group A rats post-treatment when compared with rats in Group B (diabetic control). No hypoglycaemic effect was observed in normal rats administered with the extract ($p > 0.05$) when compared with the control animals. Therefore, the ethanolic leaf extract of *G.latifolium* has the potentials of lowering blood glucose and cholesterol levels in alloxan–induced diabetes and could be useful in the management of diabetes and its associated complications.
Keywords: *Gongronema latifolium*, diabetes, glucose, cholesterol.

Introduction

Diabetes is a metabolic disorder of multiple aetiology, characterized by chronic hyperglycaemia with disturbances of carbohydrates, fat and protein metabolism owing to overproduction and / or underutilization of glucose (1). Diabetes is the most common endocrine disorder that impairs glucose homeostasis resulting in severe complications including retinopathy, angiopathy, nephropathy and neuropathy (2). The disease could either be as a result of insufficient production of the hormone, insulin, owing to the destruction of beta cells of the islet of Langarhans (in which case it is referred to as Insulin Dependent Diabetes Mellitus/ Type I diabetes) or as a result of the cells' inability to recognize insulin, owing to possible defective insulin receptors (in which case it is referred to as Non-Insulin Dependent Diabetes Mellitus/ Type II diabetes). Alloxan (2,4,5,6-tetraoxypyrimidine 5, 6 -dioxyuracil), an oxygenated pyrimidine derivative is a toxic glucose analogue, which selectively destroys insulin producing beta cells in the pancreas when administered to rodents and many other animal species (3). Alloxan is administered parenterally: intravenously, intraperitoneally or subcutaneously, while the dose necessary to induce diabetes is a function of the animal species, nutritional status and route of administration. This diabetogenic agent causes an insulin-dependent diabetes mellitus with characteristics similar to type I diabetes in humans (4, 5). It has been employed in experimental models for the study of diabetes.

Plants are sources of potential therapeutic agents against various diseases due to their biodiversity and the presence of a wide array of bioactive phytochemicals and secondary metabolites (6). The beneficial uses of medicinal plants in traditional system of medicine of many cultures are extensively documented. Several plants have been used as dietary adjuvant and in treating the number of diseases even without any knowledge of their proper functions and constituents. This practice may be attributed to the high cost and side effects of synthetic hypoglycemic agents (7). Plants with medicinal properties are enormously used in treating diabetes throughout the world. Many recent scientific investigations have also confirmed the efficacy of plant preparations, few of which are remarkably effective (8).

According to an estimate, 80% of the world's population depend on plants for their medication. The use of the medicinal plants is increasing in many countries where 35% of drugs contain natural products and ethno botanical information indicates that plant species are used in the traditional management of diabetes (9, 10, 11). Several investigations into the chemical and biological activities of plants have yielded compounds with properties useful for the development of modern synthetic drugs for management of several diseases including diabetes (12). In fact, there has been increasing demand for the use of plant products with antidiabetic activity due to low cost, easy accessibility and lesser side effects (13). *Gongronema latifolium* is a leafy vegetable of the family, *Asclepiadaceae* widely employed in Nigeria for various medicinal and nutritional purposes (14). In Nigeria, it is known by the Igbos as Utazi, the Efik/ Ibibio call it Utasi while the Yorubas call it Arokeke (15). Nutritionally, *G.latifolium* is a good source of protein, minerals and vitamins. Previous researches have shown

that the leaves are suitable for use in food production due to their high amino acid contents (16). The leaves can be eaten fresh, dried and used as local powdery spice or as vegetable for food preparations such as unripe plantain porridge, white soup, sauces and salads in which they add a bitter-sweet flavor (17). Scientific studies have established the cardio-protective, anti-inflammatory and antioxidative effects of aqueous and ethanolic extracts of *G.latifolium* leaf (18, 19). This study evaluates the antidiabetic effects of *G.latifolium* in alloxan–induced diabetic rats.

Materials and Methods

Plant Materials

Fresh leaves of *Gongronema latifolium* were harvested from a bush in Umuozu in Nwangele Local Government Area, Imo State, Nigeria, during the month of June. The leaves were authenticated at the Department of Plant Biology and Biotechnology of the University of Benin, Benin City, Nigeria.

Preparation of Ethanolic Extracts

The leaves of *Gongronema latifolium* were washed, air dried at room temperature and pulverized into powder. 500 grams of the powder was extracted with 5 litres of absolute ethanol (BDH, England) with repeated stirring for 72h. The mixture was first filtered using cheese cloth and later with Whatman filter paper No. 1. The recovered filtrate was concentrated at 60ºC using a rotary evaporator (Buchi Labortechnik, Flawil, Switzerland) and the yield determined. Five (5) grams of the extract was dissolved in 100ml of distilled water to form the extract stock solution from which estimated doses were administered. The doses of the extract administered was estimated using the method described by Tedong *et al.* (20).

Experimental Animals

Thirty (30) male albino wistar rats weighing between 100-150g were used for the study. The animals were housed in clean disinfected galvanized cages in the animal house (Department of Biochemistry, University of Benin) and maintained under a 12h light and dark cycle. They were allowed to feed (standard pelletized growers feed from Bendel Feed and Flower Mill, Ewu, Edo State) *ad libitum* and given water throughout the duration of the study.

Induction of Diabetes

Diabetes was induced in the rats by a single intraperitoneal injection of freshly prepared alloxan (Aldrich, Germany) solution of 150mg of alloxan/ kg body weight of animal. The rats were allowed to adapt for 48h before fasting blood sugar was determined using a glucometer (Accu–Check, Mannheim, Germany). Only animals having blood glucose levels ≥ 200 mg/dl were selected for the study.

Experimental Design

The experimental animals were randomized into four groups (A to D). Groups A and C had 10 animals each, while Groups C and D had 5 each.

Group A was injected with 150 mg alloxan/kg body weight of animal and treated with 400 mg of *G. latifolium* extract /kg body weight animal twice daily using a gavage.

Group B which served as positive control was injected with 150 mg alloxan/kg body weight of animal and given distilled water.

Group C received by means of a gavage 400 mg of *G.latifolium* extract /kg body weight animal twice daily.

Group D which served as negative control was given distilled water.

A period of two weeks was allowed for acclimatization of animals, after which they were fasted overnight (14h) and blood collected from the tail tips of each animal for the estimation of baseline parameters for glucose and cholesterol (Pre-Alloxan), followed by the induction of diabetes in some of the animals.

Administration of Extracts

After the 48h adaptation period, following alloxan administration, animals were fasted overnight (14h) and blood samples collected (Post-Alloxan). The extract was then administered orally with the aid of a gavage, for 10 consecutive days at a dose of 400 mg/kg body weight twice daily. Blood samples were collected (Post–Treatment) at intervals of 2 days thereafter.

Sample Collection

Whole blood was collected from the tail tips of each animal into clean plain bottles every two days, until the last doses of extracts were administered. The blood was allowed to stand on ice for about 15 min and centrifuged (B. Bran Scientific, 80-2, England) at 10,000 r.p.m. for 5min. Serum was separated from the clot with Pasteur pipette into sterile sample tubes for biochemical assays.

Biochemical Assays

The blood glucose level was estimated using One Touch Glucometer (Accu–Check, Mannheim, Germany). Serum Cholesterol concentration was determined spectrophotometrically after enzymic hydrolysis and oxidation as described in the Randox reagent kit (Randox Laboratory Ltd, UK). 10µl of sample, standard and distilled water (which served as reagent blank) were dispensed into 3 different test tubes, and 1000 µl working reagent (4 – aminoantipyrine) was added to each of the tubes. The mixture was allowed to stand for 10minutes at 25˚C.

Absorbance of the sample and of the standard were measured against the reagent blank at 500nm within 60minutes. The indicator quinoneimine is formed hydrogen peroxide and 4 – aminoantipyrine in the presence of phenol and peroxidase.

Statistical Analysis

Data are expressed as mean ± standard error of mean. Significance of difference was tested by ANOVA and Duncan's multiple comparison test, using SPSS Advanced Statistics Version 21.0. Statistical significance was set at $p \leq 0.05$.

Results and Discussion

Tables 1 and 2 shows the mean values of fasting blood glucose and cholesterol of controls, normal and diabetic treated animals. The results revealed significant ($p < 0.05$) increases in levels of blood glucose and cholesterol in the rats administered alloxan (Groups A and B) when compared with the normal control rats (Group D). Alloxan, a derivative of uric acid and other substances of different chemical origin, is a diabetogenic drug (in the same class with streptozotocin, ditizona, etc.) which selectively degranulate and consequently degenerate the ß-cells of islet of Langerhans through a process mediated by reactive oxygen species ROS (21). It has been effectively used to induce diabetes in animal experimentation (3, 4), thus, corroborating the approach used in this study.

A major index of assessment of diabetes mellitus is persistent hyperglycemia with its attendant effect on cardiovascular diseases, thus leading to an increase in cholesterol especially LDL-cholesterol "bad" fat (22). No significant differences were seen in blood glucose and cholesterol concentrations of the treated normal rats compared with their controls. A similar finding was reported by Nimenibo-Uadia and Osagie (23) with *Ficus exasperata* leaf extract which decreased blood glucose concentration in alloxan-induced diabetic rats but not in the normal treated rats. However, there were significant ($p < 0.05$) reductions in blood glucose and cholesterol levels in animals injected with alloxan and administered the extract of *G.latifolium* (Group A) when compared with animals injected with alloxan but not administered the extracts (Group B). This indicates anti-hyperglycaemic and anti-hypercholesterolaemic effects for the extract.

Conclusion

This study has demonstrated that ethanolic leaf extract of *G.latifolium* exhibited significant ($p < 0.05$) antihyperglycaemic and antihypercholesterolaemic effects in alloxan-induced diabetic rats. Thus, suggesting a possible ameliorative effect and confirming its usage as a herbal remedy in the management of diabetes mellitus, and its associated complications. Several authors have reported that some secondary metabolites, such as saponins, flavonoids, phenolic compounds and triterpenoids possess hypoglycaemic and hypolipidaemic activity (24, 25). Hence the observed decreases in blood glucose and cholesterol in the diabetic rats treated with *G.latifolium* extract may be due to any of the different types of active secondary metabolites, which warrants further investigation.

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Table 1: **Effect of ethanolic leaf extract of *G. latifolium* on levels of Blood Glucose in diabetic and non-diabetic male rats (mg/dl).**

Group	Pre-Alloxan	Post-Alloxan	Post-Treatment → FASTING BLOOD GLUCOSE LEVELS CONCENTRATION (mg/dl)								
			Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15	Day 17
1	92.30	450.70	394.75	338.12	307.12	275.11	246.11	181.91	183.91	160.13	147.80
n=10	± 7.66^a	± 22.7^c	± 4.80^b	± 9.00^b	± 3.90^b	± 7.20^b	± 9.50^b	± 9.80^b	± 0.00^b	± 8.10^b	± 2.60^b
2	81.20	401.00	424.73	388.14	409.14	395.12	394.16	424.20	430.36	422.30	417.25
n=10	± 9.11^a	±19.82^b	± 4.40^c	± 6.80^c	± 4.50^c	± 5.50^c	± 3.00^c	± 6.20^c	± 3.00^c	± 4.00^c	± 6.60^c
3	87.80	90.10	91.06	92.40	80.80	79.30	80.88	81.50	81.20	82.30	62.30
n=5	± 5.54^a	± 0.66^a	± 4.74^a	± 6.20^a	± 3.30^a	± 9.72^a	± 5.90^a	± 0.30^a	± 7.60^a	± 5.05^a	± 0.05^a
4	76.00	82.33	93.40	81.40	75.30	73.20	71.29	86.20	66.80	86.30	76.30
n=5	± 11.42^a	± 7.45^a	± 3.98^a	± 0. 71^a	± 6.69^a	± 3.85^a	± 3.30^a	± 3.00^a	± 7.90^a	± 0.00^a	± 7^a

Data represent mean ± SEM; n = number of rats. Values with different superscripts along the same column, are significantly different ($p \leq 0.05$).

Table 2: **Effect of ethanolic leaf extract of *G. latifolium* on levels of Serum Cholesterol in diabetic and non-diabetic male rats (mg/dl).**

Group Pre-Alloxan Post-Alloxan Post-Treatment →

SERUM CHOLESTEROL LEVELS CONCENTRATION (mg/dl)

			Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15	Day 17
1	138.70	240.70	228.30	216.20	208.40	194.00	176.50	162.60	143.10	134.30	146.20
n=10	± 13.18^a	± 2.53^b	± 2.47^b	± 3.60^b	± 8.00^b	± 5.99^b	± 8.00^b	± 6.30^b	± 0.09^a	± 9.26^a	± 13.62^a
2	153.30	260.50	262.80	254.14	250.10	265.10	290.10	224.20	250.30	264.30	262.30
n=10	± 11.94^a	±5.01^c	± 4.40^c	± 4.68^c	± 4.45^c	± 5.55^c	± 6.20^c	± 6.20^c	± 6.30^c	± 0.04^c	± 0.12^c
3	156.20	144.00	147.60	152.00	136.30	148.70	147.90	148.40	144.70	150.20	144.10
n=5	± 9.27^a	± 6.99^a	± 5.22^a	± 6.04^a	± 9.03^a	± 7.00^a	± 0.56^a	± 5.55^a	± 3.29^a	± 7.00^a	± 3.15^a
4	142.20	146.80	146.60	147.90	140.30	142.80	131.30	145.30	139.20	140.30	137.40
n=5	± 14.50^a	± 0.58^a	± 7.11^a	± 7.30^a	± 5.63^a	± 5.85^a	± 3.55^a	± 0.00^a	± 3.50^a	± 7.80^a	± 1.75^a

Data represent mean ± SEM; n = number of rats. Values with different superscripts along the same column, are significantly different ($p \leq 0.05$).

References

1. Inbaraj, S. D., and Inbaraj, G. J. (2014). "Comparative study of lipid and glycemic effects of pioglitazone, rosiglitazone with glibenclamide in patients with type 2 Diabetes and Dyslipidemia". *Global Journal of Pharmacology*. 8(1): 107 - 113.
2. Vivek, K. S., Suresh, K., Hitesh, J. P., and Shivakumar, H. (2010). "Hypoglycaemic activity of ficus glomerata in alloxan induced diabetic rats". *International Journal of Pharmaceutical Sciences Review and Research*. 1(2): 18 – 22.
3. Lenzen, S. (2008). "The mechanisms of alloxan and streptozotocin induced diabetes". *Diabetologia* 5(2): 216 – 226.
4. Carvalho, E. N., Carvalho, N. A. S., and Ferreira, L. M. (2003). "Experimental model of induction of diabetes mellitus in rats. *Acta Cir Bras*. 8 (9): 60 - 64.
5. Szkudelski, T. (2001). "The Mechanism of Alloxan and Streptozotocin Action in Beta Cells of the Rat Pancreas". *Physiology. Research*. 50: 536 - 546.
6. Farombi, E. O. (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. Taylor, B. J., and Triggle, J. D. (2006). "Comprehensive Medicinal Chemistry – II. Global Perspective", Text Book, vol. 1. Elsevier, pp. 357.
8. Marles, R.J., and Farnsworth, N.R. (1995). "Antidiabeticplants and their active constituents". *Phytomed.* Vol.2, no.2 pp.137 - 189.
9. Abo, K.A., Fred-Jaiyesimi, A.A., and Jaiyesimi, A.E.A. (2008). "Ethnobotanical studies of medicinal plants used in the management of diabetes mellitus in South Western Nigeria". J. Ethnopharmacol. 115: 67 – 71.
10. Li, W.L., Zheng, H.C., Bukuru, J., and Kimpe De, N. (2004). "Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus". J. Ethnophamacol. 92: 1 - 21.
11. Van Huyssteen, M., Milne, P.J., Campbell, E.E., and Van de Venter, M. (2011). "Antidiabetic and Cytotoxicity Screening of Five Medicinal Plants Used by Traditional African Health Practitioners in the Nelson Mandela Metropole, South Africa". Afr. J.Trad., Complement. and Alt. Med. (AJTCAM). 8(2): 150 - 158.
12. Malviya, N., Jain, S., and Malviya, S. (2010). "Antidiabetic potential of medicinal plants". *Acta Poloniae Pharmaceutica*. 67(2): 113 - 118.
13. Modak, M., Dixit, P., Londhe, J., Ghaskadbi, S., Devasagayam, T.P.A. (2007). "Indian Herbs and Herbal Drugs Used for the Treatment of Diabetes". *J Clin Biochem Nutr*, **40**(3): 163 - 173.
14. Morebise, O., Fafunso, M. A., Makinde, J. M., Olajide, O. A., and Awe, E. O. (2002). "Antiinflammatory property of the leaves of Gongronema latifolium". *Phytotherapy Research.* 16: 75 - 77.
15. Edim, E. H., Egomi, U. G., Ekpo, U. F., and Archibong, E. U. (2012). "A review on Gongronema latifolium (Utasi): A novel antibiotic against Staphylococcus aureus related infections". *International Journal of Biochemistry and Biotechnology*. 1(8): 204 - 208.

16. Osuagwu, A.W., Ekpo, I.A., Okpako, E.C., Out, P., and Ottoho, E. (2013). "The Biology, Utilization and phytochemical composition of the fruits and leaves of Gongronema latifolium Benth". Agr. Technol., 2: 115.
17. Morebise, O., and Fafunso, M.A. (1998). "Antimicrobial and phytotoxic activities of saponin extracts from two Nigerian edible medicinal plants". Biokemistri., 8(2): 69 - 77.
18. Ogundipe, O. O., Moody, J. O., Akinyemi, T. O., and Raman, A. (2003). "Hypoglycaemic potentials of methanolic extracts of selected plant foods in alloxanized micec. *Plant Food for Humun Nutrition*. 58: 1 - 7.
19. Edet, E. E., Akpanabiatu, M. I., Eno, A. E., Umoh, I. B., and Itam, E. H. (2009). "Effect of Gongronema latifolium crude leaf extract on some cardiac enzymes of alloxan-induced diabetic rats". *African Journal of Biochemistry Research*. 3(11): 366 - 369.
20. Tedong, L., Dzeufiet, P. D. D., Dimo, T., Asongalem, E. A., Sokeng, S. N., Flejou, J. F., Callard, P., and Kamtchouing, P. (2007). "Acute and sub-chronic toxicity of Anacardium occidentale leaves hexane extract in mice". *African Journal of Traditional, Complementary and Alternative Med*icine. 4(2): 140 - 147.
21. Covington, D. S., Xue, H., Pizzini, R., Lally, K., and Andrassy, R. J. (1993). "Streptozotocin and aloxan are comparable agents in the diabetic model of impaired wound healing". *Diabetic Res*. 23: 47 - 53.
22. Howard, B. V. (1987). "Lipoprotein metabolism in diabetes mellitus". *Journal of Lipid Research* 28: 613 - 628.
23. Nimenibo-Uadia, R. I., and Osagie, A. U. (2001). "Effect of Ficus exasperata (Vahl) Aqueous leaf extract on normal and alloxan-diabetic rats". *Nigerian Journal of Biochemistry and Molecular Biology* 16(1): 67 – 71.
24. Ukwe, C.V., Ubaka, C.M., Adibe, M.O., Okonkwo, C.J. and Akah, P.A. (2010). Antiulcer activity of roots of zapoteca portoricensis. J. Basic Clin. Pharmacol., 1: 185-186.
25. Punitha, R., Shirwaika, A. and Shirwaikar, A. (2005). Anti-diabetic activity of benzyl tetraisoquinoline alkaloid, berberine, in streptozocin-nicotinamide induced type 2 diabetic rats. *Diabetologian Croat*., 34 (4): 117-121.