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Evaluation of Body Weight, Serum Glucose Level and Oxidative Stress Parameters of Streptozotocin-Induced Diabetic Rats Administered Insulin and Alkaloid Leaf Extract of *Jatropha tanjorensis*

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Abstract

Diabetes mellitus (DM) is known to reduce body weight and induce oxidative stress. Hence, this study evaluates the effects of alkaloid leaf extract of *Jatropha tanjorensis* (ALEJT) on body weight, serum glucose, and oxidative stress parameters in streptozotocin (STZ)-induced DM rats to delineate its possibility as a better therapeutic agent compared to insulin in the treatment of DM. Forty female albino rats (150–200 g) were grouped into four (n = 6). Group 1 served as normal control. Diabetes was induced in Groups 2, 3, and 4 rats by intraperitoneal (i.p.) administration of a single dose of STZ (80 mg/kg) dissolved in citrate buffer (pH 4.5) to overnight-fasted rats. After 72 hours of injection, rats with fasting blood glucose ≥ 200 mg/dL were deemed diabetic. Group 2 served as diabetic control; Group 3 diabetic rats were treated with ALEJT (500 mg/kg orally), while Group 4 was treated with insulin (2 IU/kg/day, i.p.) for 21 days. Changes in body weight and serum glucose levels

were assayed within 21 days, after which the rats were sedated with 60 mg/kg of ketamine-hydrochloride, and blood samples were collected via cardiac puncture; serum was used for antioxidant (glutathione [GSH], superoxide dismutase [SOD], glutathione peroxidase [GPx], catalase [CAT], total antioxidant capacity [TAC], and malondialdehyde [MDA]) assays using standard methods. There was no variation in the weight of diabetic rats treated with ALEJT. Percentage growth rate was significantly reduced in the untreated and treated diabetic rats. Glucose levels increased ($p < 0.001$) in diabetic rats compared with the control. Diabetic control showed a significant decrease in GSH, SOD, TAC, and GPx, while MDA levels increased ($p < 0.001$) compared with the control. The levels of SOD, GPx, and TAC increased, while MDA decreased significantly ($p < 0.001$) in the treated groups compared with the diabetic control. GSH increased in insulin ($p < 0.01$) and ALEJT ($p < 0.001$) treated rats compared with the diabetic control. GSH and GPx increased significantly in ALEJT-treated rats ($p < 0.001$) compared with insulin-treated rats. ALEJT was more effective in protecting against weight loss and reversed the levels of GPx and GSH better than insulin. ALEJT could serve as a potential natural and safe remedy for the management of DM.

Keywords: Diabetes Mellitus; Streptozotocin Model; Oxidative Stress; *Jatropha tanjorensis*; Antioxidant Enzymes

INTRODUCTION

Diabetes mellitus (DM) is a serious health problem as based on the World Health Organization report (Pournaghi *et al.*, 2012). It is a chronic endocrine disorder characterized by hyperglycemia from complete or relative lack of insulin secretion or tissue insensitivity to it (Essiet *et al.*, 2020). DM are of two types, type 1 termed insulin-dependent diabetes mellitus is categorized by blood hyperglycemia due to deficit in insulin production by the β-cells of the islets of the pancreas and type 2 termed non-insulin dependent diabetes mellitus which may be categorized by ineffective use of insulin by the body cells resulting in hyperglycemia (Yahia *et al.*, 2016).

The streptozotocin (STZ) rat model of diabetes is one of the most commonly used models of human disease (Marta *et al.*, 2012) because it mimics many of the acute and chronic complications of human diabetes and the model has the advantage of being highly reproducible. Since the discovery of it diabetogenic property by Rakieten *et al.*, (1963) it has been widely used to induce DM in experimental rodents. A single, rapid injection of STZ

causes selective destruction of the insulin-producing β-cells of the pancreas (Al-Attar and Alsalmi, 2019). In addition to its ability to induce type 1 DM, STZ was also found capable of developing peripheral insulin resistance or impairing insulin secretion from these cells. Such effects are usually sufficient to induce type 2 DM in animals (Qinna and Badwan, 2015).

It was estimated that the prevalence of people with DM worldwide by the year 2025 will be over 300 million (Shirin *et al.*, 2011; Pournaghi *et al.*, 2012; Al-Attar and Alsalmi, 2019). This is because in modern medicine, there is no satisfactory effective therapy to cure DM (Al-Attar and Alsalmi, 2019). DM has been estimated to be the 7th leading cause of death in 2030 (Yahia *et al.*, 2016). Asia's and Africa's rural areas have been identified as greatest potential of the disease, which could rise up to two or three fold above the estimated level (Yahia *et al.*, 2016).

The consequence of DM thus manifests in multiple systems (Essiet *et al.*, 2020). Oxidative stress is currently proposed as mechanism underlying diabetes and diabetic complications (Ali *et al.*, 2012). During diabetes, continuous hyperglycemia causes increased free radical production in the tissues creating oxidative stress (Pournaghi *et al.*, 2012). Oxidative stress results from increased free radical production or reduced activity of antioxidant defences or both these phenomena (Ali *et al.*, 2012).

In recent years, there has been increasing interest in finding natural antioxidants from medicinal plants because synthetic antioxidants have been connected to cellular toxicity (Obasi *et al.*, 2019). *Jatropha tanjorensis* is one of such plants that have attracted substantial attention due to its numerous pharmacological and biological actions. *Jatropha tanjorensis* popularly known as 'Hospital too far' have been found valuable to cure various ailments such as lowering blood cholesterol level and of cardiovascular diseases due to hyperlipidemia (Ukoh *et al.*, 2022), improve haematological indices which revealed an enhancement of bone marrow function, increases the amount of iron available for erythropoiesis, increase packed cell volume and hemoglobin concentration in rats (Antai *et al.*, 2023). The leaves of *Jatropha tanjorensis* was reported rich in antioxidant properties (Ukoh *et al.*, 2022). Phytochemical screening of *J. tanjorensis* leaf revealed that it contains bioactive principles such as alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinones and saponins (Antai *et al.*, 2023; Ukoh *et al.*, 2024). There have been claims that *Jatropha tanjorensis* is used as a traditional remedy in the treatment of diverse health

condition including DM (Ukoh *et al*., 2024), but there are no scientific proofs to back this up. Hence, the present study was performed to evaluate the impact of alkaloid extract of *Jatropha tanjorensis* leaves on body weight, serum glucose level and oxidative stress parameters of STZ induced diabetic rats. The outcome of the study will be used for public campaign to encourage or discourage its use.

METHODOLOGY

Collection and identification of Jatropha tanjorensis leaves

Fresh leaves of *Jatropha tanjorensis* were collected within Calabar South local government area of Cross River State, Nigeria. The plant was identified and authenticated by a botanist in University of Calabar, with voucher number (Herb/Bot/UCC/182) kept in the botanical garden.

Alkaloid extraction

The methods of Pertot *et al*., (1984) were modified for alkaloid extraction. 100g of dried, ground and pulverized plant samples were defatted with n-Hexane in a Soxhlet apparatus to remove fats, oils, terpenes, waxes etc and the extract was discarded. The marc was dried and re-extracted with methanol for 24 hours and the methanol solution was evaporated to about ¼ of its original volume. The solution was washed thrice with n-Hexane to further remove lipid soluble substances after which it was diluted with a 2% solution of sulphuric acid up to pH 2 in order to ensure complete retrieval of alkaloids, the original marc after methanolic solution in order to retrieve any alkaloidal salt that only methanol cannot extract and the two acidic methanolic solutions were pooled. Drops of concentrated ammonium hydroxide were added up to a pH of 10 – 12 in the solution and kept for 3 hours. The solution was then added to chloroform and shaken vigorously in a separating flask. The chloroform layer was decanted, dried over anhydrous sodium sulphate and evaporated to dry in a vacuum.

Experimental animals

Forty (40) female albino rats of Wistar strain weighing averagely 150 -200g were purchased from the animal farm of University of Uyo, Akwa-Ibom State and were allowed one week acclimatization in the animal house, with free access to standard feeds (Pfizer feed PLC, Lagos, Nigeria), and water ad libitum in Physiology Department, University of

Calabar. The Ethical Committee for the use of animals in research was obtained from the University of Calabar College Ethical Committee on the use of experimental animals with protocol number (093PHY3321). Handling of the rats and other experimental protocols were in accordance with laid - down ethics for animal care approved by the National Committee for Research Ethics in Science and Technology (NENT), 2018.

Induction of diabetes mellitus

The rats were fasted for 16 hours, and the initial fasting serum glucose concentrations (FSGC) were measured using a blood glucose test meter (Double G glucometer, USA) with blood drawn from the tail vein. Experimental DM was induced by intra-peritoneal injection of a single dose of streptozotocin (Sigma- Aldrich Corp, St. Louis, MO, USA) at 80 mg/kg dissolved in citrate buffer pH 4.5 (Qinna and Badwan, 2015). After 72 hours of injection, to demonstrate that diabetes has been produced, blood was extracted from the tail vein for blood sugar level analysis, using a blood glucose test meter (Double G glucometer, USA). Rats with blood glucose level ≥ 200 mg/dl and above were considered to be diabetic and selected for the diabetic group.

Table 1. Study Design and Drug and Extract administration

Groups	No. of rat	Treatment
Group 1 (Normal Control)	**6**	**Normal Saline**
Group 2 (Diabetic Control)	6	DM-rats without treatment
Group 3 (ALEJT Treated)	6	DM-rats + ALEJT at 500mg/kg orally/day
Group 4 (Insulin Treated)	6	DM-rats + insulin 2units/kg/day intra-peritoneal route.

Alkaloid extract of *Jatropha tanjorensis* was administered for 21 days. Diabetic rats were treated daily with human insulin (Humulin N; Lilly, Sao Paulo, SP, Brazil).

Body Weight

Rats initial body weight were measured at the start of the study and final body weight on day 21 using electronic weighing balance {Digital Precision Weighing Balance (JCS-QC03) – China}.

Change in body weight: Final body weight – Initial body weight

Percentage growth rate: $\frac{\text{Change in body weight}}{\text{Experimental duration}} \times 10$

Blood collection

After expiration of the experiment, the rats were sedated with 60 mg kg^{-1} of ketamine-hydrochloride and blood samples collected via cardiac puncture and allowed to clot in sterile vials. Serum samples were collected from clotted blood using a centrifuge operated at 2000 rpm for 10 min. The serum was collected by aspiration using a Pasteur pipette, and stored in the refrigerator at 4◦C prior to biochemical analyses.

Biochemical parameters

Serum glucose

Serum blood glucose (SBG) levels were determined on days 1, 7, 14 and 21 in fasting condition.

Antioxidant Enzymes Activities: Determination of oxidative stress markers

Abcam reagent kits (UK) was used for the quantitative in-vitro determination of levels of malondialdehyde (MDA), superoxide dismutase (SOD) glutathione peroxidase (GPX) catalase (CAT) and total antioxidant capacity (TAC).

Serum Malondialdehyde (MDA) level

Serum MDA concentration was measured according to the method of Placer *et al.*, (1966).

Procedure: 1ml serum was homogenized on ice in 300ml of the MDA lysis buffer and then centrifuged at 13,600xg for 10min. 200ml of the supernatant from homogenized sample was placed into a micro centrifuge tube. Graded doses of 0,2,4,6,8,10 ml of the 2mmol MDA standard added into separate micro-centrifuge tubes and the final volume adjusted to 200ml with distilled water to generate 0,4,8,12,16 and 20nml standard per well. 600ml of thiobabituric acid (TBA) solution was added into each vial containing standard sample and incubated at 95^0c for 60 minutes. It was then cooled to room temperature in ice bath for 40 minutes and 200ml (from each 800ml reaction mixture) was pipette into a 96 – well micro-plate for analysis. The absorbance was read at 532nm.

Serum Superoxide Dismutase (SOD) activity

Serum SOD activity was measured according to the methods of Nishikimi *et al.*, (1972).

Procedure: Serum was mixed with 150mml to remove any red blood cells. 1ml of serum was homogenized in ice cold. The crude serum homogenate was centrifuged at 14000xg for 5 minutes at 40^0c and the cell debris discarded. Plates were incubated at 37^0c for 20 minutes and absorbance read at 450nm using a microplate reader.

Serum Glutathione level

Serum GSH concentration was measured according to the methods Sedlak and Lindsay (1968). **Procedure:** Based on the development of a yellow colour when 5,5-dithiobis-2-nitrobezoic acid (DTNB), was added to compounds containing sulfhydryl groups. The absorbance was read spectrophotometrically at 412 nm. Total GSH content was expressed as mmole GSH/g of tissue.

Serum Glutathione Peroxidase (GPX) activity

Serum GSH peroxidase concentration was measured according to Rotruck *et al.*, (1973) method

Procedure: 0.5ml of tissue was homogenized on ice in 0.2ml cold assay buffer and centrifuged at 10,000 xa for 15 minutes at 40^0c. The supernatant was collected for assay. 40ml of the samples was added into a 96 well plate and the volume made up to 50ml with assay buffer. The standard curve was prepared by diluting 25ml of the 40nm NADPH solution into 975ml distilled water 10ml of the 1mm NADPH standard. 0, 20, 40, 60, 80 and 10ml of the 1mm NADPH standard was added into 96 well plate in duplicate to generate 0, 20,40,60,80 and 100 nmol/well standard. The final volume was brought to 100ml with assay buffer. The optical density at 340nm was measured and the NADPH standard curve was plotted. The positive control was prepared putting 5 – 10ml of the GPX positive control into the desired wells and adjusted to 50ml with assay buffer. 50ml of assay buffer was added into the wells as a reagent control (Rc). 40 ml of the reaction mix was added to each test samples, positives controls and reagent control were mixed well and incubated for 15minutes to deplete all GSSG in the sample. 10ml cumene hydroperoxide solution was added to start GPX reaction and then mixed well. The OD at 340nm at T_1, was measured to read A, OD at 340nm was measured again at T_2 after incubating the reaction at 25^0c for 5 minutes to read A_2.

A340nm [(Sample A_1 – sample A_2) – (RcA_1 – RcA_2).

Serum Catalase (CAT) Activity)

Serum CAT activity was measured using the methods of Luck (1963).

Procedure: 0.5ml serum was homogenized in 0.2ml cold assay buffer and centrifuged at 10,000xg for 15 minutes at 40^0c. The supernatant was collected for assay and 40ml of samples solution was added into each well and volume adjusted to total 78ml with assay buffer. High control (Hc) was prepared with the same amount of sampled in separate wells then made up to 78ml with assay buffer. 10ml of stop solution was added into the sample Hc, mixed and incubated for 5mintues at 25^0c. 12ml of fresh 1mmol H_20_2 is added into each well of both samples and sample Hc to start the reaction, then incubated at 25^0c for 30 minutes. 10ml stop solution was then added into each sample vial to stop the reaction. 50ml of the developer mix was added to seach test sample, controls and standard. It was mixed well and incubated at 25^0c for 10minutes. The OD at 570nm is read in a Mindray chemistry analyzer BS – 120.

Serum total antioxidant capacity (TAC)

Serum TAC was measured according to the method of Benzie and Strain (1998).

Procedure: A working solution of FRAP (ferric reducing ability of plasma) was provided by mixing buffer acetate with TPTZ (2, 4, 6 tripyridyl -S- tiazine) solution in HCl. After that $FeCl_3$ was added and mixed. This process is founded upon the ability of plasma in reducing Fe_3 to Fe_2 in the presence of (TPTZ). 8µL of serum and 240µL of mentioned working solution were mixed and incubated for 10min at room temperature. The optical density of samples was measured at 532nm. Total antioxidant capacity was expressed as mmol/L.

Statistical Analysis

Values for the result were expressed as mean ± SEM. The statistical analysis was done using the analysis of variance (ANOVA) and the post hoc Turkey's multiple comparison tests. The computer software was Microsoft excel and SPSS for window. Differences between means was tested at $p<0.05$ levels of significance.

RESULTS

Body weight change

Initial and final body weight among the different groups: The initial versus final mean body weights (g) for the normal control, diabetic control, diabetic + extract and diabetic + insulin are: 162.50 ±4.31 versus 168.50 ±4.04, 144.50 ±3.72 versus 133.67 ±2.84, 157.83 ±7.48 versus 152.00 ±6.01 and 150.92 ±4.59 versus 141.40 ±4.30 respectively. The final mean body weight was significantly ($p<0.001$) lower in the diabetic control group compared to the initial mean body weight. The final mean body weight was significantly ($p<0.05$) lower in the diabetic + insulin group compared to the initial mean body weight (Figure 1).

Mean body weight change among the different groups: The mean body weight changes (g) for the normal control, diabetic control, diabetic + extract and diabetic + insulin are: 6.00 ±1.37, -10.83 ±3.12, -5.83 ±2.39 and -9.20 ±2.01 respectively. The mean body weight change was significantly ($p<0.001$) lower in the diabetic control and diabetic + insulin groups compared to the control. It was significantly ($p<0.01$) lower in the diabetic + extract group compared to the control (Figure 2).

Percentage growth rate among the different groups: The percentage growth rates (%) for the normal control, diabetic control, diabetic + extract and diabetic + insulin are as shown in Figure 3. The percentage growth rate was significantly ($p<0.001$) lower in the diabetic control and diabetic + insulin groups compared to the control. It was significantly ($p<0.01$) lower in the diabetic + extract group compared to the control.

Blood glucose levels

Blood glucose levels before and after induction of diabetes: The mean blood glucose levels (mg/dl) before induction of diabetes in normal control, diabetic control, diabetic + extract and diabetic + insulin are: 92.83 ±1.92, 89.17 ±2.17, 93.00 ±1.88 and 92.17 ±1.90 respectively. There was no significant difference in the mean blood glucose concentration before the induction of diabetes among the different groups (Figure 4).

The mean blood glucose levels (mg/dl) after induction of diabetes for normal control, diabetic control, diabetic + extract and diabetic + insulin are: 92.17 ±1.01, 360.17 ±28.37, 376.00 ±38.06 and 368.40 ±22.94 respectively. The mean blood glucose concentration after the induction of diabetes was significantly ($p<0.001$) higher in the

diabetic control, diabetic + extract and diabetic + insulin groups compared to normal control (Figure 4).

Blood glucose levels after treatment with extract and insulin: The mean blood glucose (mg/dl**)** after treatment for normal control, diabetic control, diabetic + extract and diabetic + insulin are: 93.83 ±1.30, 239.83 ±18.84, 180.00 ±9.82 and 112.80 ±9.84 respectively. After 21 days of treatment the mean blood glucose concentration was significantly ($p<0.001$) higher in the diabetic control and diabetic + extract rats compared to the control. It was significantly ($p<0.01$) lower in the diabetic + extract and ($p<0.001$) diabetic + Insulin groups compared to the diabetic control. Similarly, the mean blood glucose concentration after 21 days of treatment with extract was significantly ($p<0.001$) higher compared to the diabetic + insulin (Figure 4).

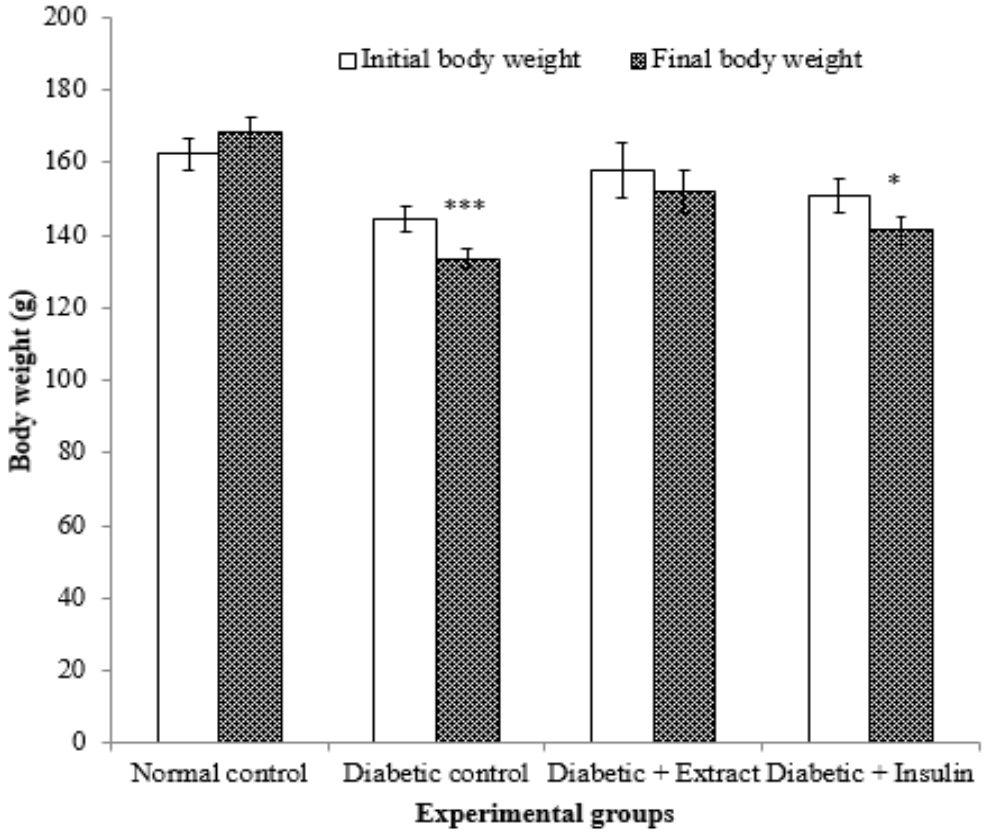

Figure 1: Comparison of initial and final body weight change of the different experimental groups.

Values are expressed as mean +SEM, n = 6.
*** = $p<0.001$, * = $p<0.05$ vs initial body weight.

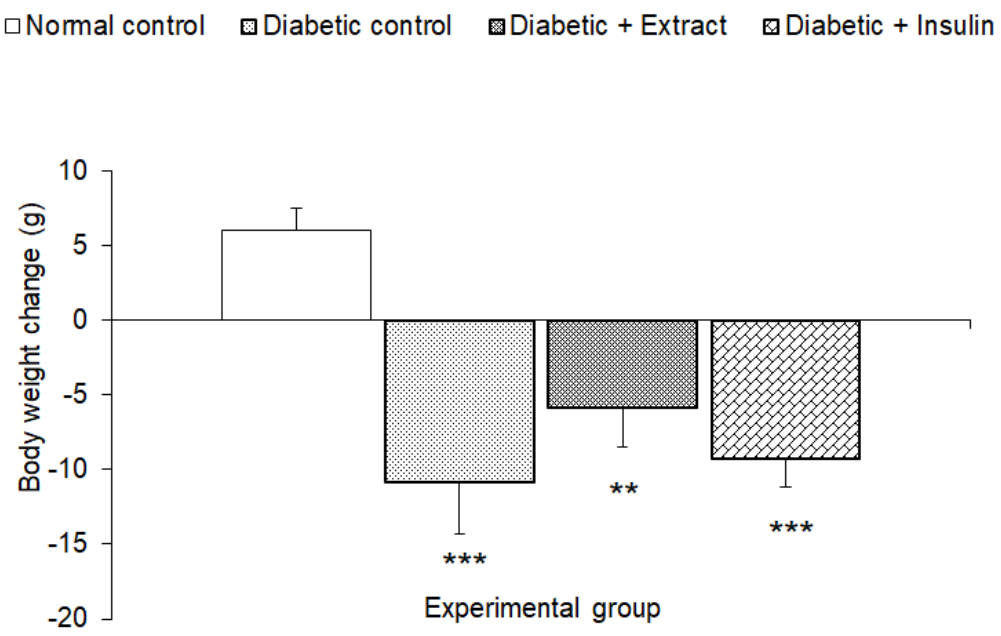

Figure 2: Comparison of body weight change of the different experimental groups.

Values are expressed as mean +SEM, n = 6.
** = p<0.01, *** = p<0.001vs normal control.

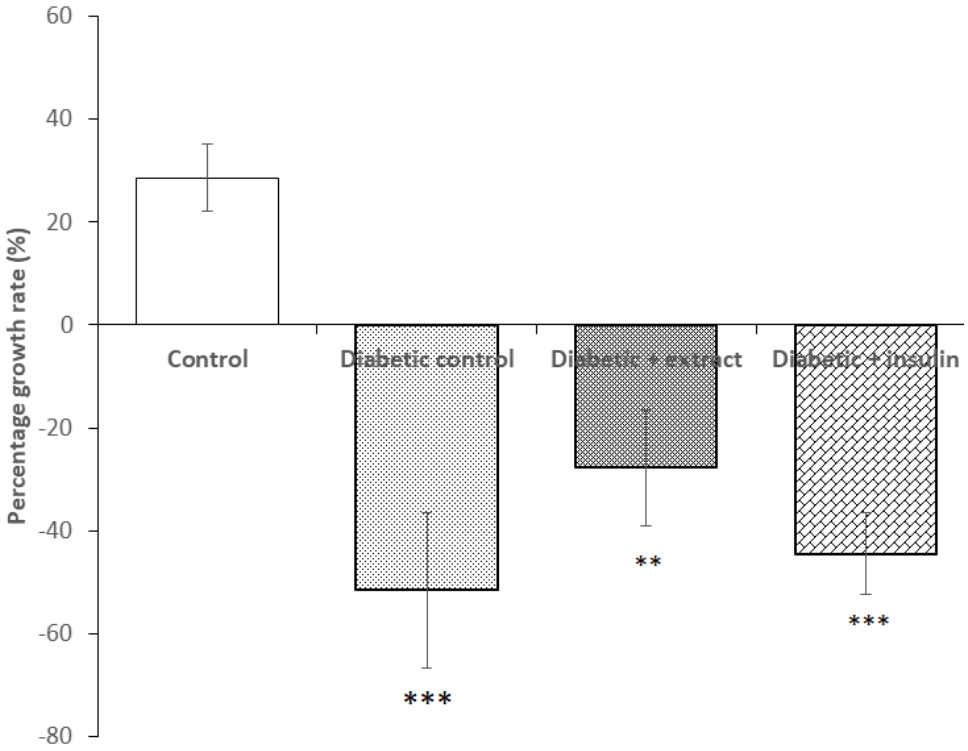

Figure 3: Comparison of percentage growth rate of the different experimental groups

Values are expressed as mean +SEM, n = 6.
** = $p<0.01$, *** = $p<0.001$ vs normal control

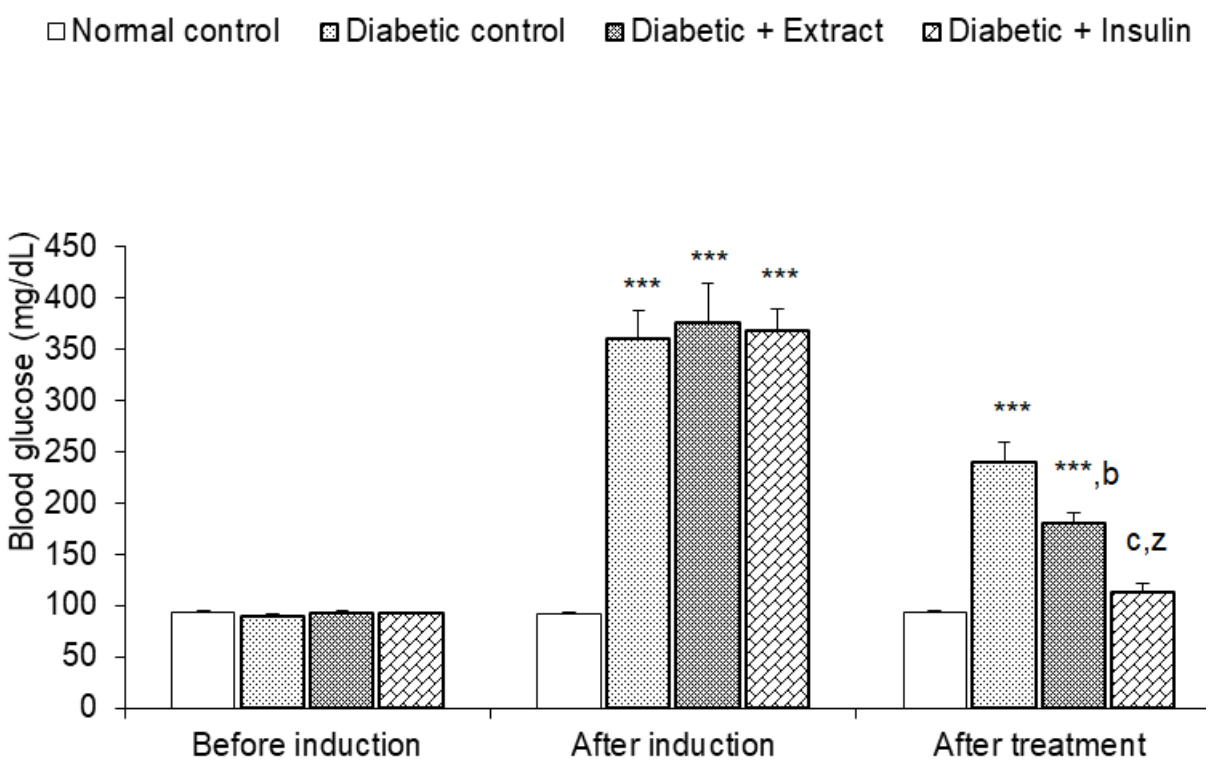

Figure 4: Comparison of fasting blood glucose levels of different experimental groups before and after induction of diabetes, and treatment with extract

Values are expressed as mean +SEM, n = 6.
*** = $p<0.001$ vs normal control
b= $p<0.01$, c= $p<0.001$ vs diabetic control
z= $p<0.001$ vs diabetic + extract

The mean blood glucose after 7 hours of induction of diabetes was significantly ($p<0.001$) higher in Group 2-4 compared to Group 1. This trend was similar for mean blood glucose concentration after 1, 2, 3, 5 and 7 hours of induction of diabetes as shown in Figure 5.

The mean daily blood glucose after 21 days of induction of diabetes is as shown in Figure 6.

On day 1, the mean blood glucose level was higher ($p<0.001$) in Group 2, 3 and ($p<0.01$) Group 4 against Group 1. It was also higher ($p<0.01$) in Group 2 and 3 against Group 4 (Figure 6).

On day 7, the mean blood glucose level was higher ($p<0.001$) in Group 2, 3 and ($p<0.05$) Group 4 against Group 1. It was also ($p<0.01$) higher in Group 2 and 3 against Group 4 (Figure 6).

On day 14 and 21, the mean blood glucose concentration was significantly ($p<0.001$) higher in Group 2 and 3 compared to Group 1 respectively (Figure 6).

The mean change in fasting blood glucose after induction of DM is as shown in Figure 7. This was higher ($p<0.001$) in Group 2-4 against Group 1. Also shown in Figure 7 is the mean change in fasting blood glucose after 21 days of treatment. This was lower ($p<0.001$) in Group 2-4 against Group 1. It was also lower ($p<0.05$) in Group 3 and ($p<0.001$) Group 4 against Group 2.

Serum GSH concentration of Group 2 and 4 was lower ($p<0.001$) compared to Group1, while Group 3 was not significantly reduced (Figure 8). GSH was higher in Group 3 and 4 ($p<0.01$) compared to Group 1, and was significantly higher in Group 3 compared to Group 4 (Figure 8).

There was no significant difference in CAT concentrations among the groups (Figure 9).

SOD concentration was lower in Group 2 ($p<0.001$) but higher in Group 4 ($p<0.05$) against Group 1. It was significantly ($p<0.001$) higher in Group 3 and 4 against Group 2 (Figure 10).

MDA concentration was significantly higher in Group 2 ($p<0.001$) compared to Group 1. It was lower in Group 3 ($p<0.001$) and Group 4 ($p<0.01$) compared to Group 2 (Figure 11).

TAC concentration was significantly lower in Group 2 ($p<0.001$) against Group 1. It was significantly higher in Group 1, 3 ($p<0.001$) and Group 4 ($p<0.01$) against Group 2 (Figure 12).

GPx concentration was significantly lower in Group 2 ($p<0.001$) compared to Group 1. It was significantly higher in Group 3 ($p<0.001$) and Group 4 ($p<0.001$) compared to Group 2. GPx was also significantly higher in Group 3 ($p<0.001$) compared to Group 4 (Figure 13).

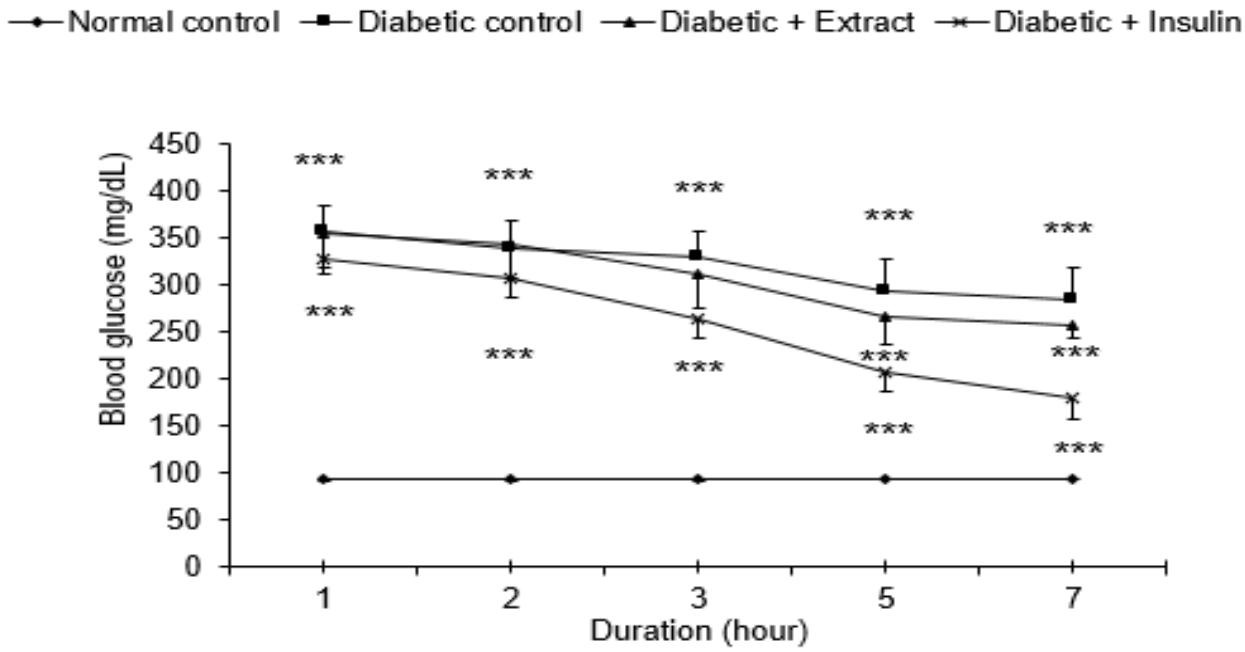

Figure 5: Serial blood glucose concentration of different experimental groups recorded after induction of diabetes

Values are expressed as mean +SEM, n = 6.
*** = p<0.001 vs normal control

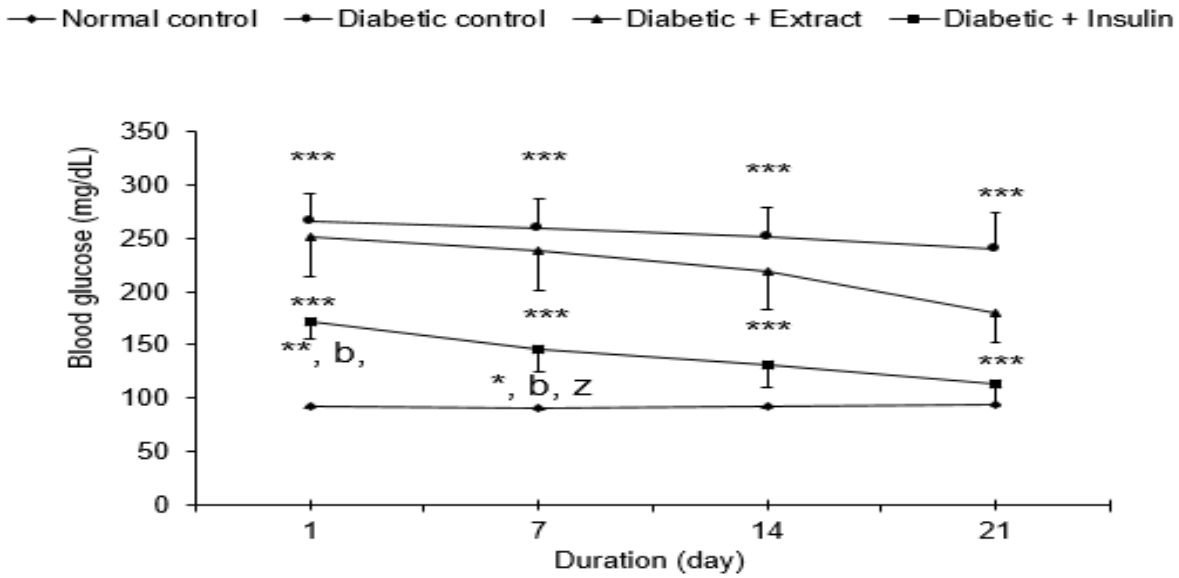

Figure 6: Daily blood glucose concentration of different experimental groups recorded after induction of diabetes

Values are expressed as mean +SEM, n = 6.
* = p<0.05, ** = p<0.01, *** = p<0.001 vs normal control
b = p<0.01 vs diabetic control
z = p<0.001 vs diabetic + extract

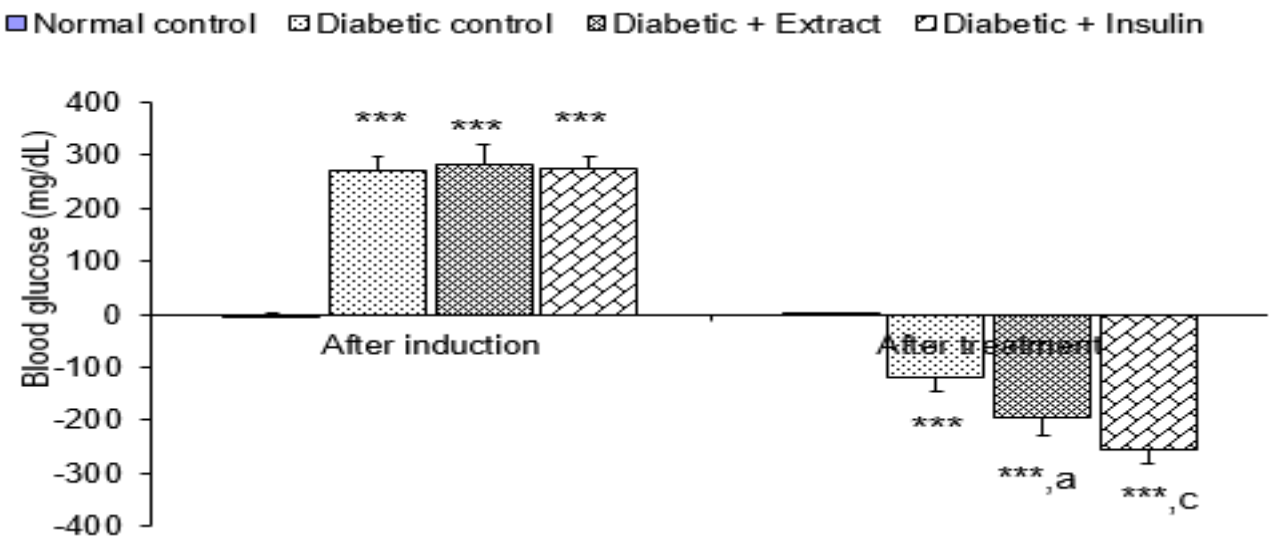

Figure 7: Change in fasting blood glucose levels of different experimental groups after induction of diabetes and treatment with extract

Values are expressed as mean +SEM, n = 6.
*** = p<0.001 vs normal control
a = p<0.05, c = p<0.001 vs diabetic control

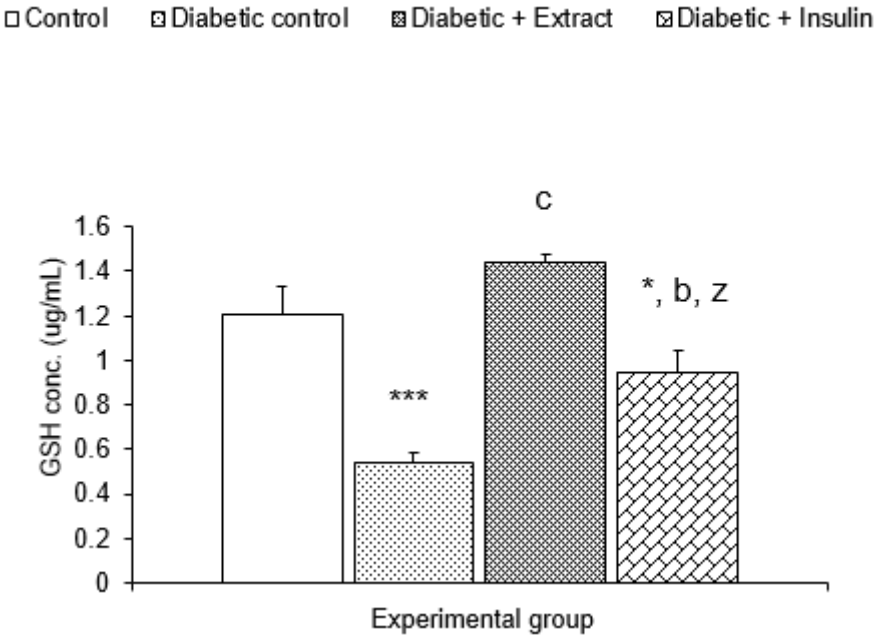

Figure 8: Reduced glutathione concentration in the different experimental groups

Values are expressed as mean +SEM, n = 6.
* = p<0.05, *** = p<0.001 vs normal control
b = p<0.01, c = p<0.001 vs diabetic control
z = p<0.001 vs diabetic + extract

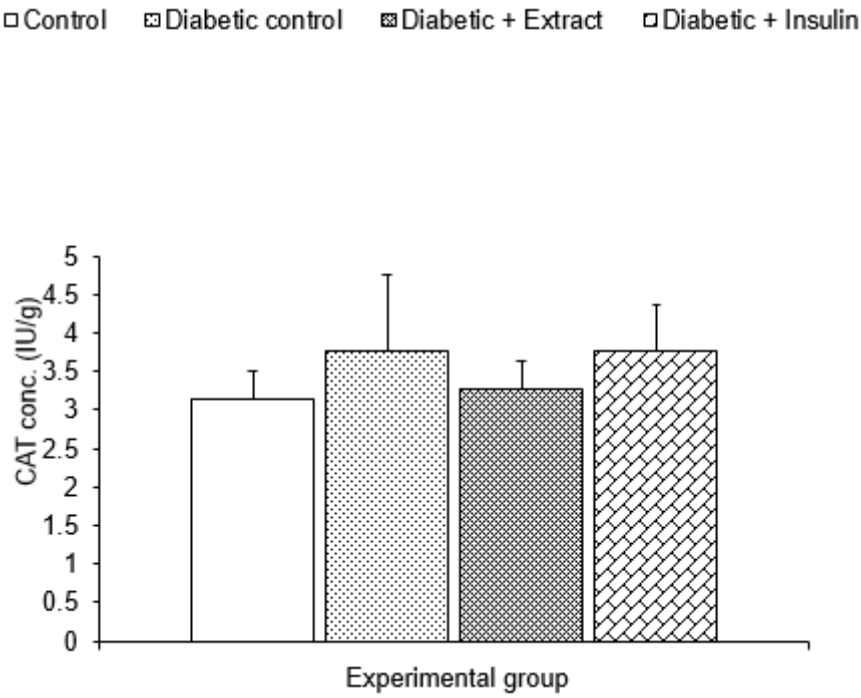

Figure 9: Catalase concentration in the different experimental groups

Values are expressed as mean +SEM, n = 6.
No significant difference among groups

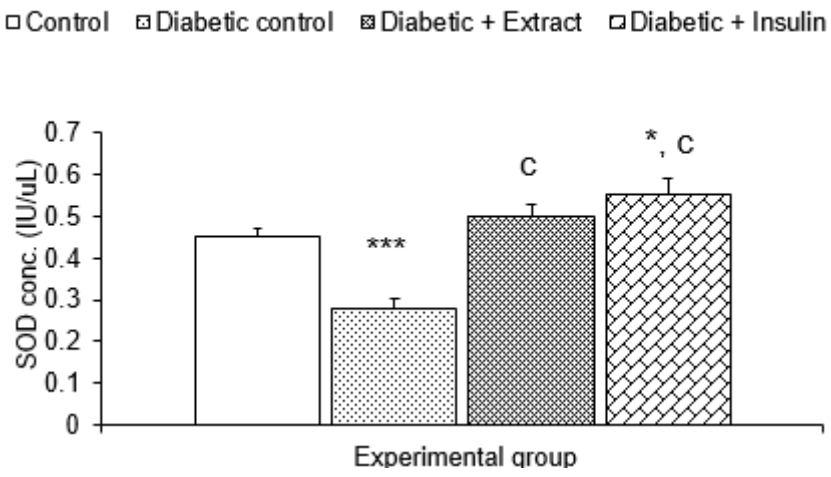

Figure 10: Superoxide dismutase concentration in different experimental groups

Values are expressed as mean +SEM, n = 6.
* = $p<0.05$, *** = $p<0.001$ vs normal control
c = $p<0.001$ vs diabetic control

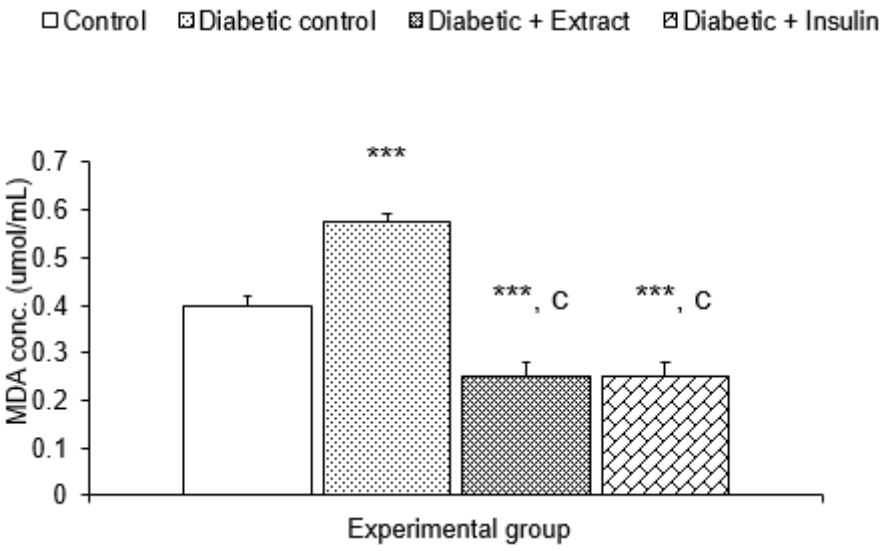

Figure 11: Malondialdehyde concentration in different experimental groups

Values are expressed as mean +SEM, n = 6.
*** = p<0.001 vs normal control
c = p<0.001 vs diabetic control

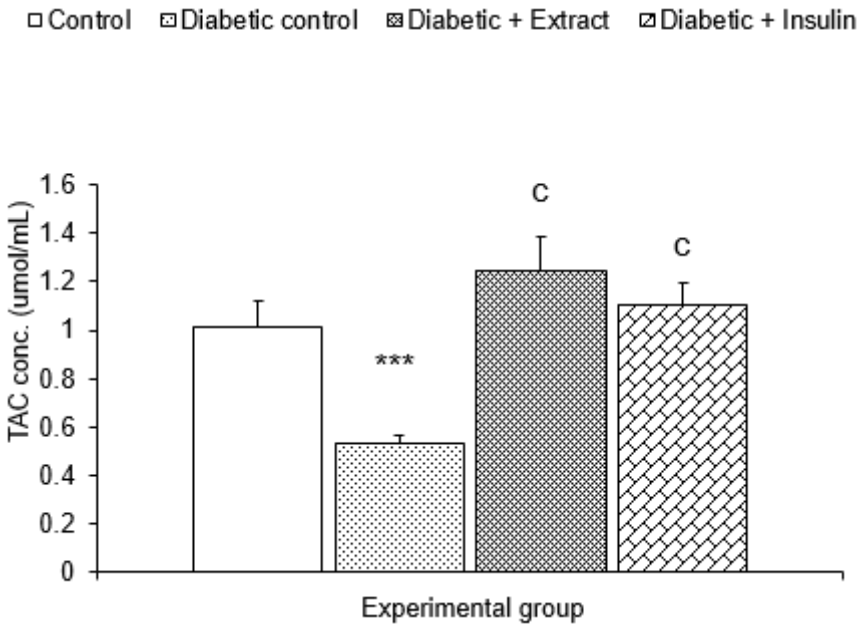

Figure 12: Total antioxidant capacity concentration in different groups

Values are expressed as mean +SEM, n = 6.
*** = p<0.001 vs normal control
c = p<0.001 vs diabetic control

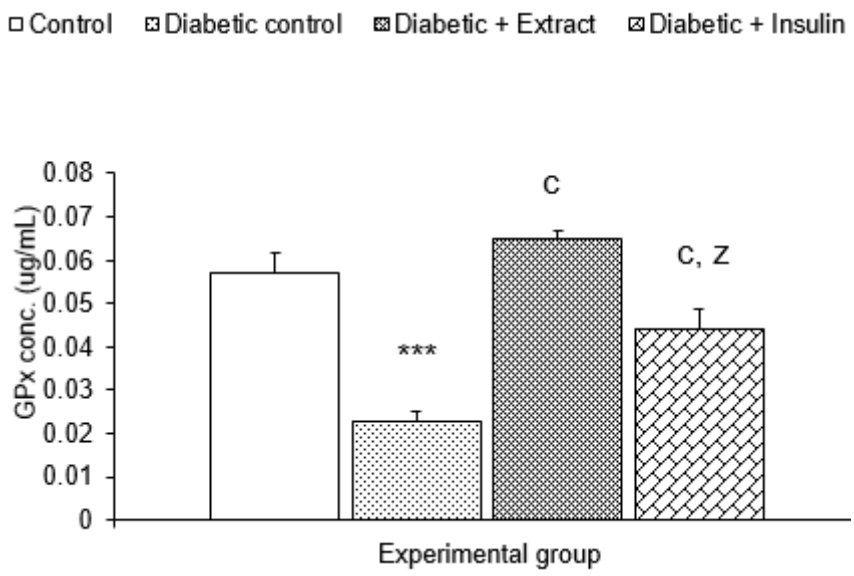

Figure 13: Glutathione peroxidase concentration in different experimental groups

Values are expressed as mean +SEM, n = 6.
*** = p<0.001 vs normal control
c = p<0.001 vs diabetic control
z = p<0.001 vs diabetic + extract

DISCUSSION

DM is a metabolic disorder associated with prolonged hyperglycemia with loss in body weight (Asagba et al., 2019) and may directly or indirectly contribute to oxidative stress (Obasi, 2019). Excessive oxidative stress has been implicated in the pathology and complications of DM (Ugwu *et al*., 2019). This study was designed to evaluate the effect of ALEJT in comparison to insulin on STZ-diabetic Wistar rats.

The results from this study showed a drastic reduction in initial versus final body weight in the diabetes control and insulin treated rats, but there was no variation in the ALEJT treated rats, signifying protection against weight loss. This is contrary to the findings of Ukubuiwe *et al*., (2019) which showed a final increase in weight following the administration of *J. tanjorensis*. There was a significant loss in body weight change in diabetic rats untreated and treated compared to control. Similar loss in weight has been observed in STZ-diabetic rats (Asagba *et al*., 2019). These reductions in body weight simply mean that catabolism has persisted. The destruction of β-cells and disorder of insulin secretion in STZ-induced diabetic rat causes physio-metabolic abnormalities resulting in tissue protein

wasting due to unavailability of carbohydrate as an energy source (Al-Attar and Alsalmi, 2019).

The study showed an elevation in both change in fasting blood glucose levels and blood glucose concentration after 1, 2, 3, 5 and 7 hours and after 1, 7, 14 and 21 days of induction of DM using STZ in the treated and untreated diabetic rats compared to control. The elevation is due to the hyperglycemic activity triggered by STZ administration. This observation is similar to a study by Szkudelski, (2012) which reported the cellular toxicity and local immune responses, triggered by STZ in the pancreatic β cell, consequently resulting into hypoinsulinemia and hyperglycemia in animals. The study also showed a decrease in both change in fasting blood glucose level and blood glucose concentration after 21 days of treatment with ALEJT and insulin compared to diabetic control. This explains the anti-diabetic action of ALEJT and the blood glucose regulating function of insulin. These observations are similar to the findings by Ukubuiwe *et al*., (2019) who reported on the hypoglycemic potential of ALEJT; and by Diabetic.co.uk (2019) on the role of insulin in controlling how the body uses and stores blood glucose.

A proven pathway in the pathogenesis of diabetic complications is oxidative stress (Giacco and Brownlee, 2010). There has been increased interest in oxidative stress and its role in the development of complications of diabetes mellitus (Asmat *et al*., 2016). This study showed that STZ induced oxidative stress which is confirmed by the decrease in serum GSH, SOD, TAC and GPx levels, and an increased in MDA level. A dysfunction of the beta cell in diabetes causes disruption of insulin secretion which create an imbalance between oxidant and antioxidant systems characterized by excessive production of reactive oxygen species (ROS) and a reduction in enzymatic (SOD and GPx) and non-enzymatic (GSH and TAC) antioxidant systems, resulting in the appearance of exaggerated oxidative status characterized by macromolecular damage due to oxidative stress.

The enzymes (SOD, CAT and GPx) are responsible for the breakdown of the highly ROS to less reactive ones. The levels of SOD and GPx were significantly higher in ALEJT and insulin treated group compared to the diabetic control. SOD plays a significant role in catalyzing the breakdown of highly reactive O_2^- to less reactive hydrogen peroxide (H_2O_2) and oxygen (Dasuri *et al*., 2013), while GPx catalyses the reduction of H_2O_2 and lipid peroxides utilizing GSH as an electron donor (Gandghi and Abramov, 2012; Dasuri *et al*., 2013). In this study the levels of GPx was higher in ALEJT compared to insulin indicating the protective effect of ALEJT against oxidative stress.

Catalase is responsible for the conversion of H_2O_2 to water and oxygen using either iron or manganese as a cofactor (Gandghi and Abramov, 2012; Dasuri *et al*., 2013). This study showed no variation in catalase levels among the groups, but ALEJT showed a recovery trend from diabetic control and restoration of CAT concentration towards normal control level.

GSH antioxidant role is in two phases; first, GSH reacts with ROS such as O^{2-} and OH^- non-enzymatically to aid their removal (Dringen *et al*., 2000; Gandghi and Abramov, 2012). Secondly, GSH reaction is the electron donor for the reduction of peroxides in the GPx reaction (Dringen *et al*., 2000). The results from this study showed that GSH concentration was significantly lower in the diabetic control compared to that of normal control. Treatment with ALEJT and insulin significantly increased the level of GSH, and GSH level was significantly higher in ALEJT group compared to insulin treated group. This signifies the importance of the extract protecting against ROS injuries.

The results from this study showed that MDA concentration was significantly lower in the ALEJT and insulin treated groups compared to normal control and diabetic control. The reduced production of MDA could also be a result of the increased antioxidant elements such as GSH and SOD, scavenging excess ROS before excessive oxidation takes place (Yuan *et al*., 2007).

In this study, the TAC concentration was significantly higher in the alkaloid extract and insulin treated group compared to diabetic control group. Results from this study showed that *J. tanjorensis* is rich in antioxidant property and was effective in fighting against ROS following STZ-induced diabetes in rats. One of the possible mechanism by which the extract alleviates the toxic effect of STZ-induced DM could be due to it rich phyto-constituents and antioxidant properties. Ukoh *et al.,* (2024) reported the rich phyto-constituents of *Jatropha tanjorensis* such as alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinones and saponins and Ugwu *et al*., (2013) reported that flavonoids are known for protecting against free radicals attack

CONCLUSION

Administration of 500mg/kg of ALEJT and 2 IU/kg of insulin alleviated the observed oxidative stress in diabetic rats by reversing the antioxidant activities in STZ-induced DM. In this study ALEJT was more effective in protecting against weight loss and reversal of the levels of GPx and GSH compared to insulin. Therefore, ALEJT could serve

as a potential natural and safe remedy for the management of DM and/or delay in DM complications.

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Competing Interests

The authors declare that no competing interests exist.

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