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Thaumatococcus daniellii Extract Modulates Glibenclamide Activity and Ameliorates Heamatological Disorders, Oxidative Stress and Dyslipidemia Associated with Diabetes Mellitus in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author OTA designed the study, performed the statistical analysis and managed the analysis of the study. Author GOO managed the literature search and its review. Authors JAB and GEA wrote the protocol and the draft of the manuscripts. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Native practices in poor regions of Africa takes local herbs with orthodox medicine for treatment of diabetes. This study investigates the roles of *Thaumatococcus daniellii* methanol leaves extract in combination with Glibenclamide.
Study Design: 35 male Wistar rats averagely weighing 150 g treated for 15 days, were randomly selected into seven groups; A (Control), B (Streptozotocin-induced diabetic rats), C (Diabetic rats administered 100 mg/kg/day body weight of extract), D (Diabetic rats administered therapeutic dose of Glibenclamide), E (Diabetic rats treated with therapeutic dose of Glibenclamide and 100 mg/kg/day bodyweight of extract), F (Extract only) and G (Glibenclamide only).

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Methodology: Haematological, lipid profile and antioxidant parameters were evaluated using international standardized methods.
Results: Results showed that Streptozotocin-induced diabetic rats (group B) elicits significant (*P*=.05) increases in haematological parameters such as fasting blood glucose level, white blood cell counts, lymphocytes and platelets with corresponding decreases in red blood cell counts and serum protein while it also exhibits significant (*P*=.05) increases in serum total cholesterol, triglycerides and a significant (*P*=.05) decrease in high density lipoprotein (HDL) Cholesterol with corresponding decreases in liver reduced glutathione level, superoxide dismutase and catalase activities as well as elevated levels of malondialdehyde. However, extract and Glibenclamide showed anti-diabetic effects as levels and activities of these markers were restored nearly to controls in groups C, D and E with significant effects exhibited by group C and in the order C>D=E.
Conclusion: Results are suggestive of the anti-diabetic and modulatory effects of the extract on Glibenclamide activity and its use could be encouraged.

Keywords: Anti-diabetic; modulatory; ameliorative; glibenclamide; Thaumatococcus daniellii.

1. INTRODUCTION

Diabetes mellitus describes the combination of numerous disorders commonly presenting with episodes of hyper glycaemia and glucose intolerance, as a result of lack of insulin, defective insulin action, or both [1]. These complications results due to derangements in the regulatory systems for storage and mobilization of metabolic fuels, including the catabolism and anabolism of carbohydrates, lipids and proteins emanating from defective insulin secretion, insulin action, or both [2,3]. Four classes of diabetes mellitus have been identified; Type 1 diabetes mellitus, Type 2 diabetes mellitus, Gestational diabetes mellitus, and other specific types [1].

Type 1 diabetes mellitus accounts for only a minority of the total burden of diabetes in a population although it is the major type of the diabetes in younger age groups. The incidence of type 1 diabetes mellitus is increasing in both rich and poor countries. Furthermore, a general shift towards type 1 diabetes mellitus occurring in children at earlier ages is imminent [1]. However 85 to 95% of all diabetes in high-income countries are of type 2 diabetes mellitus accounting for an even higher dominance in developing countries. It is intimately associated with improper utilization of insulin by target cells and tissues. According to World Health Organization [4], this problem has been aggravated by rapid cultural and social dynamics, ageing populations, increasing urbanization, dietary changes, reduced physical activity and other unhealthy lifestyle and behavioural patterns.

Diabetes mellitus and lesser forms of glucose intolerance, particularly impaired glucose tolerance, can now be found in almost every population in the world and epidemiological evidence suggests that, without effective prevention and control programmes, diabetes will likely continue to increase globally [4]. Major type 1 diabetes is of the immune mediated nature, involving cell mediated autoimmune attack due to beta cell loss [5]. Most affected people are otherwise healthy and of a healthy weight when onset occurs while sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes mellitus can also affect children or adults which was traditionally termed “Juvenile diabetes” because it represents a majority of the diabetes cases in children.

Type 2 diabetes mellitus is the most common type and commonly affects people who are obese and insulin resistant, but these two factors alone are insufficient to cause diabetes unless they are accompanied by impaired beta cell function. This type of diabetes is also associated with genetics and environmental factors such as lifestyle, malnutrition in-utero age and pregnancy [6].

Gestational diabetes mellitus is similar to type 2 diabetes mellitus in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2%-5% of all pregnancies and may improve or disappear after delivery. Gestational diabetes mellitus is fully treatable but requires careful medical supervision throughout the pregnancy. About 20%-50% of affected women develop type 2 diabetes mellitus later in life [7].

Diabetes due to genetic defects of beta-cell function under which are several forms of the diabetic state associated with monogenic defects

in beta-cell function, frequently characterized by onset of mild hyper glycaemia at an early age (generally before age 25 years). They are usually inherited in an autosomal dominant pattern. Patients with these forms of diabetes, formerly referred to as Maturity-onset diabetes of the young (MODY), have impaired insulin secretion with minimal or no defect in insulin action [8].

The metabolic abnormalities associated with mutations of the insulin receptor may range from hyper insulinaemia and modest hyper glycaemia to symptomatic diabetes. Also diabetes due to Endocrinopathies with several hormones (e.g. growth hormone, cortisol, glucagon, epinephrine) antagonize insulin action while diseases associated with excess secretion of these hormones can cause diabetes (e.g. Acromegaly and Cushing's Syndrome) [9]. As a consequence of the metabolic derangements in diabetes, various complications develop including both macro and micro-vascular dysfunctions, while It is well accepted that oxidative stress results from an imbalance between the generation of oxygen derived radicals and the organism's antioxidant potential [10]. Various researches have shown that diabetes mellitus is associated with increased formation of free radicals and decrease in antioxidant potential and due to these various events, the balance normally present in cells between radical formation and protection against them is disturbed. This leads to oxidative damage of cell components such as proteins, lipids and nucleic acids. Increased oxidative stress can induce both type 1 and type 2 diabetes mellitus as well as its complications [11].

Also, Conflicting results have been reported for the role of free radical induced oxidative stress in diabetes mellitus. F2-isoprostanes are prostaglandin like compounds formed *in-vivo* from free radical catalyzed peroxidation of arachidonic acid and have emerged as novel and direct measures of oxidative stress. F2-isoprostane levels have been reported to be increased in the plasma of type 2 diabetes mellitus and in the urine of type 2 and type 1 diabetic subjects [12]. A correlation between impaired glycemic control and enhanced lipid peroxidation has been reported and this has shown that oxidative stress exists in diabetic patients as evidenced by increased total antioxidant capacity in saliva and blood sample of patients [13].

In addition, oxidative stress is increased in diabetes because of multiple factors, dominant among these factors is glucose auto-oxidation leading to the production of free radicals. Other factors include cellular oxidation or reduction imbalances and reduction in antioxidant defenses (including decreased cellular antioxidant levels and a reduction in the activity of enzymes that disposes free radicals). Levels of some pro-oxidants such as ferritin and homocysteine are elevated in diabetes. Another important factor is the interaction of advanced glycation end products (AGEs) with specific cellular receptors called advanced glycation end products receptor (RAGE). Elevated levels of advanced glycation end product are formed under hyperglycemic conditions. Their formation is initiated when glucose interacts with specific amino acids on proteins forming a compound which undergoes further chemical reactions. Glycation of protein alters protein and cellular/immune function, and binding of advanced glycation end products to their receptors can lead to modification in cell signaling pathways and further production of free radicals [14].

The prevalence of diabetes is increasing rapidly worldwide and the World Health Organization [4], has predicted that by 2030 the number of adults with diabetes would have almost doubled worldwide, from 177million in 2000 to 370 million. Experts project that the incidence of diabetes is set to soar by 64% by 2025, meaning that a staggering 53.1 million citizens will be affected by the disease [15]. The estimated worldwide prevalence of diabetes among adults in 2010 was 285 million (6.4%) and this value is predicted to rise to around439 million (7.7%) by 2030 [16].

Treatment of diabetes mellitus with orthodox approach have faced several challenges over the years with varieties of drugs in the market with various anti–diabetic potentials and mode of action: a variety of recombinant human insulin and insulin analogues are available and these products serve as the primary basis for treating the glucose metabolic defects in type 1diabetes. Insulin and its analogues also have an important role in the treatment of type 2 diabetes, particularly as the disease progresses. These products are used in different combinations according to the pharmacokinetic profile of each insulin type, and some are available in premixed combinations of different proportions of short and long–acting agents. These insulin can also be used in conjunction with oral agents to achieve the control of blood glucose. The first oral

products for the treatment of diabetes mellitus were the sulfonylureas which are long–acting insulin secretagogues. The meglitinides constitute another class of insulin secretagogues with short-term effects which act primarily on the postprandial elevation of plasma glucose. Metformin exerts its effects on endogenous hepatic glucose production while Peroxisome Proliferator–Activated Receptors (PPAR) agonists enhance insulin sensitivity. Alpha glucosidase inhibitors prevent intestinal glucose absorption and have primary effects on the excursion of postprandial glucose. The newer classes of therapeutic products recently approved for the treatment of type 1 and type 2 diabetic patients as an adjunct to meal-time short–acting or rapid–acting insulin are analogue of human amylin and pramlintide [17].

Glibenclamide (5-chloro-N-(4[N-(cyclohexyl-carbamoyl) sulfamoyl] phenethyl)-2-methoxy-benzamide, is an oral hypoglycaemic drug (sulphonyl urea-second generation) which acts by inhibiting ATP-sensitive potassium channels in pancreatic beta cells. This inhibition causes cell membrane depolarization, which causes voltage dependent calcium channels to open and causes an increase in intracellular calcium in the beta cell to stimulate insulin release from the pancreas, thus its wide usage in the treatment of type 2 diabetes [17,18].

In recent years, bioactive agents derived from natural sources mainly plants have been intensively used to prevent, manage and treat diseases because of their advantages over synthetic ones; as they are easily obtained, economical and have slight or negligible effects compared with synthetic drugs [19].

Diabetes have been treated with plant medicines since antiquity as diabetes was treated with plant medicines before the advent of insulin, while recent scientific investigations has confirmed the efficacy of many anti diabetic plant preparations, some of which are very effective and relatively non-toxic. In 1980, the World Health Organization urged researchers to examine whether traditional medicines produced any beneficial clinical results, while in the last 10 to 20 years, scientific investigations have confirmed the efficacy of many of these preparations as alternative approach sought by diabetic patients and health care professionals as over 400 traditional plants treatment for diabetes have been reported till date [20]. Although the major pathogenic events of diabetes mellitus includes the increased generation of free radicals, cellular oxidative stress, impaired antioxidant defence system with concomitant imbalance of the oxidant/antioxidant status [21,22]. The inhibition of these cascades of oxidative processes has been reported to prevent the onset and development of diabetic complications as during this cascade of events ROS are produced resulting in tissue damage [23].

Anti-diabetic plants have been documented to scavenge free radicals, quench electronically excited compounds, reduce hydroperoxide formation, attenuate ROS production, exhibit anti-hyperglyceamic activities and attenuation of hyper lipideamia through modulation of several enzymes [24,25].

Thaumatococcus daniellii is a plant species from Africa, natives to the rain forest of Western Africa, known for being the natural source of thaumatin, an intensely sweet protein which is of interests in the development of sweetners. It is a large rhizomatous flowering herb growing three to four meters in height with large papery leaves up to 46 cm long. It bears pale purple flowers and a soft fruits containing a few shiny black seeds. The fruit is covered in a fleshy red aril containing thaumatin [26]. It is one of the underutilized plants in Nigeria, where it grows predominantly in the cocoa-growing areas of the South-West, where it is locally called “Ewe-Eran” or “Adundunmitan”. The seed is relatively high in protein, starch and minerals, especially calcium and magnesium [27]. Traditionally, the leaves of *Thaumatococcus daniellii* are used for wrapping various types of foods such as bean cake, yam flour, rice and pounded yam for both domestic use and commercial enterprise. They are also used for preserving kolanuts and as food supplement to some ruminants including goat [26]. The fibrous nature of the leaves enhance its use in combination with some other materials as roof thatching in hamlet and as resorts. The stalk is used for weaving mat, fish traps, ornamental bag and it is also used as sponge and for pulping roll; while the roots, seeds and leaves have many traditional medicinal uses which may be attributed to its many useful bioactive constituents [28,29]. It is also used in traditional medicines in Ivory Coast and Congo where the fruit is used as a laxative and the seed as an emetic and for pulmonary problems [30]. The leaf sap is used as an antidote against venoms, strings and bites. Leaf and root sap are used as sedative and for treating diabetes mellitus and in sanity [31].

However, despite the considerable progress in the treatment of diabetes by oral hypo glyceamic agents, search for newer drugs continues since the existing synthetic drugs have several limitations. Interestingly, numerous herbal drugs with anti-diabetic activity are yet to be commercially formulated as modern medicines, even though they have been acclaimed for their therapeutic properties in the traditional systems of medicines. In Nigeria several medicinal plants have been used for treatments of diabetes mellitus as a remedy in some poor areas who may not afford orthodox drugs or engaged in combination of both therapies. This study was designed to investigate the effects of methanol leaves extract of *Thaumatococcus danielli* in streptozotocin–induced diabetic rats as well as its combinative effects with glibenclamide (standard drug) by evaluation of some biochemical parameters.

2. MATERIALS AND METHODS

2.1 Materials

Some of the materials and equipment used includes: measuring cylinders, spectrophotometer, micropipettes, centrifuge, water-bath, serum bottles, dissecting set, disposable gloves, thumb pin, thermometer, stopwatch, syringes and needles, pH meter, glucometer, glucometer strip, spatula, hand gloves, conical flasks, test-tubes, test tube racks, spatula, beakers, refrigerator, weighing balance, and homogenizer.

2.2 Reagents

Distilled water, washing buffer, homogenizing buffer, methanol, Tris buffers, potassium chloride, normal saline, Chemical Laboratory diagnostic Kits for the quantitative determination of total protein, lipid profile and some haematological parameters. Sodium hydroxide, hydrogen peroxide, trichloroacetic acid (TCA), thiobarbituric acid (TBA), adrenalin, sodium bicarbonate (Na_2CO_3), Dichromate/acetic acid, Glacial acetic acid, Phosphate, Ellmans reagents. All reagents, kits and chemicals used were of high quality analytical grade obtained from Sigma USA.

2.3 Plant Collection and Extract Preparation

Thaumatococcus danielli leaves was obtained from the University farm and were identified and authenticated at the Botany Unit of the Department of Pure and Applied Biology, Ladoke Akintola University of Technology Ogbomoso with herbarium voucher number LHO479 deposited. The leaves were air dried in the laboratory and powdered. 400 g of the powdered leaves were soaked in 2500 ml of aqueous 70% methanol for 72 hours, kept in a dark cupboard and filtered using a filter paper. The filtrate was dried between 30-40°C in a water bath to obtain a dry residue. The residue was placed in a desiccator to remove methanol remaining in the residue and stored in the refrigerator for subsequent use.

2.4 Experimental Animals and Grouping

Thirty five male albino rats of Wistar strain used for this study were obtained from the Biomedical Laboratory Science animal house of Ladoke Akintola University of Technology Ogbomoso, Oyo State, Nigeria. The rats were acclimatized in the laboratory for two weeks, fed with rat pellets and water *ad-libitum* and their weights monitored. The animals were handled based on Ladoke Akintola University of Technology Ogbomoso, Oyo state Nigeria, ethics and conduct for handling experimental animals which conforms with the international standards and made available in the laboratories. The animals were randomly selected into seven groups (A, B, C, D, E, F, and G) with average weight of 150g and treated as shown:

Group A: Control animals fed rats pellets and water throughout the experiment.

Group B: Streptozotocin–induced diabetic rats (50 mg/kg. body weight of streptozotocin made up to 0.2 ml in citrate buffer administered as a single large dose subcutaneously.

Group C: Streptozotocin-induced diabetic rats treated with extract only at 100 mg/kg. body weight orally, daily.

Group D: Streptozotocin-induced diabetic rats treated with theurapeutic dose of Glibenclamide only (5 mg/kg body weight, administered orally per day).

Group E: (Combinative therapy), Streptozotocin-induced diabetic rats treated with extract (100 mg/kg body weight, orally per day) and theurapeutic dose of Glibenclamide (5 mg/kg body weight, administered orally per day) throughout the experimental period of 15 days.

Group F: Animals treated with extract only (100 mg/kg. body weight, orally per day).

Group G: Animals treated with theurapeutic dose of Glibenclamide only (5 mg/kg body weight, administered orally per day).

2.5 Preparation of Blood Serum and Liver Homogenate

The experimental animals were sacrificed 24 hours after the last day of the administration by cervical dislocation with the blood collected from the heart using syringe and needle. Part of the collected blood was transferred into a small serum bottles and centrifuged at 4000 rpm for 10 minutes to collect the serum while the rest were used for the determination of other haematological parameters. The liver was excised and placed in a pre-weighed beaker containing 5 ml of washing buffer. It was thoroughly washed in cold washing buffer and weighed. 1 g of the liver was homogenized using 4 mls of homogenizing buffer. The serum and the homogenate were stored at 4°C.

2.6 Biochemical Studies

Various samples were prepared from the blood and the liver for different biochemical assays. Serum and liver total proteins were determined according to the Biuret method [32]. The fasting blood glucose level was measured using a glucometer (Accucheck) as fresh blood were carefully dropped on the glucometer strip and read. Total white blood cell (WBC) counts was determined by acetic acid dilution of the whole blood which facilitate haemolysis of mature erythrocytes and enhance leukocyte counting. Lymphocytes counts was estimated by flow cytometry using antibodies as the procedure for CD4 enumeration. Platelets counts were estimated using a hemocytometer with an automated analyser. Counts were estimated during blood smear examination on automated hemograms while the red blood cell counts were obtained by dilution of the blood specimen (usually 200 times) using red cell diluting fluid which allows red cells to be counted under magnification in a known volume of fluid while this is calculated and reported as the number of red cells/µl of whole blood.

Total serum cholesterol concentrations were determined spectrophotometrically according to the methods of [33], based on the principle of enzymatic end point upon enzyme hydrolysis of cholesteryl esters while triglycerides concentration was determined by an enzymatic colorimetric method using Fortress diagnostic triglycerides kit with standard working reagents. The quantitative determination of high density lipoprotein (HDL) cholesterol was based on HDL-cholesterol (HDL-C) precipitating method of [34] and [35].

The liver homogenates were used to assay for the followings; Malondialdehyde (MDA); estimated spectrophotometrically by thiobarbituric acid-reacting substances (TBARS) as described by the procedure of [36]. Determination of reduced glutathione (GSH) concentration was carried out using the method described by [37], while superoxide dismutase (SOD) and catalase (CAT) activities were determined by the methods of [38] and [39] respectively.

2.7 Statistical Analysis

Data obtained were analysed using analysis of Variance (ANOVA), value of P=.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

Diabetes mellitus is a multifactorial, multi systemic endocrine disorder common and very prevalent disease affecting the citizens of both developed and developing countries. It is associated with the production of reactive oxygen species (ROS) and consequently oxidative stress, reduction in natural antioxidant defense systems which promotes not only an alteration in the cellular redox state but also reduces the ability of tissue to utilize carbohydrates, leading to disturbances in the metabolism of fat and protein [40,41]. Oxidative stress may occur as a consequence of abnormalities in glucose and lipid metabolism, which may favor hyperglycemia and dyslipidemia with long-term damage, dysfunction, and failure of different organs [41].

Blood glucose level is of great importance in the diagnosis of diabetes mellitus. From the results obtained in Table 1, there was a significant (P=.05) increase in blood glucose level in Group B (streptozotocin-induced diabetic rats) compared with Group A (control). This is a strong indication of diabetes mellitus (hyperglycemia). However, Group C (Diabetic rats + extract), Group D (Diabetic rats + Glibenclamide) and Group E (Diabetic rats + extract + Glibenclamide) showed significant (P=.05) decreases in the blood glucose levels (hypoglycemia) compared

with Group B (streptozotocin-induced diabetic rats). Moreover, Group F (extract only) and Group G (Glibenclamide only) significantly (*P*=.05) decreased the blood glucose levels (hypoglycemia) compared with Group A (control), showing the possible hypoglycemic and anti–diabetic potential of the plant extract and the standard drug.

Protein studies and its determination as a biochemical test for measuring the total amount of protein in the blood plasma or serum is useful for monitoring gross changes in protein levels caused by various disease states. In Table 2, total protein concentrations in the serum and the liver were significantly (*P*=.05) decreased in group B (streptozotocin-induced diabetic rats) compared with controls and other treated groups. However in group E (combinative therapy involving the streptozotocin–induced diabetic rats treated with the extract and glibenclamide), concomitant significant (*P*=.05) increases in both serum and liver protein concentrations compared with group B were obtained with similar trends observed in groups C and D, suggestive of the possible modulatory effects of the extract as its administration alone enhances significant increases in protein concentration. The decreased serum and liver protein concentrations in the diabetic rats could be a result of cellular dysfunction in chronic liver diseases [42], protein oxidation in tissue caused by imbalance between free oxygen radicals and antioxidants due to oxidative stress [43], as well as induction of diabetes resulting from beta cell destruction through the action of streptozotocin [44].

Haematological parameters studied revealed that the white blood cells counts (WBC), lymphocytes and platelets counts were significantly (*P*=.05) increased with corresponding significant (*P*=.05) decreases in red blood cell counts in group B (Streptozotocin–induced diabetic rats) compared with the controls and other treated groups (Table 3). The haematological abnormalities observed in streptozotocin-induced diabetic rats compared with other groups could be due to the effects of streptozotocin on rapidly dividing haemopoietic cells and suppression of haemopoiesis as a result of insulin deficiency occasioned by the selective destruction of the cells in the Islets of Langerhans of the pancreas by streptozotocin [45]. However in group E (combinative therapy), the white blood cell counts, lymphocytes and platelets counts were significantly (*P*=.05) decreased nearly to controls coupled with significant (*P*=.05) increases in red blood cells with similar effects shown by group C,D,F and G. Properties exhibited are indication that the plant extract and the standard drug possess anti-inflammatory and anti-anaemic properties which are necessary in diabetes treatments and management with the performance of the extract highly competitive with the standard drug i.e (group C compared with D), and a possible modulatory effects on the drug in the combined therapy. The elevated levels of WBC, lymphocytes and platelets may be due to increased ROS generated by streptozotocin in the serum [46]. Lymphocyte is a type of white blood cell constituting the immune system while the platelets contributes to cellular homeostasis' at the site of interrupted endothelium. The increased lymphocytes, also may accounts for excess ROS and inflammation which impairs insulin signaling and promotes beta cell death [47], while the extract ability to reverse these trends are suggestive of its antioxidant properties which are able to neutralize the ROS effects.

The results of the lipid profile for the various treatment groups (Table 4), shows that total serum cholesterol and triglycerides were significantly (*P*=.05) increased with corresponding decreases in serum high density lipoprotein (HDL) cholesterol in streptozotocin–induced diabetic rats (group B) compared with other treated groups. These results were in accordance with previous studies as marked increases in total cholesterol ,triglycerides and decreased HDL-cholesterol have been reported in streptozotocin–induced diabetic rats, an indication of hyperlipidemia [48,49]. However, in group E(combinative therapy), this was strongly reversed with significant (*P*=.05) decreases in total cholesterol and triglycerides coupled with significant (*P*=.05) increases in HDL-cholesterol compared with group B. Similar results were obtained in group C and D, suggestive of the hypolipideamic property of the extract. The abnormal high concentrations of serum lipids in diabetic animals are due mainly to an increase in the mobilization of free fatty acids from the peripheral fat depot, since insulin inhibits the hormone sensitive lipase [50,51]. Excess fatty acids in the serum of diabetic rats is converted into phospholipids and cholesterol in the liver. The increased level of HDL-cholesterol as shown by the extract protects against cardiovascular events associated with dyslipidemia in diabetes mellitus.

Table 1. Fasting blood glucose concentrations (Mg/dl) of various treatment groups

Groups	Fasting blood glucose concentration (Mg/dl)± SD
A. (Control)	80.200 ± 13.880
B. Streptozotocin only (diabetic rats)	460.000 ± 90.600*
C. Diabetic rats + Extract only	145.000 ± 40.700**
D. Diabetic rats + Glibenclamide	185.800 ± 83.600**
E. Diabetic rats + Extract + Glibenclamide (combinative therapy)	107.400 ± 26.900**
F. Extract only	70.030 ± 10.130**
G. Glibenclamide only (standard drug)	63.050 ± 16.380**

*Values are given as mean and standard deviation of six determinations, * Values differ significantly from control (P =.05), ** Value differ significantly from streptozotocin-induced diabetic rats Group B (P=.05)*

Table 2. Total protein concentrations in the serum and the liver of various treatment groups

Groups	Total protein concentration (Serum) Mg/dl ± SD	Total protein concentration (liver) Mg/dl ± SD
A. (Control)	11.564 ± 1.432	3.010 ± 0.110
B. Streptozotocin only (diabetic rats)	7.746 ± 0.721*	2.650 ± 0.050*
C. Diabetic rats + Extract only	9.500 ± 0.970	3.700 ± 0.410
D. Diabetic rats + Glibenclamide	11.000 ± 0.551	3.900 ± 1.310
E. Diabetic rats + Extract + Glibenclamide (combinative therapy)	9.670 ± 1.706	3.460 ± 0.770
F. Extract only	11.872 ± 0.5538**	4.130 ± 1.090**
G. Glibenclamide only (standard drug)	12.210 ± 0.570**	3.770 ± 0.820

*Values are given as mean and standard deviation of six determinations, * Values differ significantly from control (P =.05), ** Value differ significantly from streptozotocin-induced diabetic rats Group B (P=.05)*

Table 3. Values of some haematological indices (White blood cell (WBC), Lymphocytes, Platelets and Red blood cell counts) of various treatment groups

Groups	WBC count (x 10^9/L) ± SD	Lympocytes count (x10^9/L) ± SD	Platelets count (x10^9/L) ± SD	Red blood cell count (x10^{12}/L) ± SD
A. (Control)	3.060± 0.763	2.180 ± 0.192	348.800±32.400	9.146± 0.431
B. Streptozotocin only (Diabetic rats)	9.100± 0.396*	3.400 ± 0.358*	614.200±30.200*	3.980± 0.653*
C. Diabetic rats + Extract only	5.380± 0.550**	2.240 ± 0.581	432.000±16.560**	7.956± 1.023**
D. Diabetic rats + Glibenclamide	4.180± 0.179**	2.140 ± 0.835	398.800±73.700**	7.074± 1.606**
E. Diabetic rats + Extract + Glibenclamide (combinative therapy)	5.627± 1.110**	1.680 ± 0.217**	303.200±84.900**	7.528± 1.649**
F. Extract only	5.440± 0.841**	1.920 ± 0.497	268.200±39.700**	8.556± 0.428**
G. Glibenclamide only (standard drug)	5.400 ±1.064**	1.960 ± 0.321	220.000±15.310**	8.494± 0.399**

*Values are given as mean and standard deviation of six determinations, * Values differ significantly from control (P =.05), ** Value differ significantly from streptozotocin-induced diabetic rats group B (P=.05)*

Studies of some antioxidant indices in the liver of various treatment groups shows significant (*P*=.05) decreases in GSH concentrations, SOD and Catalase activities as well as significant (*P*=.05) increases in MDA concentrations in group B (streptozotocin-induced diabetic rats), compared with other treatment groups (Tables 5 and 6). Interestingly, administration of the extracts (group C), standard drug (group D) and the combined treatment (group E) all elicits significant increases in GSH concentration, SOD and Catalase activities with significant decreases in MDA concentrations in the order C>D=E, suggestive of the extract ability to boost the antioxidant status of the animal. The increased MDA level in group B, indicates the free radical

Table 4. Results of the serum lipid profile of various treatment groups

Groups	Total serum cholesterol concentration (mg/dl) ± SD	Serum triglycerides concentration (mg/dl) ± SD	Serum HDL cholesterol concentration (mg/dl) ± SD
A. (Control)	81.667 ± 11.750	230.910 ±12.930	27.760 ± 3.810
B. Streptozotocin only (diabetic rats)	120.250± 10.750*	386.667 ± 10.78*	19.000 ± 1.250*
C. Diabetic rats + Extract only	74.000 ± 12.750**	307.000 ± 27.000**	35.900 ± 2.520**
D. Diabetic rats + Glibenclamide	66.000 ± 11.250**	279.760 ± 47.5000**	32.430 ± 5.390**
E. Diabetic rats + Extract+Glibenclamide (combinative therapy)	73.970 ± 6.500**	327.120 ± 12.400**	35.890 ± 6.152**
F. Extract only	72.660 ± 5.750**	265.020 ± 74.100**	30.900 ± 5.320**
G. Glibenclamide only (standard drug)	67.333 ± 6.300**	247.980 ± 10.020**	31.680 ±4.979*

*Values are given as mean and standard deviation of six determinations, * Values differ significantly from control (P =.05), ** Value differ significantly from streptozotocin-induced diabetic rats group B (P=.05)*

Table 5. Liver Malondialdehyde (MDA) and Reduced glutathione (GSH) concentrations of various treatment groups

Groups	Malondialdehyde (MDA) concentration (Mg/dl) ± SD	Reduced glutathione (GSH) concentration (Mg/dl protein) ± SD
A. (Control)	1534.500 ± 74.100	45.300 ± 5.460
B. Streptozotocin only (diabetic rats)	2118.333 ± 30.700*	39.000 ± 1.597*
C. Diabetic rats + Extract only	1228.600 ± 64.700**	54.333 ± 3.500**
D. Diabetic rats + Glibenclamide	1250.750 ± 81.800**	47.000 ± 9.870**
E. Diabetic rats + Extract+Glibenclamide (combinative therapy)	1231.760 ± 61.000**	46.400 ± 1.249**
F. Extract only	1345.520 ± 35.000**	49.333 ± 1.210**
G. Glibenclamide only (standard drug)	1267.930 ± 36.500**	46.300 ± 1.396**

*Values are given as mean and standard deviation of six determinations, * Values differ significantly from control (P =.05), ** Value differ significantly from streptozotocin-induced diabetic rats group B (P=.05)*

Table 6. Superoxide dismutase (SOD) and Catalase (CAT) activities in the liver of various treatment groups

Groups	SOD activity (U/mg protein) ± SD	CAT activity (U/mg protein) ± SD
A. (Control)	4.874 ± 1.115	0.661 ± 0.015
B. Streptozotocin only (diabetic rats)	1.310 ± 0.326*	0.328 ± 0.010*
C. Diabetic rats + Extract only	3.037 ± 0.977	0.566 ± 0.062
D. Diabetic rats + Glibenclamide	3.791 ± 1.102	0.667 ± 0.023**
E. Diabetic rats + Extract+Glibenclamide (combinative therapy)	3.663 ± 0.813	0.653 ± 0.050**
F. Extract only	5.071 ± 1.213	0.612 ± 0.040**
G. Glibenclamide only (standard drug)	5.004 ± 1.101	0.600 ± 0.040**

*Values are given as mean and standard deviation of six determinations, * Values differ significantly from control (P =.05), ** Value differ significantly from streptozotocin-induced diabetic rats group B (P=.05)*

generating power of streptozotocin to induce lipid peroxidation, decreases antioxidant defense and an indication of the susceptibility of pancreas to streptozotocin induction of oxidative stress [52], while its consequent amelioration nearly to control level by the extract and the standard drug are indicative of their antioxidant properties [53].

4. CONCLUSION

The results from the evaluation of the various biochemical parameters studied are indicative of a possible hypoglycaemia, hypolipidaemia, anti-inflammatory, antioxidative and hepatoprotective potentials of the extract and in combined treatment with the standard drug, an indication that the extract contain certain bioactive agent with anti-oxidant properties which may act by probably inhibiting or mopping ROS formation in diabetes, a property which may be exploited in drug discovery, while the extract behavior in the combined treatment are indicative of its modulatory effects on the standard drug or possibly working synergistically with the drug. Hence usage of the plant in folk medicine as anti–diabetic agent could be encouraged in poor regions of Africa; either singly or in combination with orthodox anti-diabetic drugs when available. While further work is required on the safety of such combination with orthodox drugs as currently practiced by some natives.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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