AQUEOUS EXTRACT OF *SOLANUM MACROCARPON* LEAVES AMELIORATES DYSLIPIDEMIA, OXIDATIVE STRESS, RENAL HISTOPATHOLOGY AND DYSFUNCTION IN ALLOXAN-INDUCED DIABETES

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ABSTRACT
This research investigated the effect of aqueous extract of *Solanum macrocarpon* leaves on antioxidant enzymes and histology of the kidney, lipid profile, and blood electrolytes in alloxan-induced diabetic male Wistar rats. For the study, 30 male Wistar rats weighing between 150 g and 200 g were randomly categorized into six groups of five rats each. Normal (A) and diabetic control (B) groups were given distilled water. The diabetic treated groups were given 250 mg/kg, 500 mg/kg, and 750 mg/kg body weight of *Solanum macrocarpon* orally for 14 days. Significant reversal of the altered levels of total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were observed in the results. Amelioration of the altered blood glucose and electrolyte levels also occurred. The histological study showed damage to the renal tubules of the untreated rats, which was absent in diabetic rats treated with the aqueous extract. The findings suggest that the aqueous *Solanum macrocarpon* leaves extract can be utilized as a source of natural antioxidants with anti-diabetic and anti-hyperlipidemic potential.

Keywords: Antioxidant enzymes, blood electrolyte, diabetes mellitus, histology, lipid profile, *Solanum macrocarpon*.
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INTRODUCTION

Diabetes mellitus is a long-term health problem with devastating consequences that, in critical cases, can lead to death [1]. This disease is characterized by increased blood glucose levels resulting from defects in insulin production, insulin action, or both [2]. It is mostly associated with progressive damage of various organs, such as the eyes, kidneys, liver, nerves, heart, and blood vessels. It is also marked by changes in the plasma lipid and lipoprotein profile [1].

Globally, recent data from the International Diabetes Federation estimated that 381 million people have diabetes [2]. The use of conventional drugs in the management of diabetes mellitus has not improved the situation as its prevalence is on the increase; this number is predicted to double by 2030, with the major part of this increase expected to occur in developing countries [3]. Diabetes mellitus is one of the leading causes of death and is the third when its complications are considered [3]. The 2016 estimate for the prevalence of diabetes in Nigeria by the World Health Organization (WHO) was 4.3%. Local studies estimate the prevalence to range from 0.8%-11% among rural and urban populations [2]. In Nigeria, the common type of DM is Type 2 (T2DM), accounting for about 90-95% of all cases [4]. The WHO Assessment of the national response to diabetes in 2016 revealed that policies, guidelines, and monitoring were only partially implemented [4].

Unlike other pharmacological strategies for treating diabetes, plant usage is more efficient and affordable. Research journals indicate that more than 800 plant species show hypoglycemic activity [5-8]. They have been reported to be non-toxic, accessible, and serve as the go-to treatment method for much of the world's population. Some of the plants used for the treatment of diabetes include the fruits of *Momordica charantia* and the leaves of *Veronia amygdalina* [6].

Solanum macrocarpon, also known as African eggplant, is a tropical plant widely used for medicinal, ornamental, and nutritional purposes. It has been used in Nigeria for the management of diabetes mellitus. In South-Western Nigeria, the fruit and leaves are used as a laxative and also to treat cardiac diseases [7]. Its uses also range from detoxifying the kidneys to treating several ailments such as nasal congestion and inflammation, skin infections, body pain, and injuries [8]. The pharmacological properties have been attributed to the presence of phytonutrients, such as phenols, ascorbic acid, alkaloids, anthocyanin, and α-chaconine [7].

Studies on the extracts of *Solanum macrocarpon* leaves have reported its anti-diabetic effects on diabetes in experimental models [9]. However, there has been little research to evaluate the anti-dyslipidemic, antioxidant and renal protective potential of the leaves. This study's aim was to compare the effects of aqueous extract of *Solanum macrocarpon* leaves to a standard anti-diabetic drug, glibenclamide, on these parameters in diabetic rats.

MATERIALS AND METHODS

Leaf collection and extract preparation
Fresh leaves of *Solanum macrocarpon* were purchased from the Awolowo market in Sagamu-Remo, Ogun State, Nigeria. The leaves were cleaned and air-dried for 3 days under shade. The dried leaves were then processed into powder form using a dry

mill. For 2 days, 25 g of the powdered leaves were macerated in 400 ml of distilled water in a container, with shaking at intervals. The solution was filtered, and the filtrate obtained was evaporated at 450 °C to get a crude extract, which was refrigerated until needed.

Animal housing

Thirty (30) male Wistar rats weighing between 150 g and 200 g were obtained from a reputable animal house in Ibadan, Oyo State and allowed to acclimatize for 2 weeks. The rats were sheltered in clean plastic cages and given free access to pelletized diet and water under suitable laboratory conditions. Animal handling was in accordance with the university's guidelines and ethical standards for the use of laboratory animals.

Groups of animals and treatment

Group A - Normal control.
Group B - Diabetic control.
Group C - Diabetes + Glibenclamide (5mg/kg) only.
Group D - Diabetes + Aqueous extract of *Solanum macrocarpon* leaves (250mg/kg).
Group E - Diabetes + Aqueous extract of *Solanum macrocarpon* leaves (500mg/kg).
Group F - Diabetes + Aqueous extract of *Solanum macrocarpon* leaves (750mg/kg).

Diabetes induction using alloxan

Diabetes was induced by a single alloxan monohydrate intraperitoneal injection (150 mg/kg body weight) to overnight fasted rats. 20% glucose solution was administered orally 48 hours post-induction to prevent hypoglycemia. Blood was obtained by the tail-snipping method, and the fasting blood glucose level of the rats was determined 72 hours after diabetes induction using an Accu-Chek glucometer [10]. Rats with fasting blood glucose (FBG) levels ≥ 200 mg/dl were considered diabetic and used for the study [11].

Administration of doses of the aqueous extract and glibenclamide

250 mg of the crude extract was dissolved in 100 ml of distilled water and administered orally to the rats in groups D, E, and F as 2.5 ml, 5 ml and 7.5 ml respectively to get the required dosage.
20 mg of glibenclamide was dissolved in 10 ml of distilled water to form a solution. 0.5 ml of the solution was given to 150 g rats, while 1 ml of the solution was given to 200 g rats daily to obtain 5 mg/kg dosage.

Collection of blood and organs

After the administration period of 14 days, blood samples for biochemical analysis (lipid profile, blood electrolytes, urea and creatinine) were collected from the orbital sinus of the rats, and plasma was obtained by centrifugation. The rats were sacrificed by cervical dislocation, and the kidneys of each rat were excised.

Determination of antioxidant enzymes

The kidneys were homogenized in phosphate buffer. Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx) activities were determined as described by Eze *et al.* (2012) [12].

Determination of lipid profile

Blood plasma was analyzed using an automated biochemical analyzer. The method explained by Eze *et al.* (2012) was used to determine Total Cholesterol (TC), Triglycerides (TG), High-Density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL) levels [12].

Histology of the kidney

The method described by Okoye *et al.* (2017) was used to carry out histological processing. The kidney tissue sections were stained with Haematoxylin-Eosin using standard procedures. Photomicrographs were taken under a light microscope [13].

Statistical analysis

The results were analyzed using one-way analysis of variance (ANOVA) with the Microsoft Office Excel 2013 software version. Values were considered statistically significant at p value < 0.05, and were presented as the mean ± standard error of the mean (M ± SEM).

RESULTS

The total cholesterol, triglyceride, high- and low-density lipoprotein levels in alloxan-induced diabetic rats treated with glibenclamide and aqueous extract of *Solanum macrocarpon* leaves is shown in Table 1. A decrease in TC level was observed in groups D (58.8 ± 1.71), E (66.6 ± 6.37), and F (64.0 ± 5.53) when compared with groups B (73.8 ± 3.20) and C (71.6 ± 0.24), but it was significant only in group D. TG level was reduced in groups D (28.4 ± 0.98), E (34.8 ± 0.73), and F (46.4 ± 3.16) upon comparison with group C (47.0 ± 2.45), and significant in groups D and E. There was an increase in HDL in groups E (22.4 ± 2.20) and F (29.6 ± 2.93) when compared to groups B (21.8 ± 1.83) and C (21.2 ± 0.49) which was significant only in group F. LDL levels showed no significant decrease in groups C (41.6 ± 0.24), D (45.2 ± 3.18), and E (34.8 ± 4.16) in relation to group B (45.8 ± 3.67).

Table 1: The effect of SM and glibenclamide on total cholesterol, triglyceride, high- and low-density lipoprotein levels in diabetic rats.

GROUP	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
A	66.0 ± 2.45	23.2 ± 0.20	16.8 ± 1.96	23.8 ± 2.80
B	73.8 ± 3.20	35.6 ± 2.93[a]	21.8 ± 1.83	45.8 ± 3.67[a]
C	71.6 ± 0.24	47.0 ± 2.45[a b]	21.2 ± 0.49	41.6 ± 0.24[a]
D	58.8 ± 1.71[a b c]	28.4 ± 0.98[a b c]	14.4 ± 0.60[b c]	45.2 ± 3.18[a]
E	66.6 ± 6.37	34.8 ± 0.74[a c]	22.4 ± 2.20	34.8 ± 4.16
F	64.0 ± 5.53	46.4 ± 3.16[a b]	29.6 ± 2.93[a c]	46.6 ± 6.37[a]

Table 2 shows the results of blood electrolytes, urea and creatinine levels in alloxan-induced diabetic rats treated with glibenclamide and aqueous extract of *Solanum macrocarpon* leaves. There was no significant increase in sodium levels in groups C (141.2 ± 0.92) and F (143.2 ± 2.40) when compared with group B (140.4 ± 1.29). A significant increase in potassium level is shown in group C (4.38 ± 0.25) when compared with group B (3.82 ± 0.07), and when groups D, E, and F were compared with group B (3.82 ± 0.07), there was no significant decrease in group D (3.26 ± 0.56) and increases in groups E (4.04 ± 0.10) and F (4.46 ± 0.09), but it was significant only in group F. Chloride levels were reduced in groups D (97.6 ± 0.60) and E (101.6 ± 0.24) when compared with groups B (104.2 ± 2.54) and C (105.4 ± 2.09) which was significant only in group D. Urea level showed a significant decrease in groups C (59.6 ± 2.20) and D (66.0 ± 3.67) when compared with group B (81.2 ± 2.94), while there was a decrease in creatinine level in groups C (1.88 ± 0.07), D (1.92 ± 0.07), and E (2.30 ± 0.17) when compared with group B (2.40 ± 0.20) but significant only in groups C and F (2.96 ± 0.10).

Table 2: The effect of SM and glibenclamide on blood electrolytes, urea and creatinine levels in diabetic rats.

GROUP	Na^+ (mg/dl)	K^+ (mg/dl)	Cl^- (mg/dl)	UREA	CREATININE
A	144.6 ± 1.94	3.74 ± 0.10	103.0 ± 0.55	51.2 ± 3.18	1.34 ± 0.21
B	140.4± 1.29	3.82 ± 0.07	104.2 ± 2.54	81.2 ± 2.94[a]	2.40 ± 0.20[a]
C	141.2 ± 0.92	4.38 ± 0.25[a]	105.4 ± 2.09	59.6 ± 2.20[b]	1.88 ± 0.07[a b]
D	139.2± 2.56	3.26 ± 0.56	97.6 ± 0.60[a b c]	66.0 ± 3.67[a b]	1.92 ± 0.07[a]
E	138.0 ± 2.45	4.04 ± 0.10	101.6 ± 0.24[a]	83.4 ± 5.88[a c]	2.30 ± 0.17[a c]
F	143.2 ± 2.40	4.46 ± 0.09[a b]	114.4 ± 3.96[a]	108.0 ± 6.12[a b c]	2.96 ± 0.10[a b c]

Table 3 shows the result of glutathione peroxidase, catalase and superoxide dismutase levels in alloxan-induced diabetic rats treated with glibenclamide and aqueous extract of *Solanum macrocarpon* leaves. There was a significant increase in GPx activity in groups D (7.66 ± 1.39), E (38.07 ± 0.69), and F (11.10 ± 1.02) when compared to group C (3.12 ± 0.26). The increase was also significant in groups E (38.07 ± 0.69) and F (11.10 ± 1.02) when compared to group B (6.31 ± 1.15). CAT activity was reduced in groups C (17.33 ± 1.03), D (12.63 ± 1.26), E (10.14 ± 0.19), and F (16.86 ± 2.71) when compared to group B (19.04 ± 1.27) and was significant in groups D and E. The decrease in groups D and E was significant in relation to group C. Reduced SOD activity was observed in groups D and E in comparison with group B, but significant only in group E. There was a significant decrease in groups D and E when compared to group C.

Table 3: The effect of SM and glibenclamide on glutathione peroxidase, catalase and superoxide dismutase levels in diabetic rats.

GROUP	GPx (Units)	CAT (U/mg)	SOD (Units)
A	11.82 ± 1.21	16.34 ± 0.72	1.99 ± 0.15
B	6.31 ± 1.15[a]	19.04 ± 1.27	1.81 ± 0.26[a]
C	3.12 ± 0.26[a b]	17.33 ± 1.03	2.06 ± 0.01
D	7.66 ± 1.39[c]	12.63 ± 1.26[a b c]	1.36 ± 0.15[a c]
E	38.07 ± 0.69[a b c]	10.14 ± 0.19[a b c]	0.75 ± 0.03[a b c]
F	11.10 ± 1.02[b c]	16.86 ± 2.71	2.00 ± 0.28

Histological changes of the kidney in diabetic rats treated with aqueous extract of *Solanum macrocarpon* leaves and glibenclamide

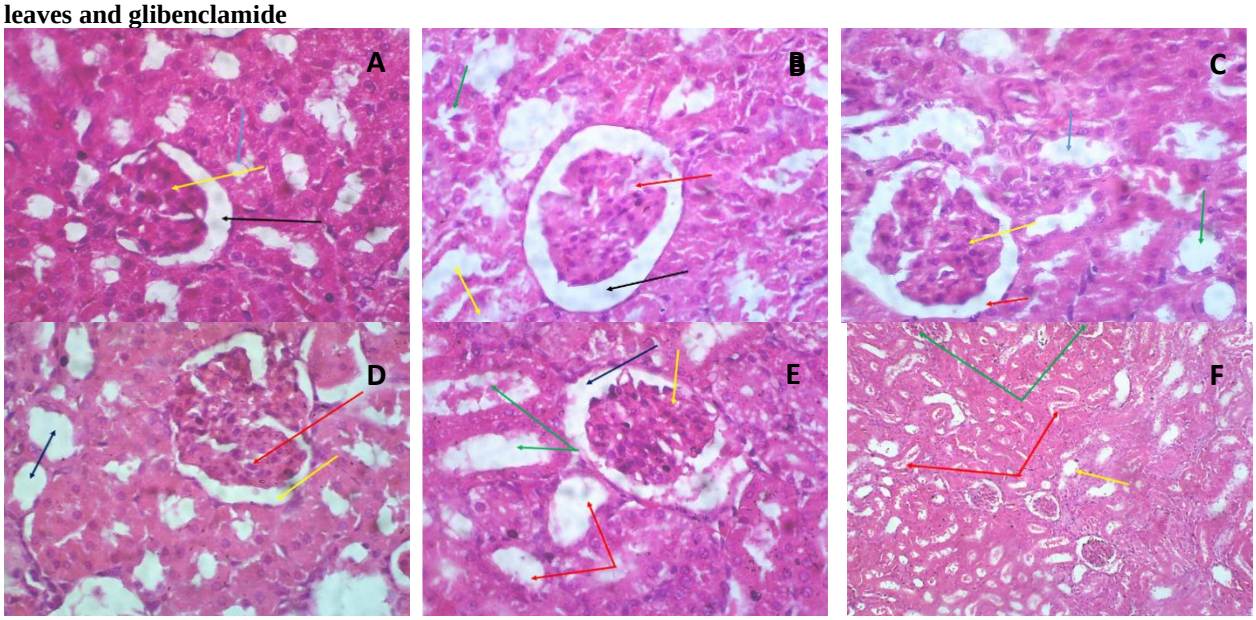

Plate I: Photomicrographs of the kidney histology of Wistar rats (×400) stained with H & E.

Slide A represents the kidney of normal control group A, showing the typical cellular structures of the renal glomerulus (yellow arrow) with Bowman's space (black arrow). The proximal convoluted tubules (blue arrow) have a distinct regular lumen. Slide B showed areas of vascular degeneration, tubular necrosis, glomerular inflammation (red arrow) and epithelial lining desquamation (black arrow) in diabetic control group B. The slowly regenerating structure of the Bowman's space (red arrow) can be seen in slide C for group C. The epithelial lining of the glomerular capsule (yellow arrow) and the convoluted tubules (green and blue arrows) can be seen demonstrating a slight reversal of the damage. Slide D represents diabetic rats + 250 mg/kg SM extract (group D), and it shows cellular rearrangement of the renal capsule (red and yellow arrows). The convoluted tubules (dark blue arrow) still possess irregular but distinct lumens with desquamated cells. Observations from slides E and F, groups E & F, show recovering structures of the renal glomerulus and the epithelial lining of the glomerular capsule (yellow arrow). The proximal convoluted tubules (red arrow) still possess an irregular lumen with mild desquamation of cells recovering from the damage (green arrow).

DISCUSSION

Medicinal plants are widely used to manage diseases worldwide [14, 15]. In Nigeria, many plant species have been claimed to possess medicinal properties and have been employed to treat many illnesses [16]. Alloxan induces diabetes in experimental animals by destroying the beta cells in the pancreas, leading to reduced synthesis and release of insulin, thereby inducing hyperglycemia and leaving residual or less active beta cells.

It was shown that administration of aqueous extract of *Solanum macrocarpon* leaves to diabetic groups for 14 days significantly reversed the altered TC, TG, LDL, and HDL levels in a dose-dependent manner relative to the diabetic untreated group and glibenclamide-treated group. Significant decreases ($p < 0.05$) were observed in sodium, potassium, urea and creatinine levels, and an increase in chloride level was observed among the diabetic untreated rats compared to the control group. Administration of the aqueous extract to the diabetic groups showed significant increases ($p< 0.05$) in sodium, potassium, urea and creatinine levels, and a significant decrease in chloride level compared to the group given glibenclamide and the untreated group.

In alloxan-induced diabetic rats, the aqueous extract of *Solanum macrocarpon* leaves significantly restored the altered level of antioxidant enzymes. Oxidative stress resulting from increased reactive oxygen species (ROS) and decreased antioxidant enzyme activity and chronic hyperglycemia potentiates the increased risk of cardiovascular diseases through changes in blood lipid levels. Furthermore, the histological study performed on the kidney sections of the diabetic rats showed damage to the glomerulus, thickened basement membrane, and edema in the proximal convoluted tubule with an increase in crystal-like deposits present in diabetic and glibenclamide-treated rats, which were absent in the diabetic kidneys treated with the aqueous extract.

The results of this work support the findings of Mendez and Balderas (2001) and Mitra *et al.* (1995), who have reported increased TC and TG, low LDL, and increased HDL in streptozotocin-induced hyperglycemia in rats [17, 18]. Daisy *et al.* (2009)

reported insulin-deficient associated hypercholesterolemia and hypertriglyceridemia in streptozotocin-induced diabetes in rats [19]. The observed increase in the HDL level after administration of the aqueous extract in alloxan-induced diabetic rats indicates that the extract has an HDL boosting effect. In addition, the stabilization of serum triglyceride and cholesterol levels in rats by the plant extract may be attributed to glucose utilization and hence depressed mobilization of fat [16, 20]. Previous studies on diabetic rats also reported similar histological findings [21, 22]. The decrease in sodium, potassium, urea and creatinine levels, and increased chloride levels among the diabetic untreated rats compared to the normal control group, were consistent with those reported by previous studies [23, 24].

CONCLUSION

The study results indicate that the aqueous extract of *Solanum macrocarpon* leaves has a greater protective potential in alloxan-induced diabetic rats, with the extract administration exhibiting significantly higher modulation action on kidney histological changes than glibenclamide-treated rats. The extract also protected beta-cells from ROS-mediated damage in plasma and significantly improved the activities of antioxidant enzymes at the tissue level, thus ameliorating hyperglycemia. The aqueous extract of *Solanum macrocarpon* leaves can be utilized as a source of natural antioxidants with anti-diabetic and anti-hyperlipidemic properties. It can also be employed as a nutraceutical for treating diabetes mellitus and its complications.

RECOMMENDATION

It is recommended that extensive investigations be carried out on this extract at a higher concentration to determine its toxic levels. The adverse effects associated with the long-term usage of this medicinal plant should also be considered.

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