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Protective Effects of Combined Aqueous Leaf Extract Of Gnetum Africanum and Gongronema Latifolium in Alloxan-Induced Diabetic Wistar Rats

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ABSTRACT
This study evaluated the antidiabetic effects of combined aqueous leaf extract of Gnetum africanum and Gongronema latifolium in alloxan-induced diabetic Wistar rats. Fresh leaves extract of Gnetum africanum and Gongronema latifolium were prepared using distilled water in appropriate stock concentrations and used in a 14-day treatment. Experimental animals were acclimatized for 14 days while diabetes was induced using 150mg/kg body weight alloxan monohydrate administered intraperitoneally. After 14 days of treatment of diabetic rats with 15%, 20% and 25% concentration of combined aqueous leaf extract of G. africanum and G. latifolium extracts respectively as well as with 100mg/kg metformin, blood glucose levels decreased significantly ($p<0.05$) when compared to untreated diabetic rats. Also, there was a significant ($p<0.05$) reduction in serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) compared to the diabetic control. A dose-dependent effect was observed and the combined extract had a close similar effect as the standard drug metformin. Hence, the combined aqueous leaf extract of Gnetum africanum and Gongronema latifolium exhibit antidiabetic and hepatoprotective which can be attributed to the presence of phytochemical constituents in the leaf part of the plants.

Key Words: Gnetum africanum, Gongronema latifolium combined aqueous leaf extract, diabetes, hepatotoxicity, Bioactive constituents

I. INTRODUCTION

Diabetes is a condition that impairs the body's ability to process blood glucose, otherwise known as blood sugar. In the United States, the estimated number of people over 18 years of age with diagnosed and undiagnosed diabetes is 30.2 million. The figure represents between 27.9 and 32.7 percent of the population. Without ongoing careful management, diabetes can lead to a buildup of sugars in the blood, which can increase the risk of dangerous complications, including stroke and heart disease. Different kinds of diabetes can occur, and managing the condition depends on the type as not all forms of diabetes stem from a person being overweight or leading an inactive lifestyle. In fact, some are present from childhood [1].

Over the years, plants apart from serving as food have also been known to exhibit medicinal properties. A large and increasing number of patients use medicinal herbs or seek the advice of their physician regarding their uses. It has been estimated roughly that presently more than half of the total population of the world use herbal drugs. Extract from herbal plants is being revealed through biochemical methodology to be of medicinal benefits, playing a vital role in daily living in almost every culture. Herbal medicine which was practiced in the Asia and Africa is still useful and gaining momentum in recent time as source of medicinal raw materials, and has provided very effective treatment to many different diseases and ailments [2].

Medicinal plants are the potential source of various secondary metabolites, which are critical for their existence and also to defend the plants from fungi, bacteria, animals and even other plants. These metabolites serve as pharmaceutics, cosmetics, nutraceutics, food additives, flavours and industrially important biochemicals. Many modern medicines are derived indirectly from medicinal plants. The current search for biologically active principles from plants has all the more enhanced the importance of medicinal plants [3].

This research is based on the implementation of herbal products which involves the effect of aqueous extract of Gnetum africanum

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and Gongronema latifolium on biochemical markers of alloxan-induced Wister rat.

Gnetum africanum (eru or African jointfir) is a vine gymnosperm species found natively throughout tropical Africa [4]. Though bearing leaves, the genus Gnetum are gymnosperms, related to pine and other conifers [5-7]. Gnetum africanum has also been referred to as a form of wild spinach in English [8]. It is a perennial that grows approximately 10 meters long, with thick papery-like leaves growing in groups of three. The leaves are used as a vegetable for soups and stews, commonly called eru soup or afang soup [9]. The leaves of the vine are sold in markets throughout the year and may be used in soups and stews or eaten raw. The leaves may further be used as a remedy for nausea, sore throats, or as a dressing for warts. The stem of the plant may also be eaten for medicinal purposes, including the reduction of pain during childbirth (Styslinger, Matt.). Gnetum africanum is a good source of protein and is strong in essential and non-essential amino acids. It is high in glutamic acid, leucine, and aspartic acid, with low levels of histidine, and cysteine, while there appears to be trace amounts of tryptophan in the plant. Some of the most important bioactive phytochemical constituents present in this plant include alkaloids, flavonoids, phenolics, essential oils, tannins saponins, phytosterols, glycosides, and anthocyanidins [10]. These phytochemicals are known to perform several general and specific functions in plants, and may exhibit different biochemical and pharmacological actions in different species of animals when ingested [11,12]. These actions range from cell toxicity to cell protective effects [13]. Gongronema latifolium belongs to the group of plants known as spices. It is of the family of Asclepiadacea, genus Gongronema and species of latifolium, and the vernacular name is Bush Buck. Gongronema latifolium is a climber with woody hollow glaborous stems below and characterized by greenish yellow flowers [14]. Gongronema latifolium, commonly called 'utazi' by the Igbos in South-Eastern Nigeria and 'Arokeke' or 'madumaro' by the Yorubas of South-Western Nigeria [15]. Utazi is used in small quantity in preparing soups like Nsala soup, ugba sauce, and yam and also in garnishing dish like Abacha, Ncha, Isiewu, Nkwobi etc. The leaves are used to spice locally brewed beer. The bark contains much latex and has been tasted for exploitation [16]. The plant has been widely used in folk medicine for maintaining healthy blood glucose level [17]. The plant leaves have been found very efficacious as an anti-diarrhea, and anti-tussive [18,19]. It has been observed that the extracts of Gongronema latifolium contain phytochemical compounds including alkaloids, saponins, tannins (flavonoids), and glycosides [20]. Studies have shown that these phytochemicals found in Gongronema latifolium may influence cellular proteins with enzymic activities.

II. METHODOLOGY

Plant Material Collection

The leaves of the plants Gnetum africanum and Gongronema latifolium were collected from local farm in Aluu Community in Ikwerre Local Government of Rivers State and identified in the herbarium of the Department of Plant Science and Biotechnology in the University of Port Harcourt.

Preparation of Aqueous Extract

The leaves of the plants Gnetum africanum and Gongronema latifolium were washed and air tried for three weeks at room temperature after which the leaves were ground into fine powder using an Electric Blender. 5g of Gnetum africanum and 5g of Gongronema latifolium powder were combined and soaked in 100ml of distilled water and stirred intermittently and was sieved with white handkerchief and filtered with Whitman paper (No. 1). The extract obtained after the filtration was later diluted into 15, 20 and 25% concentration levels in different bottles of 85, 80 and 75% of distilled water respectively to obtain 100% volume of solution.

Drug Purchase and Preparation

Metformin hydrochloride (MET) by Pfizer was obtained from Alpha Pharmacy and Stores, a licensed pharmacy in Port Harcourt, Rivers State, Nigeria. In order to prepare the powder for administration to the test animals, the tablets were crushed into a fine powder and the proper concentrations produced in distilled water. Alloxan monohydrate, another substance utilized, was also bought from the same pharmacy to cause diabetes in rats. All chemicals and solvents used were of analytical grade.

Procurement of Animal

For this investigation, adult Wistar rats of either sex weighing 140–210g were used. They were acquired from the Animal House of the Department of Pharmacology at the University of Port Harcourt in River State, Nigeria, and were acclimatized for two weeks. They were kept in a

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conventional laboratory environment with 28°C temperature (28±2°C), relative humidity (46±6%), a 12-hour light/dark cycle, and adequate ventilation. The animals were given access to water and a commercial feed (Vital Feed Nig. Ltd.) ad libitum. Twelve hours prior to the experiments, food was withheld, although water was always available for free.

Induction of Diabetes

Alloxan monohydrate, freshly made with distilled water as the vehicle, was diluted to a concentration of 150mg/kg body weight and administered intraperitoneally to rats to cause diabetes. Three days later, diabetes was identified in alloxan-induced rats with Random Blood Glucose (RBG) levels ≥200mg/dL. Glucose levels were monitored using a hand held glucometer (Accu-CHEK) to test blood samples taken from the tail vein.

Experimental Layout

30 adult Wistar rats of both sexes where used in this study and were randomly selected into 5 different groups of 5 rats each. The experimental layout is as below;

Group	Treatment
Group 1	Received 1ml distilled water and rat feed only (Normal Control)
Group 2	Received 150mg/kg alloxan (Diabetes control)
Group 3	Received 150mg/kg alloxan + 100mg/kg Metformin HCL
Group 4	Received 150mg/kg alloxan + 15% concentration of extract
Group 5	Received 150mg/kg alloxan + 20% concentration of extract
Group 6	Received 150mg/kg alloxan + 25% concentration of extract

The rats in group 3 were given oral dose of 100mg/kg metformin HCl daily for 14 days. A safe oral dose of metformin HCl for rats is 100-200mg/kg [21]. All 6 groups of rats were sacrificed on last day (14th day) of treatment after 12 hours of fasting and given a chloroform anesthetic. Blood was collected by cardiac puncture into heparinized sample bottles for biochemical estimations. Blood glucose level was determined using a hand held glucometer (Accu-CHEK) before and 48 hours after alloxan monohydrate administration, for the confirmation of the diabetic state of animals.

Biochemical Assay

Glucose was determined using methods of Malloy and Evelyn [22] as modified by Tietz [23], while serum lipids including total cholesterol, total protein and triacylglycerol were determined using the method of Tietz [24]. The method of Reitman and Frankel [25] was adopted for the ALT and AST assay. ALP was estimated using the method of King and King [26] as adapted by Cheesbrough [27].

Ethical Clearance

All procedures carried out during this research were done in accordance with the guiding principles of research involving animals as recommended by the Research Ethics Committee of the University of Port Harcourt and NIH guidelines for care and use of laboratory animals.

Method of Data Analysis

Data were analyzed using SPSS version 23.0. All data obtained were expressed as Mean ± SEM. One-way analysis of variance (ANOVA) was used to compare the means between and within the groups and a p-value <0.05 was considered significant. A Tuckey's post-hoc test was also applied to assess significant differences between groups.

III. RESULTS

Effect of combined aqueous leaf extract of G. africanum and G. latifolium extract on blood glucose levels in alloxan induced diabetic rats

In untreated diabetic rats, there was a significant ($p<0.05$) rise in blood glucose level when compared with untreated non-diabetic rats. Results of this study showed that 14 days treatment with combined aqueous leaf extract of G. africanum and G. latifolium extract produced a significant ($p<0.05$) decrease in blood glucose level in experimental rats. A dose-dependent reduction in blood glucose level was observed with the 25% concentration of the combined extract as the most effective. Hence, the combined aqueous leaf extract of G. africanum and G. latifolium extract showed dose-dependent hypoglycemic effect by lowering the blood glucose (Table 1).

Effect of Metformin on blood glucose level in alloxan–induced diabetic rats

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Metformin produced significant ($p<0.05$) decrease in blood glucose levels in alloxan induced diabetic rats. With 100mg/kg dose of metformin, the blood glucose levels was 4.68±0.58mmol/L compared to blood glucose levels of 14.62±0.42mmol/L in diabetic control animals (Table 1).

Effect of combined aqueous leaf extract of G. africanum and G. latifolium extract on serum lipid parameters in alloxan induced diabetic rats

There was a decrease in total protein and total cholesterol values and an increase in triglyceride value in untreated diabetic rats. The treatment with combined aqueous leaf extract of G. africanum and G. latifolium extract (15%, 20% and 25% concentration of the combined extract) caused a significant ($p<0.05$) increase in total protein value and a significant ($p<0.05$) decrease in total cholesterol and triglyceride values when compared with untreated diabetic rats. The effect of the combined aqueous leaf extract of G. africanum and G. latifolium extract was dose-dependent and had a close similar effect as the standard drug, metformin (Table 1).

Table 1: Effect of combined aqueous leaf extract of G. africanum and G. latifolium extract on some lipid profile parameters of alloxan-induced Wistar rats

Groups	Glucose (mmol/L)	Total Protein (g/L)	T. Cholesterol (mmol/L)	Triglyceride (mmol/L)
Normal Control	4.12±0.24	63.20±2.42	3.82±0.30	1.44±0.23
Diabetes Control	14.62±0.42*	47.20±3.26*	6.32±0.30*	3.10±0.25*
100mg/kg Metformin	4.68±0.58*#	62.73±3.16*#	3.25±0.34*#	1.51±0.60*#
Alloxan + 15% Conc. of extract	13.00±0.51*#	52.00±2.61*#	5.88±0.22*#	2.48±0.21*#
Alloxan + 20% Conc. of extract	10.26±0.46*#	55.60±1.72*#	5.02±0.11*#	1.96±0.09*#
Alloxan + 25% Conc. of extract	3.92±0.10*#	67.20±2.35*#	3.70±0.22*#	1.42±0.20*#

Each value represents mean ± SEM, values marked with (*) differ significantly from normal control group (*$p<0.05$) while those marked with (#) differ significantly from diabetes control group (#$p<0.05$).

Effect of combined aqueous leaf extract of G. africanum and G. latifolium extract on Liver enzyme parameters of alloxan-induced Wistar rats

There was an increase in liver enzyme markers such as AST and ALT in untreated diabetic rats. The administration of the combined aqueous leaf extract of G. africanum and G. latifolium extract (15%, 20% and 25% concentration of the combined extract) significantly ($p<0.05$) ameliorated the levels of AST and ALT when compared with the untreated diabetic rats. The effect of the combined aqueous leaf extract of G. africanum and G. latifolium extract on liver enzyme markers was observed to be dose-dependent (Table 2).

Table 2: Effect of combined aqueous leaf extract of G. africanum and G. latifolium extract on some Liver enzyme parameters of alloxan-induced Wistar rats

Groups	AST (IU/L)	ALT (IU/L)
Normal Control	8.80±1.02	9.20±1.02
Diabetes Control	22.40±0.75*	22.52±1.83*
100mg/kg Metformin	8.76±0.55#	10.10±1.42*#
Alloxan + 15% Conc. of extract	18.40±0.86*#	17.88±0.72*#
Alloxan + 20% Conc. of extract	15.45±0.73*#	14.16±1.17*#
Alloxan + 25% Conc. of extract	8.80±1.02*#	10.80±1.02*#

Each value represents mean ± SEM, values marked with (*) differ significantly from normal control

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group (*p<0.05) while those marked with (#) differ significantly from diabetes control group (#p<0.05).

IV. DISCUSSION

Herbal medicinal researches have attained an incredible global level in the recent past as well as the continued application of some plant constituents in pharmaceutical industries thereby elevating the growing status of traditional herbal medicine in Africa in general and Nigeria in particular. There is hardly any meal prepared without the use of a plant with medicinal significance in it Port Harcourt, Rivers State of Nigeria and this tells of house important the use of plants with medicinal values are to our every day dietary life. The plants Gnetum africanum and Gongronema latifolium has been reportedly been used as treatment for diabetes and hypertension, hence the interest in assessing the antidiabetic and liver protective effects of the combined aqueous leaf extract of both plants.

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 [28]. 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 antidiabetic 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 [29]. 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 [28].

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 [30].. The results of this study showed that the combined aqueous extract (15%, 20% and 25% concentration of the combined extract) of G. africanum and 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. africanum and G. latifolium extracts on alloxan induced diabetic rats observed in this study appeared to be dose dependent with the higher percentage concentration (25% extract concentration) being the most potent. Thus, the results of this study 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 [31-33]. The possible mechanism by which the combined aqueous extract brings about its hypoglycemic action may be by induction of pancreatic insulin secretion from beta cells of islets of langerhans or due to enhanced transport of blood glucose to peripheral tissue [34,35].

The levels of serum lipid profiles; total protein (TP), total cholesterol (TC) and total triglycerides (TG) in control and experimental animals were investigated. Alloxan induced diabetic untreated rats showed significantly increased serum lipid levels of TC and TG except TP when compared with the control rats. However, treatment with combined aqueous extracts (15%, 20% and 25% concentration of the combined extract) significantly (p<0.05) reduces the total cholesterol and triglyceride levels when compared to the diabetic untreated rats. Similarly the total protein which was reduced in the diabetic untreated rats was significantly increased (p<0.05) in the groups administered the combined aqueous extracts (15%, 20% and 25% concentration of the combined extract). The elevated TC, TG and decreased TP 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 [32,33]. 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 artherosclerosis which is one of the major complications of diabetes by lowering serum lipids particularly total cholesterol, triglyceride and low density lipoprotein level [32,33].

Measurement of the activities of biomarkers in body fluids can be used in assessing the degree of assault and the toxicity of a chemical compound on organs/tissues [36,37]. Such measurements can also be used to indicate tissue cellular damage caused by a chemical compound long before it is revealed by histological techniques [38]. The observed marked increase in the activities of AST and ALT in the diabetic untreated rats is an indication of in cytotoxic injury in the liver when

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compared with the control. Conversely, a marked decrease in the activities of these liver marker enzymes was observed in the G. africanum and G. latifolium combined aqueous leaf 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 [34,35,39].

V. CONCLUSION

This study has shown that G. africanum and G. latifolium combined leaves have potentials for use in formulation of antidiabetic and antihyperlipidemic drugs in view of its hypoglycemic and hypolipidemic activities as well as the extracts ability to ameliorate the effects of 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.

Declaration

Authors declared that this is an original research and not conflicts of interest exist.

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