

Protective Effect of Ethanolic Extract of *Gongronema Latifolium* Leaves in Alloxan-Induced Diabetic Rats

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Abstract: *Gongronema latifolium* is traditionally used in folk lore medicine for the treatment of various ailments. The effect of ethanolic extract of *Gongronema latifolium* on body weight, glucose level, lipid profile and hepatic markers was investigated in alloxan-induced diabetic rats. For the investigation, rats were divided into four groups (control, diabetic untreated, diabetic treated with two different doses (200 and 400 mg/kg) extracts of *G. latifolium*) . The control rats were administered tap water and normal rat diet while alloxan monohydrate in a dose of 150 mg/kg body weight was administered once intraperitoneally to all the groups except control after the rats were confirm to be diabetic, *G. latifolium* extract in two doses of 200 mg/kg and 400 mg/kg body weight was administered for 14 days to diabetic treated rats groups C and D respectively. The results revealed that treatment with alloxan monohydrate significantly increase ($p < 0.05$) glucose, Serum total cholesterol (TC), total triglycerides (TG), low density lipoprotein (LDL), alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels and a significant decrease in body weight and high density lipoprotein (HDL) level in diabetic untreated rats when compared with the control. However, treatment with *G. latifolium* extracts significantly reduces ($p < 0.05$) or reverses the changes caused by alloxan treatment in all examined parameters. This study showed that *G. latifolium* extracts exerted a hypoglycemic effect; improved the lipid profile in diabetic rats and positively affected integrity and function of both liver and pancreas.

Keywords: Alloxan monohydrate, Diabetes, *Gongronema latifolium*, Lipid profile, Liver marker enzymes

I. Introduction

Diabetes mellitus is a predominant public health concern, affecting approximately 176 million people worldwide and there are projections that the number of diabetics will exceed 350 million by 2030 [1,2]. The disease causes substantial morbidity, mortality, and long-term complications and remains an important risk factor for cardiovascular disease [3]. Diabetes mellitus is a metabolic disorder characterized by the presence of chronic hyperglycemia resulting from a deficiency of insulin secretion, abnormalities of insulin action on target tissues, or combining the two [4,5]. It is a disease that occurs when the pancreas produces little or no insulin. An abnormal blood sugar level in the blood reflects an imbalance between the inputs of glucose in the body and good use by the cells of various organs. Prolonged hyperglycemia is now recognized as the primary casual factor in the pathogenesis of diabetic complications because hyperglycemia induces a large number of alterations in vascular tissue that potentially initiate and accelerated some diabetic complications including atherosclerosis [6].

Though the discovery of insulin and oral hypoglycemic agents has helped in the management of diabetes mellitus, the frequent occurrence of side effects and the toxicities as well as their inability to cure the disease have necessitated the search for plant based derivatives for possible use in the treatment of the disease [2]. Also, the current shift away from the use of synthetic chemicals in food processing necessitates a further evaluation of widely available but underutilized tropical medicine which bears low cost, easy availability and has lesser side effects. Hence plant materials are continuously scrutinized and explored for their beneficial effect. The phytochemicals identified from traditional medicinal plants are presenting an exciting opportunity for the development of new types of therapeutics, with accelerated global efforts to harness and harvest those medicinal plants that bear a substantial amount of potential phytochemicals showing multiple beneficial effects in combating diabetes and diabetes-related complications [7,8]. Treatment by herbal medicines may have some advantages over treatment by single purified chemicals; as herbal medicine are the mixtures of more therapeutic or preventive components, and so might have more activity than single products alone.

Gongronema latifolium is a plant that is traditionally used for a number of medicinal and nutritional purposes [9]. In Eastern Nigeria, the leaves are used to prepare soup for mothers that have recently put to bed, where it is believed to stimulate appetite, reduce post-partum contraction and enhance the return of the menstrual cycle. The traditional medicine practitioners in this region use *Gongronema latifolium* as a staple vegetable and spice in the management and treatment of a number of ailments amongst which is diabetes

mellitus [10,11] and to support the pancreas regeneration [12]. In Sierra Leone, a decoction or cold infusion of the pounded stem is used for colic and intestinal symptoms usually associated with worms [13]. In Ghana, the boiled fruits are used as laxative. In the United State, it is used as a constituent of herbal tea blend for maintenance of healthy glycemic control. Indigenous folk medicine practitioners of the Jos Plateau, Nigeria, claim that the aqueous leaf extract of *Gongronema latifolium* is also effective against diabetes mellitus. But there appears to be little or no scientific data in support of the claim. This study was designed to assess the effect of ethanolic leave extract of *Gongronema latifolium* on glyceamic responses in normal and alloxan induced diabetic rats so as to ascertain whether indeed it exhibits hypoglycemic activity in the mammalian model system.

II. Materials And Methods

2.1 Plant material

Gongronema latifolium leaves used in this research work were freshly obtained from Chobe, Jos and were botanically identified and authenticated as *G. latifolium* before usage at the Department of Plant Science, University of Jos, Plateau state, Nigeria.

2.2 Preparation of plant extract for antidiabetic studies

The *Gongronema latifolium* leaves were shade dried at room temperature under continuous ventilation and the dried leaves were pounded into fine powder using a pestle and a mortar. Sixty grams of the plant's powder was weighed and soaked or steeped overnight in 300 ml 75% ethanol. The mixture was shaken on a mechanical shaker for 3hours and filtered through a Whatman No.1 filter paper. The filtrate was concentrated on a water bath, and the concentrate was freeze-dried and stored in the dessicator pending use. The freeze-dried extract was resuspended in distilled water at appropriate concentrations for the various experimental doses using the equation of *Tedong et al.*, [14] prior to use.

2.3 Animals

Sixteen male Wistar Strain rats weighing between 150-210g b.wt obtained from the Animal House Unit, of the Department of Pharmacology, University of Jos, were used in the study. They were maintained on a standard rat diet, 'Vital Feed' (purchased from Grand Cereals and Oil Mills Ltd, Kuru, Jos, Nigeria) and tap water as drinking water, *ad libitum*.

2.4 Experimental design

In the investigation rats were distributed evenly, 4 rats/cages into four standard plastic-metal rat cages, labeled A-D, respectively and were acclimatized for 5 days prior to any procedure.

Group	Treatment
A	Normal control
B	Diabetic untreated
C	Diabetic + 200mg/kg of <i>G. latifolium</i> extract
D	Diabetic + 400mg/kg of <i>G. latifolium</i> extract

2.5 Induction of diabetics

Diabetes mellitus was induced in animals by a single intraperitoneal injection of 150 mg/kg body weight of alloxan monohydrate (Sigma, St. Louis, USA) suspended in normal saline, after an overnight fasting. Two days after alloxan injection, diabetes was confirmed using On Call Plus glucometer and glucose strips. Animal with fasting blood glucose level of 200-260 mg/100 ml were taken for the study.

2.6 Biochemical analysis

The animals were sacrificed at the end of experimental period of 14 days by decapitation. Blood was collected, sera separated by centrifugation at 3000g for 10 minutes. Serum glucose was measured by the glucose oxidase method. Serum total cholesterol (TC), total triglycerides (TG), low density lipoprotein (LDL), and high density lipoprotein (HDL) as well as the activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were evaluated using Fortress Kits (Purchased from Fortress Diagnostic LTD, Antrim, UK) in the normal, diabetic induced and extract treated rats.

2.7 Statistical Analysis

All data are expressed as mean \pm standard deviation (SD). Comparison of the data from test control groups of animals were analyzed by One Way Analysis of Variance (ANOVA) at the confidence limit of 95% and where applicable, least significant difference (LSD) was used to determine significant results; differences between groups were considered statistically significant at $P < 0.05$.

III. Results

3.1 Weight variation

The effect of administration of ethanolic leaf extract of *G. latifolium* on body weight of rats during the experimental period is summarized on Table 1. As could be seen from the table both the control group and the treated groups had significant increase ($P<0.05$) in weight while the diabetic untreated rats had significant decrease ($P<0.05$) in weight.

3.2 Glucose level

Table 2 shows the effect of the ethanolic leaf extract of *G. latifolium* on serum glucose. There was a significant increase ($P<0.05$) in the serum glucose level in all Alloxan induced diabetic groups compared to the normal control. However, serum glucose level were significantly reduced ($P<0.05$) upon administration of the extracts of *G. latifolium*. However, the effect of the extract on blood glucose level is biphasic and dose related, with the higher dose being more potent.

3.3 Lipid profile

Table 3 summarize the results of the determination of serum lipid profile; serum total cholesterol (TC), total triglycerides (TG), low density lipoprotein (LDL) and high density lipoprotein (HDL). There was a significant increase ($P<0.05$) in the levels of serum TC, TG, and LDL and a significant decrease ($P<0.05$) in the level of serum HDL of diabetic untreated rats when compared with the control rats. However, diabetic rats treated with *G. latifolium* leaf extract reversed serum lipid profiles to near normal levels with the higher dose (400mg/kg body weight) being the most effective.

3.4 Liver enzyme

The effect of *G. latifolium* leaf ethanolic extract on the activity of liver marker enzymes are shown on Table 4. The diabetic untreated rats had a significantly elevated ($P<0.05$) level of liver marker enzymes; Alkaline Phosphatase (ALP), Alanine Aminotransferases (ALT), and Aspartate Aminotransferases (AST) when compared with normal control rats. However, after treatment with *G. latifolium* leaf ethanolic extracts liver marker enzymes were significantly reversed back to near normal levels with the higher dose (400mg/kg body weight) being the most effective.

Table 1: Effect of ethanolic leaf extract of *G. latifolium* on body weight of both normal and diabetic rats.

Group	Treatment	Weight variation in g		
		Day 1	Day 7	Day 14
A	Control	160.00±1.20	198.75±1.00	226.25±1.10
B	Diabetic untreated	195.33±1.50 ^a	188.00±1.61 ^a	180.67±1.67 ^a
C	Diabetic + 200mg/kg G.lat	170.00±2.10 ^{ab}	197.50±1.20 ^{ab}	215.00±1.30 ^{ab}
D	Diabetic + 400mg/kg G.lat	186.67±1.51 ^{ab}	210.00±1.02 ^{ab}	225.00±1.25 ^b

Values are expressed as mean ± SD, n = 4, where n is the number of rats in each group.

^a values are significantly different from control ($p<0.05$)

^b values are significantly different from the diabetic untreated group ($p<0.05$)

G.lat = *G. latifolium*

Table 2: Effect of ethanolic leaf extract of *G. latifolium* on serum glucose level of both normal and diabetic rats.

Group	Treatment	Glucose (mmol/L)
A	Control	4.35±0.56 ^{ab}
B	Diabetic untreated	8.05±0.39 ^{ab}
C	Diabetic + 200mg/kg G.lat	7.22±0.22 ^{ab}
D	Diabetic + 400mg/kg G.lat	5.56±0.45 ^{ab}

Tabulated values are mean ± SD, n = 4, where n is the number of rats in each group.

^a values are significantly different from control ($p<0.05$)

^b values are significantly different from the diabetic untreated group ($p<0.05$)

G.lat = *G. latifolium*

Table 3: Effect of ethanolic leaf extract of *G. latifolium* on serum lipid profile level of both normal and diabetic rats.

Group	Treatment	Lipid profile (mmol/L)			
		TC	TG	LDL	HDL
A	Control	5.57±0.26	3.15±0.10	1.42±0.15	2.57±0.14
B	Diabetic untreated	6.39±0.34 ^{ab}	4.10±0.10 ^{ab}	3.09±0.12 ^{ab}	1.26±0.32 ^{ab}
C	Diabetic + 200mg/kg G.lat	5.76±0.36 ^{ab}	2.62±0.00 ^{ab}	2.02±0.80 ^{ab}	2.42±0.54 ^{ab}
D	Diabetic + 400mg/kg G.lat	4.86±0.28 ^{ab}	1.07±0.00 ^{ab}	0.54±0.14 ^{ab}	3.54±0.10 ^{ab}

Tabulated values are mean ± SD, n = 4, where n is the number of rats in each group.

^a values are significantly different from control (p<0.05)

^b values are significantly different from the diabetic untreated group (p<0.05)

G.lat = *G. latifolium*

Table 4: Effect of ethanolic leaf extract of *G. latifolium* on tissue marker enzymes in the liver of both normal and diabetic rats.

Group	Treatment	Tissue marker enzymes (IU/L)		
		ALP	ALT	AST
A	Control	132.50±4.51	45.25±5.19	149.75±12.26
B	Diabetic untreated	142.67±0.58 ^a	93.67±12.90 ^a	187.00±55.07 ^a
C	Diabetic + 200mg/kg G.lat	137.00±4.58 ^{ab}	64.00±2.00 ^{ab}	149.67±2.52 ^{ab}
D	Diabetic + 400mg/kg G.lat	133.00±5.66 ^b	73.50±2.12 ^{ab}	148.50±12.02 ^{ab}

Values are expressed as mean ± SD, n = 4, where n is the number of rats in each group

^a values are significantly different from control (p<0.05)

^b values are significantly different from the diabetic untreated group (p<0.05)

G.lat = *G. latifolium*

IV. Discussion

Diabetes mellitus is a major public health problem in the developed as well as developing countries and it is among the leading causes of death in the world [15]. The management of diabetes mellitus is considered a global problem because a successful and effective treatment is yet to be discovered. Most of the modern anti-diabetic drugs, including insulin and oral hypoglycemic agents only control blood sugar levels as long as they are regularly administered and are associated with a number of undesirable effects [16]. This generates the need for better, convenient and less toxic treatment options. Traditional antidiabetic plants might provide a useful source of new oral hypoglycemic compounds for development as pharmaceutical entities or as simple dietary adjuncts to existing therapies [15].

The results from this study revealed a significant loss in body weight of the rats in the diabetic untreated group when compared to the control whereas the rats in the *Gongronema latifolium* ethanolic leaf extract treated diabetic groups gained weight. This finding appears to be consistent with the previous report of World Health Organization, that diabetes mellitus is often characterized by rapid and significant weight loss leading to fatigue [17,18]. Other researchers have also reported that diabetes mellitus is characterized by a progressive weight loss and some plant extracts are known to reverse the weight loss to near normal [19,20,21]. The weight loss observed may be attributed to the loss in muscle and adipose tissue resulting from excessive breakdown of tissue protein and fatty acids [21,22].

Prolonged exposure to hyperglycemia is now recognized as the primary casual factor in the pathogenesis of diabetic complications as well as induces a large number of alterations in vascular tissue that potentially promote or accelerated atherosclerosis [6]. The results of the antidiabetic test shows that the doses of 200 and 400 mg/kg b.wt of *G. latifolium* extracts significantly (p<0.05) decrease the blood glucose levels in the diabetic treated animals when compared with the diabetic untreated animals. The effect of administration of *G. latifolium* extracts on alloxan induced diabetic rats observed in this study appear to be dose dependent with

the higher dose being the more potent. Thus, the results of this study of the ethanolic extract of *G. latifolium* on the blood glucose levels of normal and alloxan induced diabetic rats are in consonant with the findings of earlier researchers that plant extracts have hypoglycemic and insulin release stimulatory effects which in turn reversed alloxan induced hyperglycemia in [2,23]. The possible mechanism by which ethanol extract brings about its hypoglycemic action may be by induction of pancreatic insulin secretion from β cells of islets of langerhans or due to enhanced transport of blood glucose to peripheral tissue.

The levels of serum lipid profiles; total cholesterol (TC), total triglycerides (TG), low density lipoprotein (LDL), and high density lipoprotein (HDL) in control and experimental animals were investigated. Alloxan induced diabetic untreated rats showed significantly increased serum lipid profiles except HDL when compared with the control rats. However, treatment with 200 and 400 mg/kg b.wt of *G. latifolium* extracts significantly ($P<0.05$) reduces the total cholesterol, triglyceride and low density lipoprotein when compared to the diabetic untreated rats. Similarly the high density lipoprotein which was reduced in the diabetic untreated rats was significantly increased ($P<0.05$) in the groups administered the *G. latifolium* extracts. The elevated TG, TC, LDL level and decreased HDL level in alloxan-induced diabetic rats observed in this study is in agreement with the previous reports regarding alteration of these parameters under diabetic condition [23]. The diabetes-induced hyperlipidemia might be due to excess mobilization of fat from the adipose tissue because of underutilization of glucose. The result of our study is in accord with the findings of other researchers who reported that Many plants extracts have potential therapeutic value in combating atherosclerosis which is one of the major complications of diabetes by lowering serum lipids particularly total cholesterol, triglyceride and low density lipoprotein level [23].

The observed marked increases in the activities of aminotransferases (AST and ALT) and alkaline phosphatase (ALP) in the diabetic untreated rats are indications that the liver resulted in cytotoxic injury when compared with the control. Conversely, a marked decrease in the activities of these marker enzymes was observed in the *G. latifolium* extract treated diabetic rats when compared to the diabetic untreated rats which imply a decrease in the rate and magnitude of tissue cell injury and it is also in accord with the observed protective effect of plant extracts against alloxan-induced diabetes in rats. Measurement of the activities of “marker” enzymes or biomarkers in body fluids can be used in assessing the degree of assault and the toxicity of a chemical compound on organs/tissues [24,25]. Such measurements can also be used to indicate tissue cellular damage caused by a chemical compound long before it is revealed by histological techniques [26].

V. Conclusion

This study has shown that *G. latifolium* leaves have potentials for use in formulation of antidiabetic and anti-hyperlipidemic drugs in view of its hypoglycemic and hypolipidemic activities as well as the extracts ability to ameliorate the effects of weight loss and tissue marker enzymes as observed in diabetic rats. Its full potential for utilization in these systems is however dependent on the full characterization of biologically active components in the plant.

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