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Ethanol leaves extract of *Mangifera indica* (L.) exhibits protective, antioxidative, and antidiabetic effects in rats

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ABSTRACT

Objective: Many orthodox drugs used for treatment of diabetes have shown reported deleterious side effects. This study investigates the effects of ethanol leaves extract ofMangiferaindica(L) on alloxan-induced diabetic rats as an attempt to investigate herbal remedy. **Methods:** Male Wistar rats treated for fifteen days with average weight of 150g were randomlyselected into seven groups; 1(controls, received normal saline),2 (Alloxan-induced diabetic rats, 400mg/kg.bw subcutaneously),3 (Diabetic rats treated with extract,100mg/kg.bw),4 (Diabetic rats treated with glibenclamide,5mg/kg.bw),5 (Diabetic rats treated with combination of extract and glibenclamide),6 (extract only),7 (glibenclamide only).Biochemical and anti-oxidant indices were determined with standardized methods. **Results:** Results from indices studied revealed that alloxan-induced diabetic rats showed significant($p<0.05$) increases in fasting blood sugar concentrations, white blood cell counts, lymphocytes counts, platelets counts, serum total cholesterol, triglycerides, liver malondialdehyde levels and percentage DNA fragmentation with corresponding decreases in serum total protein concentrations,red blood cell counts,high density lipoprotein concentrations and liver superoxide dismutase and catalase activities. Howeverin diabetic rats treated with extract(Group 3),levels and activities of these markers werereversed significantly and comparativelywith the standard drug (Group 4). **Conclusions:** These findings indicatepossible anti-oxidative,anti-diabetic, hypoglycemic,hypolipidemic and hepatoprotective effects of the extract and or a synergistic interaction with the standard drug .

Keywords: Alloxan, Anti-diabetic, Anti-oxidative,Hepatoprotective,MangiferaIndica (L)

INTRODUCTION

Diabetes mellitus is a metabolic disorder of multiple causes known for chronic hyperglycemia and disturbances of carbohydrate, protein, and fat metabolism as a result of defects in insulin secretion, insulin action, or both.[1] Common symptoms associated with high blood sugar include frequent urination, increased thirst, and increased hunger. However, if left untreated, some long-term complications such as cardiovascular disease, stroke, chronic kidney failure, foot ulcer, and damage to the eyes are possible.[2] A functioning cell in the human body requires a regular source of energy; however, the primary and easily available energy source is glucose which circulates in the blood as fuel source for cells.[3] The role of insulin in regular cell supply is the regulation of the blood glucose level as the hormone binds to its receptors sites on peripheral side of the cell membranes where it enhances the entry of glucose into respiring cells and tissues through requisite channels for energy supply. Hence, a deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus with evidence of genetic involvement.[4]

Basically, glucose is obtained by the body through three main sources: The intestinal absorption of food, the breakdown of glycogen, and the storage form of glucose found in the liver with generation of glucose from non-carbohydrate sources. Since insulin is the principal hormone intimately connected with glucose metabolism vis-à-vis diabetes, the occurrence and type of diabetes mellitus will therefore depend on the critical roles of insulin.[5] In Type 1 diabetes mellitus, insulin is defective or insufficient resulting from the pancreas failure to produce enough insulin usually due to immune destruction of the beta cells of the islet of Langerhans of the pancreas referred to as insulin-dependent diabetes mellitus (IDDM), while in Type 2 diabetes mellitus, there is insulin insensitivity or resistance as cells fail to respond to insulin, and as this continued, a lack of insulin may also developed which was previously known as non-IDDM.[4] However, the net effects of these conditions are the persistence high levels of blood glucose, poor protein synthesis, and other metabolic derangements.[6] Other form of diabetes mellitus is gestational diabetes mellitus associated with pregnancies, which may improve or disappear after pregnancies while various types of known etiologies are grouped together forming the classification known as “other specific types.” Diabetes mellitus is associated with an increased risk of cardiovascular diseases mediated through oxidative stress as reactive oxygen species (ROS) generated can directly damage membrane constituents such as lipids, proteins, or DNA molecules which may result in the modulation of intracellular signaling pathways and contribute to development and progression of diabetic complications through oxidative stress.[7] The prevalence of diabetes mellitus among adults has been predicted to be on the high increase and with about 7.7% increase by 2030, especially in the urban areas.[8,9]

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Medications for the treatment of diabetes mellitus generally lower blood sugar levels and many classes of antidiabetic drugs have been identified which are taken orally or by injection. Insulin remains the principal therapy for Type 1 diabetes or typically with a combination of regular and NPH insulin or synthetic insulin analogs while metformin is generally recommended as a first-line treatment for Type 2 diabetes mellitus.[10] Although metformin is generally considered the first choice oral medication, unless contraindicated, this recommendation is based on metformin favorable effects on weight, low risk of hypoglycemia, and low cost; however, gastrointestinal intolerance is common as the need to monitor renal function may be problematic in many health systems as well as its effects on cardiovascular events and mortality.[11] It works by decreasing the livers production of glucose.[12] Alternatives to metformin include sulfonylureas or α-glucosidase inhibitors while several other groups of drugs mostly given by mouth may also decrease blood sugar in Type 2 diabetes mellitus and these agents may act by increasing insulin release, decrease absorption of sugar from the intestines, or make the body more sensitive to insulin.[10,12]

Over 300 plants species have been documented to have antidiabetic effects due to their hypoglycemic ability as most of these herbal preparations have been used to treat diabetes before insulin was discovered in various forms of traditional healing systems in different parts of the world.[13-15]

Mangifera indica is a plant native in most tropical biotopes in Asia, Africa, America, and Australia. It belongs to the genus *Mangifera*, order *Sapindales,* and family *Anacardiaceae*. *M. indica* is one of the most widespread fruit trees in Western Africa where four varieties have been identified.[16,17] It is an economically important tropical tree fruit found throughout the world and widely distributed in different parts of Africa, especially in the southern part of Nigeria where it is valued for its edible fruits with various nutritional and phytochemical compositions.[18-20] Various studies and reviews have shown that *M. indica* and its various parts have great medicinal values. The bark is employed against rheumatism and diphtheria in India, and the resinous gum from the trunk is applied on cracks in the skin of the feet and on scabies as extracts from unripe fruits, barks, stems, and leaves have shown antibiotic activities,[21] while the leaves decoction have been reported as remedy for diarrhea, fever, chest complaint, diabetes, and hypertension. The infusion of the tender leaves in water has been shown to control early diabetes.[22]

Interestingly, alternative approach using medicinal herbs has increased over the years as these preparations have been found to be very effective, cheap, and relatively non-toxic compared with standard antidiabetic drugs while most of these claims have continuously been investigated by researchers worldwide and a justification for this study. Hence, this study examined the effects of ethanol leaves extract of *M. indica* (L.) on some biochemical and antioxidant indices on alloxan-induced diabetic rats.

MATERIALS AND METHODS

Materials

The materials and equipment used includes: Measuring cylinder, electric weighing balance, beakers, test tube, test tube racks, conical flask, round bottom flask, water bath, pH meter, centrifuge, disposable glove, thermometer, tissue papers, micro pipette, washing brush, syringe (2 ml, 5 ml), retort stand, separating funnel, visible light spectrophotometer, refrigerator, dissecting sets, paper tape, cuvette, reagent bottles, sample bottles, razor blades, cotton wool, nose mask, mortar and pestle, and spatula.

Reagents and Salts

Distilled water, washing buffer, homogenizing buffer, ethanol, Tris buffer, potassium chloride, normal saline, distilled water, trichloroacetic acid, thiobarbituric acid, carbonate buffer, adrenaline, sodium hydroxide, hydrogen peroxide, phosphate buffer, Ellman's reagent, sulfosalicyclic acid, and laboratory kits were used. Chemicals and reagents are products of Sigma, USA with high quality grade.

Plant Material and Preparation of Ethanol Extract

Fresh leaves of the plant (*M. indica* L.) were obtained at the premises of the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The leaves were identified and confirmed by an angiosperm taxonomist of the botany unit of the same Department as the Ogbomoso specie which are very common in the Southwestern part of Nigeria with herbarium voucher number LHO 346 deposited.

The leaves were air-dried at room temperature for 3 weeks and powdered for solvent extraction. The powdered leaves were collected into a container and stored at room temperature while 600 g of the dried powdered leaves was transferred into a suitable container and soaked in 2500 ml of ethanol in a dark cupboard, well secured from light for 72 h. The colored ethanol solution containing the dried powdered leaves was filtered, and the filtrate obtained was concentrated between 30 and 40°C in water bath to obtain a dry residue of the ethanol extract of the leaf. The residue obtained was stored in a desiccator to remove any traces of ethanol remaining in the residue. The extract was now stored in the laboratory refrigerator for subsequent use.

Experimental Animals and Design

Male albino rats of Wistar strain used for this work were acclimatized in the laboratory for 2 weeks and handled based on international standards of handling laboratory animals as made available by my institution. With the average weight of 150 g, they were selected randomly into seven groups (1, 2, 3, 4, 5, 6, and 7) with 6 rats in each group. Standard doses administered were 100 mg/kg body weight of the extract, therapeutic dose (5 mg/kg body weight) of glibenclamide (standard drug), and 400mg/kg body weight of alloxan (a single large dose only administered for 1 day), all made into 0.2 ml solutions. Various group treatments are shown below.

- Group 1: Control animals fed rats pellets, water, and 0.2 ml normal saline intravenously throughout the experimental period.
- Group 2: Alloxan-induced diabetic rats (400 mg/kg body weight of alloxan made up to 0.2 ml in normal saline administered as a single large dose subcutaneously).
- Group 3: Alloxan-induced diabetic rats administered with extract only at 100 mg/kg body weight by intubation, throughout the experimental period.

- Group 4: Alloxan-induced diabetic rats treated with therapeutic dose of standard drug (glibenclamide, 5 mg/kg body weight, administered orally per day).
- Group 5: Combinative therapy, Alloxan-induced diabetic rats treated with extract (100 mg/kg body weight, orally per day) and therapeutic dose of glibenclamide (5 mg/kg body weight, administered orally per day) throughout the experiment.
- Group 6: Animals treated with extract only (100 mg/kg body weight, orally per day).
- Group 7: Animals treated with therapeutic dose of glibenclamide only (5 mg/kg body weight, administered orally per day).

Preparation of Tissue Homogenates and Blood Serum

The liver was excised from each rat and thoroughly washed in a 250 ml beaker half filled with the washing buffer to remove blood stains. The washed liver was weighed and value recorded while 1 g of the total weight was homogenized in a 4 ml homogenizing buffer. The obtained liver homogenate was put in a clean container and stored at 4°C. Blood was obtained through cardiac puncture using syringe and needle and transferred into a centrifuge in a serum bottle which was centrifuged at 4000 rpm for 10 min to obtain the serum. These processes were repeated for each rat in all the groups, while samples collected were stored at 4°C.

Biochemical and Antioxidant Indices Studied

Various biochemical and antioxidant indices were evaluated from the serum and the liver homogenate using various international standard methods. The blood fasting glucose levels carried out early in the morning of the various treatment groups were measured using a glucometer (Accucheck) as the rat tails were cleaned with methylated spirit and pricked with a lancet blade. Fresh blood from the tail was then carefully dropped on the glucometer strip to read the fasting blood sugar. Total serum and liver proteins were measured by the biuret method.[23]

Hematological indices such as the total white blood cell (WBC) counts, lymphocytes counts, red blood cell (RBC) counts, and platelets counts were determined by flow cytometry whose basic principle is the passage of cells in single files in front of a laser so they can be detected, counted, and sorted as blood cell components are fluorescently labeled and then excited by the laser to emit light at varying wavelengths. The fluorescence can be measured to determine the amount and type of cells present in the samples using automated machine.

Serum triglycerides and cholesterol were determined by enzymatic colorimetric test following sequential reaction steps involving triglycerides hydrolysis in the presence of lipase using standard triglycerides kits and enzymatic hydrolysis of cholesteryl esters by the method of,[24] while quantitative determination of high-density lipoprotein (HDL) concentration was done using HDL-cholesterol (HDLc) precipitating method of Naito and Grove.[25,26] Antioxidant indices such as malondialdehyde (MDA) and reduced glutathione (GSH) and concentrations as well as superoxide dismutase (SOD) and catalase (CAT) activities were determined spectrophotometrically using the liver homogenates by the standard methods of.[27-30] Liver assessment of percentage DNA fragmentation was determined as described by Wu *et al*.[31] using diphenylamine.

Statistical Analysis

Data obtained were presented as mean standard deviation of six determinations and analyzed using analysis of variance (ANOVA), while value of $P < 0.05$ was considered statistically significant.

RESULTS

Results are shown in Table 1-8.

DISCUSSION

Medicinal plants have recently been widely accepted and has been proved to be effective in various disease conditions while many of them still need laboratory-based experiment for their therapeutic validations as many orthodox drugs employed in treatment of many diseases such as diabetes have shown deleterious effects and questions are constantly been raised on their long-term uses. In this study, various biochemical indices were assessed to evaluate the possible effects of ethanol leaves extracts of *M. indica* on alloxan-induced diabetic rats using a standard drug (glibenclamide).

High blood sugar levels over a prolonged period are associated with diabetes mellitus, and in this study, a significant ($P<0.05$) increase in the fasting blood glucose concentrations was obtained in the alloxan-induced diabetic rats (Group 2) compared with the controls and other treatment groups [Table 1], suggestive of hyperglycemia and diabetes mellitus. However, diabetic rats treated with the ethanol leaves extract of *M. indica* (Group 3), glibenclamide (Group 4), and the combined treatment using the extract and the standard drug (Group 5), respectively, showed significant ($P < 0.05$) decreases in the fasting blood glucose levels compared with the untreated diabetic rats (Group 2) an indication of the possible hypoglycemic and antidiabetic property of the plant extract as it competes favorably with the standard drug. The separate performance of the extract in lowering blood sugar (Group 6) compared with Groups 4, 5, and 7 also shows its possible modulatory effects on the standard drug.

Evaluation of tissue and serum proteins is valuable in most disease conditions, and in Table 2, significant ($P < 0.05$) decreases in the total protein concentrations of both the liver homogenates and the serum of the alloxan-induced diabetic rats (Group 2) compared with controls and other groups were observed. This may be an indication of protein oxidation which may occur due to the covalent modifications of protein induced either by the direct reactions with ROS or indirect reactions with secondary by-products of oxidative stress associated with diabetes mellitus.[32] However, other treatment groups in both the liver and serum show increases in protein concentrations which were not significant compared with the controls (Group 1).

Investigation of infections and diseases following treatments requires the monitoring and evaluation of some hematological indices. The WBCs, also called leukocytes or leucocytes, are the cells of the immune system produced and derived from a multipotent cell in the bone marrow known as hematopoietic cell and found throughout the body, including the blood and lymphatic system.[33] From the results obtained in Table 3, significant ($P < 0.05$) increases in the WBC counts of alloxan-induced diabetic rats (Group 2) were observed compared with

controls and other treatment group. The increased WBC count may indicate inflammation associated with oxidative stress leading to impairment of insulin signaling and promote beta cell death in diabetes mellitus.[34] However, in the various treatment Groups 3-7, significant (P < 0.05) decreases in WBC counts were observed compared with Group 2 and nearly to controls, suggestive of the possible protective effect of the extract and the standard drug probably by their antioxidative potential. Furthermore, the RBCs, also called erythrocytes, are the most common type of blood cell and the vertebrate principal means of delivering oxygen (0_2) to the body tissues through blood flow through the circulatory system. The results obtained in Table 3 showed that the diabetic rats (Group 2) elicit significant (P < 0.05) decreases in RBC counts compared with the controls, an observation which correlates with existing literature suggestive of anemia as a common pathophysiology associated with diabetes mellitus which have been reported due to an increased non-enzymatic glycosylation of red blood cell membrane proteins which correlates with hyperglycemia.[35,36] The oxidation of these proteins and hyperglycemia in diabetes mellitus causes increased lipid peroxides production that leads to RBC hemolysis.[37] Further analysis of the RBC count results showed that diabetic rats treated with the extract (Group 3), treated with glibenclamide (Group 4), and the combinative therapy (Group 5) ameliorate these effects as they showed increases in the RBC counts.

Lymphocyte is one of the subtypes of WBC in a vertebrate's immune system. They include natural killer cells (NK cells) (which function in cell-mediated and cytotoxic innate immunity), T cells (for cell-mediated and cytotoxic adaptive immunity), and B cells (for humoral and antibody-driven adaptive immunity). They are the main types of cell found in lymph and hence the name lymphocytes.[38] In the result obtained from Table 4, significant (P < 0.05) increases in the lymphocyte counts of the alloxan-induced diabetic rats (Group 2) compared with other groups were observed. The increased lymphocytes correlate inflammation associated with diabetes mellitus.[34] However, diabetic rats treated with the extracts (Group 3), glibenclamide (Group 4), and combinative therapy (Group 5), all showed decreases in the lymphocytes counts, respectively. The results of the platelets counts in the same table also followed similar trends which imply that the plant extract poses certain antioxidative and anti-inflammatory properties which may be associated with its bioactive constituents.

Selected lipids studied in this work such as cholesterol, triglycerides, and HDLc are screening tool for studying abnormalities in lipids

Table 1: Fasting blood glucose concentration (mg/dl) of various treatment groups

Groups	Fasting blood glucose concentration (Mg/dl)± SD
1. (Control)	73.400±9.840
2. Alloxan-induced diabetic rats	466.400±94.300*
3. Diabetic rats+extract only	137.600±44.400**
4. Diabetic rats+glibenclamide	183.600±84.000**
5. Diabetic rats+extract+glibenclamide (combinative therapy)	99.600±24.500**
6. Extract only	36.800±6.100**
7. Standard drug (glibenclamide only)	48.000±12.040**

Values are given as mean and standard deviation of six determinations. *Values differ significantly from control (P<0.05). **Value differ significantly from alloxan-induced diabetic rats (Group 2), P<0.05. SD: Standard deviation

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
1. (Control)	8.890±0.540	4.330±1.050
2. Alloxan-induced diabetic rats	4.330±0.640*	1.330±0.110*
3. Diabetic rats+extract only	7.590±1.380	2.390±0.450
4. Diabetic rats+glibenclamide	8.690±1.700	2.450±0.880
5. Diabetic rats+extract+glibenclamide (combinative therapy)	10.820±1.430	3.470±1.760
6. Extract only	10.210±0.363**	3.930±1.910**
7. Standard drug (glibenclamide only)	10.840±1.130**	3.370±0.910

Values are given as mean and standard deviation of six determinations. *Values differ significantly from control (P<0.05). **Value differ significantly from alloxan-induced diabetic rats (Group 2), P<0.05. SD: Standard deviation

Table 3: WBC and RBC counts of various treatment groups

Groups	WBC count ($\times 10^9$/L)±SD	RBC count($\times 10^{12}$/L)±SD
1. (Control)	6.920±1.620	8.410±0.690
2. Alloxan-induced diabetic rats	16.667±2.000*	7.205±0.430*
3. Diabetic rats+extract only	8.620±0.025	8.110±0.340
4. Diabetic rats+glibenclamide	7.280±1.170	8.180±0.360
5. Diabetic rats+extract+glibenclamide (combinative therapy)	4.667±0.010	8.600±0.690
6. Extract only	4.320±0.850**	8.730±0.650**
7. Standard drug (glibenclamide only)	5.320±0.280**	9.290±0.140

Values are given as mean and standard deviation of six determinations. *Values differ significantly from control (P<0.05). **Value differ significantly from alloxan-induced diabetic rats (Group 2), P<0.05. SD: Standard deviation, WBC: White blood cell, RBC: Red blood cell

metabolism, especially cardiovascular risk in disease state, and have become important tool in many disease diagnosis.[39] Triglyceride is the body storage form of fat while results from Table 5 showed that triglycerides were significantly ($P < 0.05$) increased in the alloxan-induced diabetic rats (Group 2) compared with the controls (Group 1), suggestive of hypertriglyceridemia associated with diabetes, while significant decreases in tryglyceride levels were obtained in Groups 3 and 4 compared with Group 2. Interestingly, the combined treatment (Group 5) shows tremendous effects as it significantly decreased the triglycerides level compared with Groups 2-4 which may imply that both the standard drug and the extract at a particular dose may be useful in the treatment of diabetes and may work synergistically. The behavior of the extract singly (Group 6) also suggests that the plant extract produces hypoglycemic effect probably by acting as an analog of insulin and mimics some of the actions of insulin on glucose metabolism, such as enhancing uptake of glucose into the cells, inhibition of glucose absorption in the intestine as well as acting as antimetabolites that are capable of blocking the pathway of fatty acid oxidation.[39] Furthermore, cholesterol concentrations were significantly ($P < 0.05$) increased in Group 2, animals compared with the control (Group 1) while sequential decreases in cholesterol concentrations were obtained in other groups with Groups 6 and 7 eliciting the greater effect compared with Group 1 animals an indication of hypocholesterolemic effect.

HDL is one of the five major groups of lipoproteins which are complex particles composed of multiple proteins which transport all fat molecules (lipids) around the body within the water outside cells. The HDL particles remove fat molecules from cells which want to export fat molecules some of which are cholesterol, phospholipids, and triglycerides while the increasing concentrations of HDL particles have been strongly linked with decreasing accumulation of atherosclerosis within the walls of the arteries.[39,40] Results observed from Table 5 showed that HDLc was significantly ($P < 0.05$) decreased in the alloxan-induced diabetic rats (Group 2) compared with control and other treatment groups. However, other treatment groups elicit increases in HDLc sequentially compared with Group 2, in the order Group 7>6>5>4>3, respectively. Behavior shown by the extract and the standard drug are suggestive of their hypoglycemic effects and an indicative of the bioactive constituents of the plant extract which may be explore in drug discovery in the treatment of diabetes mellitus.

Lipid peroxidation and oxidative damage to cellular components associated with oxidative stress are synonymous with diabetes mellitus which generally may affect the body's antioxidant defense system. Consideration of MDA and other antioxidant defence system in this study [Tables 6 and 7] showed significant ($P < 0.05$) increases in MDA level of the alloxan-induced diabetic rats (Group 2) and concomitant decreases in the antioxidant defenses (reduced GSH level, CAT, and SOD activities) compared with controls. In diabetes, implications of hyperglycemic lead to oxidative stress and a decrease in the antioxidant enzymes activities and non-enzymatic antioxidant levels (CAT, SOD, and GSH) with increased MDA levels.[41] In contrast, this study showed significant ($P < 0.05$) decreases in MDA level followed by respective increases in the antioxidant defenses (GSH, CAT, and SOD) in the diabetic rats treated with the extract (Group 3) and the standard drug (Group 4) compared with Group 2 animals.

Table 4: Lymphocytes and platelets counts in the serum of various treatment groups

Groups	Lymphocytes count (109/L)±SD	Platelets count (109/L)±SD
1. (Control)	3.100±0.440	406.000±102.000
2. Alloxan-induced diabetic rats	4.800±0.990*	674.600±9.020*
3. Diabetic rats+extract only	3.100±0.310	552.800±39.600
4. Diabetic rats+glibenclamide	3.000±0.380	388.000±104.000
5. Diabetic rats+extract+glibenclamide (combinative therapy)	2.800±0.610	494.400±64.600
6. Extract only	2.900±0.150**	319.800±87.600**
7.Standard drug (glibenclamide only)	3.180±0.220**	316.800±85.400

Values are given as mean and standard deviation of six determinations. *Values differ significantly from control ($P<0.05$). **Value differ significantly from alloxan-induced diabetic rats (Group 2), $P<0.05$. SD: Standard deviation

Table 5: Results of some selected lipids in the serum of various groups

Groups	Total serum cholesterol concentration (mg/dl)±SD	Serum triglycerides concentration (mg/dl)±SD	Serum HDL cholesterol concentration (mg/dl)±SD
1. (Control)	8.667±1.000	249.000±57.167	43.750±7.500
2. Alloxan-induced diabetic rats	14.196±0.670*	509.500±16.530*	29.000±1.300*
3. Diabetic rats+extract only	12.440±0.760**	410.900±44.700**	41.110±5.140**
4. Diabetic rats+glibenclamide	9.60±2.370**	408.500±38.400**	48.610±1.470**
5. Diabetic rats+extract+glibenclamide (combinative therapy)	8.400±1.770**	276.000±8.000**	58.500±2.250**
6. Extract only	6.970±2.500**	279.000±6.750**	61.870±6.360**
7. Standard drug (glibenclamide only)	5.370±0.830**	334.100±52.000**	72.500±4.500*

Values are given as mean and standard deviation of six determinations. *Values differ significantly from control ($P<0.05$). **Value differ significantly from alloxan-induced diabetic rats (Group 2), $P<0.05$. SD: Standard deviation, HDL: High-density lipoprotein

Table 6: MDA and reduced GSH concentrations of various groups

Groups	MDA concentration (Mg/dl)±SD	Reduced GSH concentration (Mg/dl protein)±SD
1. (Control)	1.233±0.170	107.900±0.960
2. Alloxan-induced diabetic rats	5.000±0.500*	97.667±2.500*
3. Diabetic rats+extract only	2.920±0.340**	104.400±6.270**
4. Diabetic rats+glibenclamide	2.100±0.160**	106.900±3.100**
5. Diabetic rats+extract+glibenclamide (combinative therapy)	1.500±0.210**	109.400±9.070**
6. Extract only	1.960±0.210**	110.300±2.021**
7. Standard drug (glibenclamide only)	1.460±0.290**	118.900±2.010**

Values are given as mean and standard deviation of six determinations. *Values differ significantly from control ($P<0.05$). **Value differ significantly from alloxan-induced diabetic rats (Group 2), $P<0.05$. SD: Standard deviation, MDA: Malondialdehyde, GSH: Glutathione

Table 7: SOD and CAT activities of various groups

	SOD activity (U/mg protein)±SD	CAT activity (U/mg protein)±SD
1. (Control)	1.000±0.027	2.040±0.683
2. Alloxan-induced diabetic rats	0.320±0.003*	0.704±0.010*
3. Diabetic rats+extract only	1.850±0.030	1.380±0.067
4. Diabetic rats+glibenclamide	1.250±0.080	1.893±0.108**
5. Diabetic rats+extract+glibenclamide (combinative therapy)	2.250±0.860	2.850±0.456**
6.Extract only	3.090±0.766	2.170±0.901**
7. Standard drug (glibenclamide only)	3.000±0.901	1.013±0.013**

Values are given as mean and standard deviation of six determinations. *Values differ significantly from control ($P<0.05$). **Value differ significantly from alloxan-induced diabetic rats (Group 2), $P<0.05$. SD: Standard deviation, SOD: Superoxide dismutase, CAT: Catalase

Table 8: Liver percentage DNA fragmentation in various treatment groups

Groups	Percentage DNA fragmentation (%)±SD
1. (Control)	60.990±3.000
2. Alloxan-induced diabetic rats	82.560±5.000*
3. Diabetic rats+extract only	62.900±3.000**
4. Diabetic rats+glibenclamide	63.290±3.000**
5. Diabetic rats+extract+glibenclamide (combinative therapy)	54.610±4.000**
6. Extract only	35.680±7.000**
7. Standard drug (glibenclamide only)	37.930±3.000**

Values are given as mean and standard deviation of six determinations. *Values differ significantly from control ($P<0.05$). **Value differ significantly from alloxan-induced diabetic rats (Group 2), $P<0.05$

Furthermore, the individual behavior of the extract (Group 6) and the standard drug (Group 7) noticeably reduces MDA and increases the antioxidant enzymes and GSH levels suggestive of the antioxidant effects of the plant and the drug possibly the basis of their anti-diabetic effects.

Fragmentation of DNA which implies the breaking or separation of DNA molecules or strands into pieces may occur intentionally, accidentally, or spontaneously by chemical agents or by cells as this gradually accumulates in cells under pathologic or disease condition or mutation and an indices of programmed cell death apoptosis in diseases.[42,43] In this study, the hepatic percentage DNA fragmentation in the alloxan-induced diabetic rats (Group 2) was significantly ($P < 0.05$) increased compared with control an indication of pathologic apoptosis while other treatment groups showed decreases in percentage DNA fragmentation. Reduction in DNA fragmentation or damage as exhibited by the extract in Group 6 may be associated with a possible antiapoptotic effect and the prevention of pathologic apoptosis at this concentration usually associated with the malfunctioning of the cell cycle regulating mechanisms.[44]

CONCLUSION

The results from this study are indication of a possible tissue protective effect of the plant extract by demonstrating significant curative potential evident in the reversals of certain biochemical and antioxidant parameters by exhibition of hypoglycemic, hypolipidemic, anti-inflammatory, antianemic, antioxidative, and antiapoptotic effects. Combination of the extract with the standard drug also showed these effects suggestive of a synergistic interaction between the extract and the drug while other studies are required to identify the active component(s) of the extract, possible side effects, and the mechanism(s) underlying the beneficial effects of these combinations in treating diabetes mellitus in some poor countries in Africa.

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