

Antidiabetic and Antioxidant Effects of Methanolic Extracts of Leaf and Seed of *Tetracarpidium conophorum* on Alloxan-Induced Diabetic Wistar Rats

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ABSTRACT

Methanolic extracts of *Tetracarpidium conophorum* (TCME) leaf, seed or leaf + seed extract demonstrated high capacity for reversing hyperglycemia and oxidative stress comparable with the standard anti-diabetic drug, metformin, in alloxan-induced diabetic rats. The leaf + seed extracts showed the best activity among the extracts and also ameliorated oxidative stress-induced damage of pancreas and liver tissue to normal state.

1. INTRODUCTION

Diabetes mellitus is one of the most common metabolic disorders world-wide. It is a syndrome of chronic hyperglycemia due to relative insulin deficiency, resistance, or both [1]. Prevalence rates of diabetes mellitus have been on the increase throughout the world to an extent that the condition is considered to have reached an epidemic proportion in many countries.

In the treatment of diabetes, many oral hypoglycemic agents like sulfonylureas, meglitinides, thiazolidines, D-phenylalanine and α -glucosidase inhibitors are used in addition to insulin treatment action, along with appropriate diet and exercise [2]. However, none can be termed as ideal, due to their toxic side effects and sometimes diminution in response after prolonged use [3]. The limitations and side effects associated with existing synthetic oral hypoglycemic agents had necessitated the search for newer drugs. As a result, natural agents from plants and plant products have been the alternative target to source for new antioxidant and antidiabetic agents based on their traditional use.

Tetracarpidium conophorum (African walnut) (Euphorbiaceae) is a climbing shrub which is commonly cultivated in Nigeria, principally for the seeds (nuts), which are cooked and consumed as snacks. Aqueous extracts of the seeds, (but not the leaves) of this plant contain high levels of glycoprotein agglutinins which have been isolated, purified and characterized [4, 5]. However, preliminary studies have indicated that methanolic extracts of the leaves contain compounds with antioxidant [6, 7], as well as anti-

glycemic [8] activities. It was suggested [9] that the beneficial effects of antioxidants in diabetes are to provide protection to pancreatic β -cells against glucose toxicity.

This study was designed to evaluate the efficacy of methanolic extracts of *Tetracarpidium conophorum* seeds and leaves in reversing the hyperglycaemia and oxidative stress in alloxan induced diabetic rats, compared to a standard anti-diabetic drug, as well as to assess the effects of the extracts on the morphology of diabetic pancreatic Beta-islet and hepatic cells.

2. MATERIALS AND METHODS

2.1. Source of Plant Materials

The leaves of *Tetracarpidium conophorum* were harvested from a farm in Ijebu-ode, Ogun State, Nigeria, in March, 2015. Fresh seeds of the plant were also purchased from the market at the same time. The plant was identified and authenticated by Prof. E.B. Esan; a botanist in the Department of Bioscience and Biotechnology, Babcock University, Ilishan-Remo, Ogun state, Nigeria.

2.2. Preparation of *Tetracarpidium conophorum* Plant Extracts

The leaves and seeds were oven dried at 40°C and ground separately into powder form using a mechanical blender. The seed powder was defatted with petroleum ether at room temperature. The leaves and seeds underwent a cold extraction process using a 70% methanol solvent and a Soxhlet extractor. The extracts were then concentrated *in vacuo* with a rotary evaporator. The concentrated extracts were dried in water bath at 40°C and the dried extracts were reconstituted in normal saline for oral administration.

2.3. Experimental Animals

Forty-nine albino rats (wistar strains), weighing between 120 - 200 g were purchased from the Animal house, Babcock University, Ilishan-Remo, Ogun State and distributed into six treatment groups of seven rats each, as follows:

Group 1	Normal rats (No diabetes induction, no treatment).
Group 2	Diabetic rats but untreated
Group 3	Diabetic Rats + Tabs Metformin 25 mg/kg body weight
Group 4	Diabetic rats + <i>T. conophorum</i> leaf extract only (250 mg/Kg body weight)
Group 5	Diabetic rats + <i>T. conophorum</i> seed extract only (250 mg/kg body weight)
Group 6	Diabetic rats + <i>T. conophorum</i> leaf + seed extract (125 + 125 mg/Kg body weight)

The wistar rats (with the exception of the normal group) were subjected to a 12 h fast and induced with diabetes by intra-peritoneal injection of freshly prepared solution of 150 mg/kg body weight alloxan monohydrate (Sigma-Aldrich, USA) reconstituted in normal saline. They were kept in polypropylene cages (47 × 34 × 20 cm) lined with wood shaving, in a room with temperature regulated at 25°C ± 1°C. A 12 hr alternating light and dark cycle was maintained. The rats were fed on a standard pellet diet and water ad libitum. The wistar rats (with an exception of the normal, control and standard groups) were given *T. conophorum* leaf and seed extracts orally for a 4-week period.

2.4. Sacrificing of Animals, Collection of Blood and Harvesting of Organs

A weekly tail bleeding was done to monitor glucose levels with a glucometer throughout the 4-week period. At the end of the experiment, blood was collected from the rats by cardiac puncture under anaesthesia. Blood samples were collected into plain bottles and centrifuged to obtain serum for lipid profile and serum liver enzymes assay.

The liver tissue from various groups of animals was removed carefully and washed thoroughly with

ice cold saline. The wet liver tissue was weighed (0.5 g) and homogenized in 0.1 M Tris-HCl buffer, pH 7.4 at 4°C. The homogenate was centrifuged at 2500 rpm for 10 min at 4°C using a refrigerated centrifuge. The supernatant was used for the assay of various lipid peroxidation products such as malondialdehyde (MDA) and glutathione peroxidase (GSH-Px).

Pancreatic tissues were excised and weighed after the fat and lymph nodes had been removed. The splenic parts of the pancreas of each rat were fixed in aqueous Bouin's solution, and embedded in paraffin. Each pancreatic block was sectioned (5 µ) throughout its length to avoid any bias due to changes in islet distribution or cell composition, and thereafter mounted on slides.

2.5. Enzyme Assays

Optimum conditions were applied to all enzyme assay experiments, as follows: The wet liver tissue obtained immediately after the rats were sacrificed were placed in 0.25 M sucrose, 10mM Tris (pH 8) with 1mM EDTA buffer solution and refrigerated at a temperature of about 4°C. All solutions used were ice cold. The pestle and mortar used for the homogenization was pre-cooled in a freezer to prevent unwanted heat generation whilst homogenizing. Each liver tissue was finely minced before homogenization and the strokes of the pestle were slowly done to prevent denaturation. For Glutathione levels, the tissue homogenate was de-proteinized with 5 sulfosalicylic acid.

Assay of alanine aminotransferase (ALT) and aspartate aminotransferase were carried out using the procedure provided by the RANDOX KIT manufacturers (Crumlin, UK), according to the principle described by [10].

2.6. Antioxidant (*in Vivo*) Assays

2.6.1. Determination of Malondialdehyde (MDA)

Liver tissues were homogenized in ice cold 1.5% KCl to make a 10% homogenate. Three milliliters of 1% phosphoric acid and 1 ml of 0.6% thiobarbituric acid (TBA) aqueous solution were added to 0.5 ml of 10% homogenate. The mixture was heated for 45 min and after cooling; 4 ml of n-butanol was added and mixed. Absorbance of butanol phase was measured at 535 and 520 nm. The difference of the two measurements was used as the MDA value (µmol/g tissue) [11].

2.6.2. Determination of Activities of Glutathione Peroxidase (GSH-Px)

Liver homogenates (5% w/v) were prepared in cold 50 mM potassium phosphate buffer (pH 7.4). The unbroken cells and cell debris were removed by centrifugation at 1000 rpm for 10 mins using a refrigerated centrifuge. The supernatant was used for the estimation of Glutathione peroxidase (GSH Px), according to standard methods [12].

2.7. Lipid Profile

2.7.1. Estimation of Total Cholesterol (TC) (CHOD-PAP-Phosphotungstate Method) [13]

Cholesterol in serum was estimated by CHOD-PAP method using an Enzymatic Diagnostic Kit from Randox (Crumlin, UK). The absorbance of the sample and of the standard was measured against the reagent blank value at 546 nm.

$$\text{Total Cholesterol (mg/dl)} = \frac{\text{Absorbance of Total Cholesterol}}{\text{Absorbance of Standard}} \times 200$$

2.7.2. Estimation of Triglycerides [CHOD-PAP Phosphotungstate Method] [13]

In vitro quantitative determination of triglyceride (neutral fat) concentration in serum was done by using a diagnostic kit from Randox.

The absorbance of the test and standard was read against blank at 546 nm.

$$\text{Triglycerides} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 200$$

2.7.3. Estimation of HDL Cholesterol (CHOD-PAP-Phosphotungstate Method) [13]

In vitro quantitative determination of the activity of HDL-Cholesterol in serum was estimated by CHOD-PAP method using an Enzymatic Diagnostic Kit from Randox.

2.7.4. LDL Cholesterol [14]

VLDL Cholesterol was estimated by the standard formula and expressed as mg/dl, as follows.

$$\text{VLDL} = \frac{\text{Triglyceride}}{5} \quad [14].$$

LDL cholesterol = Total cholesterol – (HDL cholesterol + VLDL cholesterol).

2.8. Histological Procedures

Pancreatic and liver tissues were excised from sacrificed animals, weighed, and fixed in aqueous Bouin's solution for 48 h and were sequentially embedded in paraffin wax blocks according to the standard procedure, and sectioned at 5 μ thickness. They were further de-paraffined with xylol, and histologic observations were performed after staining for functional pancreatic tissues by Aldehyde fuchsin trichrome method described by Steven [15]. The slides were examined using light microscopy.

2.9. Statistical Analysis

Statistical analyses were performed using both descriptive and inferential statistics using Graph prism pad software. Results were expressed as mean $\pm$ Standard Deviation. *P* values of <0.05 was used to analyze the significance level.

3. RESULTS

3.1. Serum Glucose Levels

The mean serum glucose concentrations of groups of rats are presented in **Figure 1** and **Figure 2**. Normal (non-diabetic) and diabetic control groups recorded glucose concentrations below 100 and over 338 ± 5.692 (mg/dl.) respectively ($t = 31.74$, $p = 0.0001$). However, a progression towards the 28th day recorded significant reduction in the glycemic index of the rats in the groups treated with the standard anti-diabetic drug, metformin (89.75 ± 4.151 mg/dl), leaf + seed extracts (108.8 ± 3.652), and Leaf only extract-treated (110 ± 2.1) (all these values fall within internationally accepted range of normal sugar levels for non-diabetics). The mean serum glucose concentrations for the seed extract-treated group (182.3 ± 6.22 mg/dl) was in the diabetic range, but lower than in the untreated diabetic group, 338 ± 5.692 (mg/dl). When analyzing for the efficacy of TCME to salvage alloxan-Induced diabetes, the "seed only" extract was ineffective, whereas the leaf only and leaf + seed extracts were effective. Reactions to treatment in all the groups seemed to reach equilibrium at 21 days, since glucose levels did not show significant changes between day 21 and 28 (**Figure 1**). However, there was no significant difference between the mean sugar levels of the normal and metformin-treated group ($t = 1.13$, $p = 0.29$). Leaf + seed treatment was the most effective hypoglycaemic agent among the TCME on the diabetic rats, and also compared favourably with metformin. The purification or isolation of the active anti-diabetic component in the Leaf + seed extracts of TCME can further lower the required doses.

3.2. Serum Metabolic Enzymes

The concentrations of AST, ALT, and ALP followed the same pattern in all groups. Generally, leaf + seed extract-treated group demonstrated similar but slightly lower enzyme levels than the metformin-treated diabetic group.

3.2.1. Serum Aspartate Aminotransferase (AST)

AST concentration was highest in the untreated diabetic rat group, reaching above 100 IU/L, and

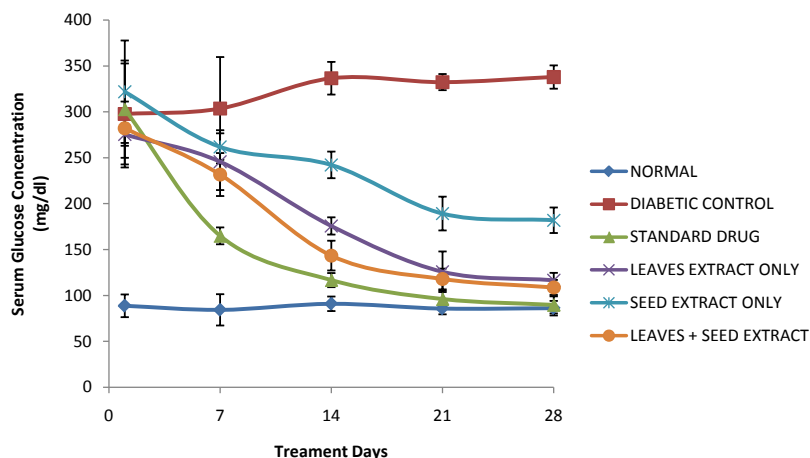


Figure 1. Serum glucose concentrations in different treatment groups of rats. Values are expressed as mean $\pm$ standard deviation. Values are expressed as mean $\pm$ standard deviation of each group values. Standard deviation was calculated using the Graph prism pad software. The data was feed into the software to obtain the S.D. Normal refers to the non-diabetic and untreated group, Control is the alloxan-induced but untreated diabetic group, Standard is the diabetic group treated with the standard drug, metformin, Leaf only is the diabetic group treated with the leaf extract of TCME (250 mg/Kg body weight), Seed only is the diabetic group treated with the seed extract of TCME (250 mg/Kg body weight), Leaf + Seed is the diabetic group treated with the seed extract of TCME (125 + 125 mg/Kg body weight).

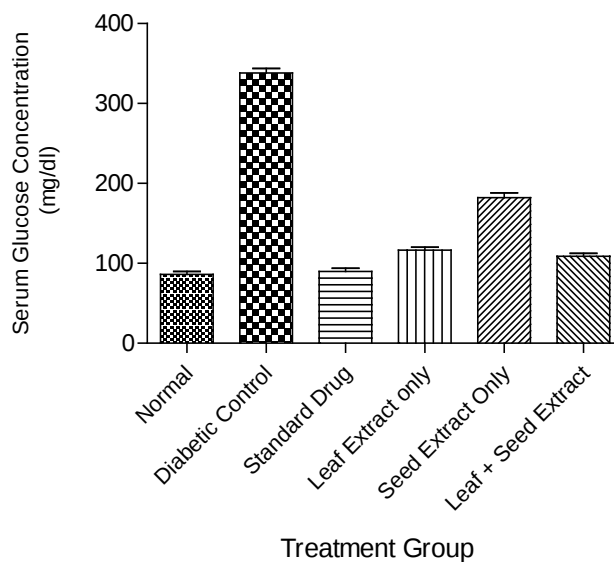


Figure 2. Mean ($\pm$ SD) of Serum Glucose Concentrations in Experimental Groups of Rats at 28 days of Treatment. Values are expressed as mean $\pm$ standard deviation of each group values. Normal refers to the non-diabetic and untreated group, Control is the alloxan-induced but untreated diabetic group, Standard is the diabetic group treated with the standard drug, metformin, Leaf only is the diabetic group treated with the leaf extract of TCME (250 mg/Kg body weight), Seed only is the diabetic group treated with the seed extract of TCME (250 mg/Kg body weight), Leaf + Seed is the diabetic group treated with the seed extract of TCME (125 + 125 mg/Kg body weight).

lowest in the normal non-diabetic group (53 IU/L). Treatment of diabetic rats with metformin, leaf, or leaf + seed extract of TCME reduced the concentrations of AST to comparatively near-normal values, whereas in the seed extract group AST activities was almost as high as in the untreated diabetic group (Figure 3).

3.2.2. Serum Alanine Aminotransferase Levels (ALT)

The results of Serum Alanine aminotransferase concentrations (Figure 4) showed the same pattern as

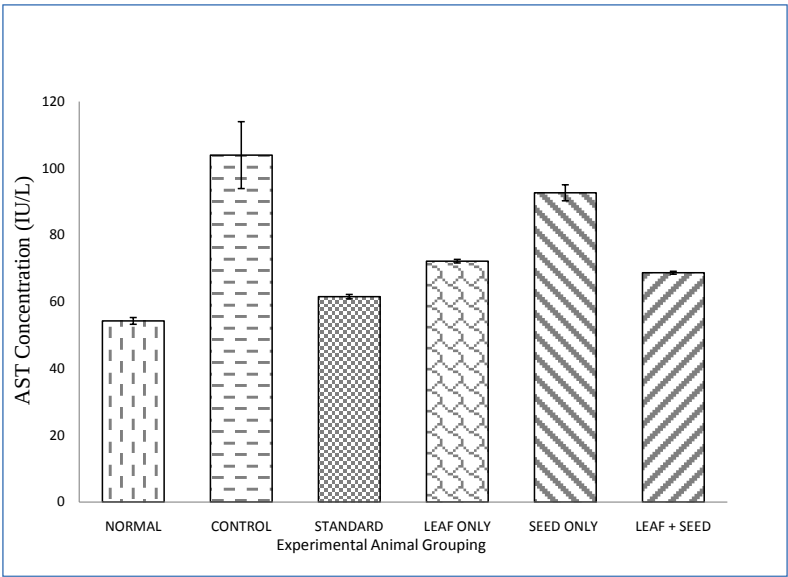


Figure 3. Serum Aspartate Aminotransferase (AST) Activity (IU/L). Values are expressed as Mean $\pm$ Standard deviation.

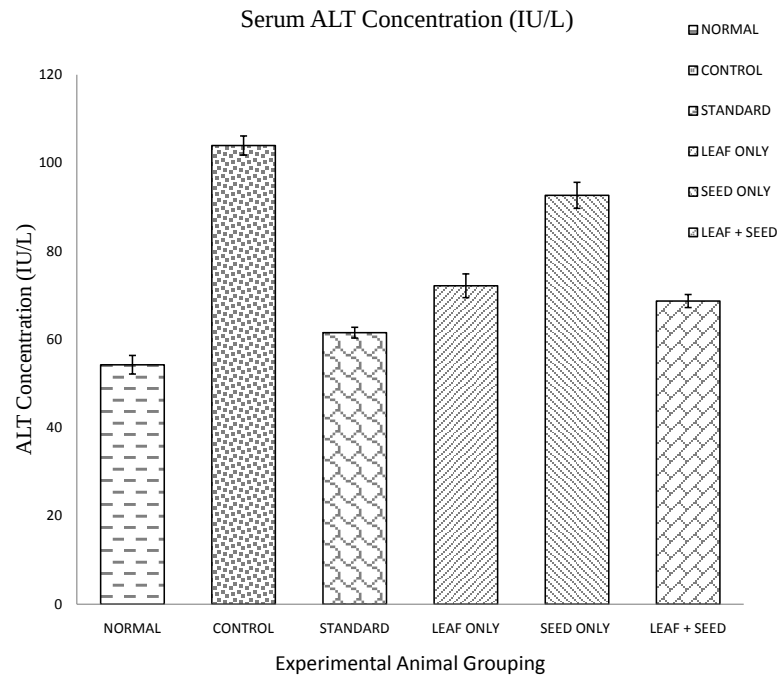


Figure 4. Serum Alanine Aminotransferase (ALT) Concentrations IU/L). Values are expressed as Mean $\pm$ Standard.

AST. Mean ALT concentrations were highest in the untreated diabetic control group almost double the concentration of the normal non-diabetic group, but reduced to near normal by metformin, and almost to metformin level by Leaf + seed extract of TCME (Figure 4).

3.2.3. Serum Alkaline Phosphatase Levels (ALP)

Serum alkaline phosphatase levels showed similar patterns as AST and ALT. The mean concentrations of this serum enzyme were very similar in the normal non-diabetic, metformin-treated diabetic, leaf-only extract-treated and leaf + seed-treated diabetic groups, whereas the seed-only extract treated group showed only minimal reduction of the enzyme concentrations (Figure 5).

3.3. Antioxidant Studies

These studies show that seed extract of TCME is very ineffective in controlling the antioxidant status of alloxan induced diabetes, but leaf + seed extract of TCME completely restored antioxidant function.

3.3.1. Malondialdehyde (MDA)

MDA concentration of untreated group of diabetic rats (diabetic control) was very high, about three times higher than the values recorded for non-diabetic rats. Treatment of diabetic rat groups with standard anti-diabetic drug metformin reduced the concentration of MDA below normal, but leaf + seed extracts of TCME brought the elevated levels down to normal values (Figure 6), while leaf extract group was slightly higher. The seed only extract did not record any significant difference when compared with the untreated diabetic control group.

3.3.2. Glutathione (GSH) Levels

Glutathione antioxidant concentration increased when MDA decreased, and vice versa. Hence, normal non-diabetic rat showed the highest concentrations of GSH. Treated diabetic rat groups with either the metformin, or leaf extract or leaf + seed extracts of TCME demonstrated similar ability to raise GSH levels to the same concentration found in non-diabetic rats (Figure 7), while the seed extract of TCME

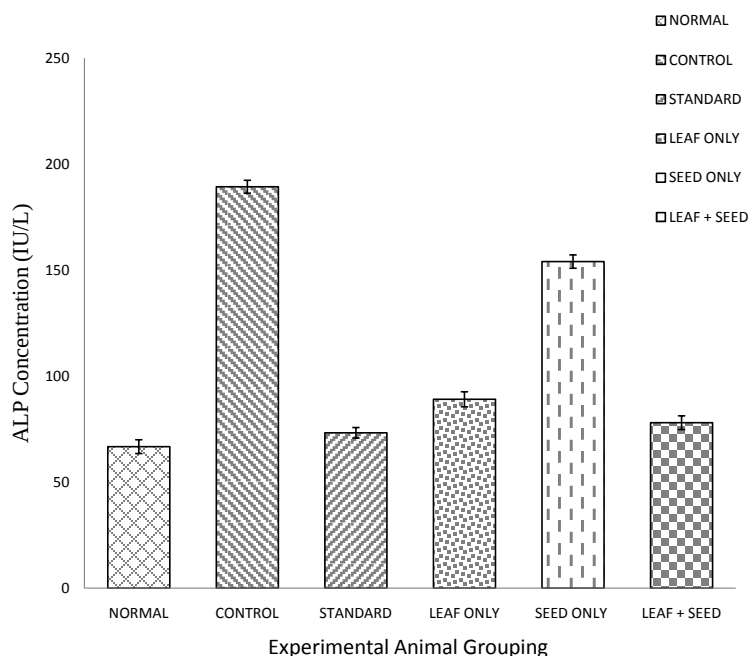


Figure 5. Serum Alkaline Phosphatase (ALP) Activity (UI/L). Values are expressed as Mean $\pm$ Standard deviation.

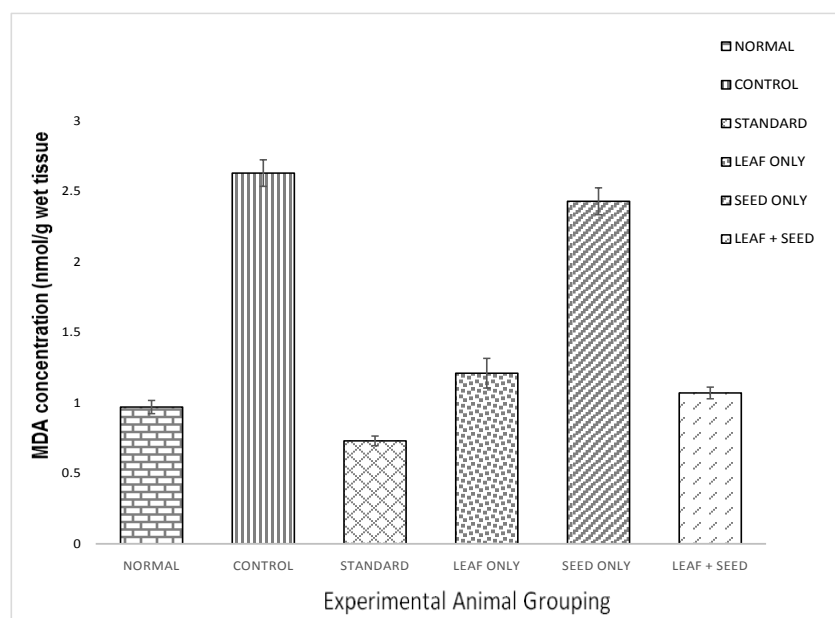


Figure 6. Malondialdehyde concentrations in the liver (nmol/g wet tissue) Values are expressed as Mean $\pm$ Standard Deviation.

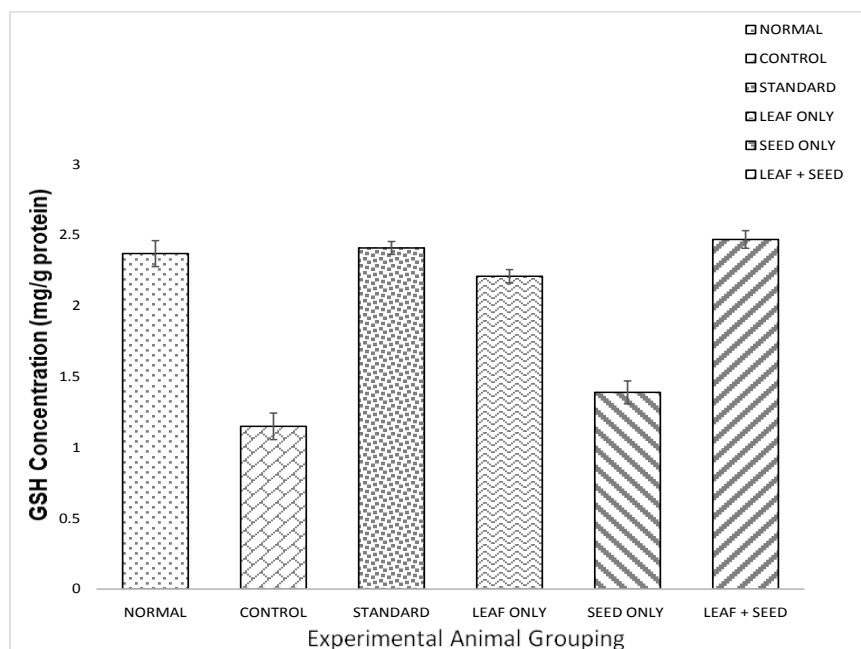


Figure 7. Reduced Glutathione (GSH) Concentration (mg/g protein) in the liver. Values are expressed as Mean $\pm$ Standard Deviation.

showed very little difference from the untreated diabetic group.

3.4. Lipid Profile

3.4.1. Total Cholesterol

Total cholesterol concentration was lowest in the non-diabetic rat group (88 ± 12 mg/dl) and highest

in the untreated diabetic group (138 ± 12 mg/dl). Leaf +seed (105 ± 12 mg/dl and leaf extract (110 ± 8 mg/dl) of TCME showed greater efficiency at reducing total cholesterol than seed extract (120 ± 10 mg/dl) and metformin (128 ± 6 mg/dl) (**Figure 8**).

3.4.2. Serum Tri-Glyceraldehyde

Serum Tri-glyceraldehyde concentration was highest in untreated diabetes group, but lowest in the non-diabetic group. Leaf + seed extract of TCME was the most efficient treatment in lowering the concentration of triglyceraldehyde, while leaf extract alone and metformin treatment were both minimally effective, and seed extract had only negligible lowering effect (**Figure 9**).

3.4.3. High Density Lipoprotein (HDL) Cholesterol

HDL-cholesterol concentrations was highest in the non-diabetic normal rat group and also in the leaf+seed extract (TCME)-treated group. Metformin and Leaf extract-treated groups recorded relatively lower concentrations while seed extract was only a little higher than in the untreated diabetic group (**Figure 10**).

3.4.4. Serum Low Density Lipoprotein (LDL) Cholesterol

The concentration of serum low density cholesterol was very high in the untreated diabetic rat group (73 ± 23 mg/dl), compared with the normal non-diabetic rat group (18 ± 5 mg/dl). TCME-Leaf + seed extract treated group had the lowest LDL concentration next to normal (23 ± 15 mg/dl), which was not significantly different from normal values. Metformin-treated (33 ± 4 mg/dl) and leaf extract TCME-treated rats (43 ± 23 mg/dl) also showed less efficient reductions in LDL concentrations, but the seed extract-treated rats (54 ± 10 mg/dl) were the least sensitive to LDL control (**Figure 11**).

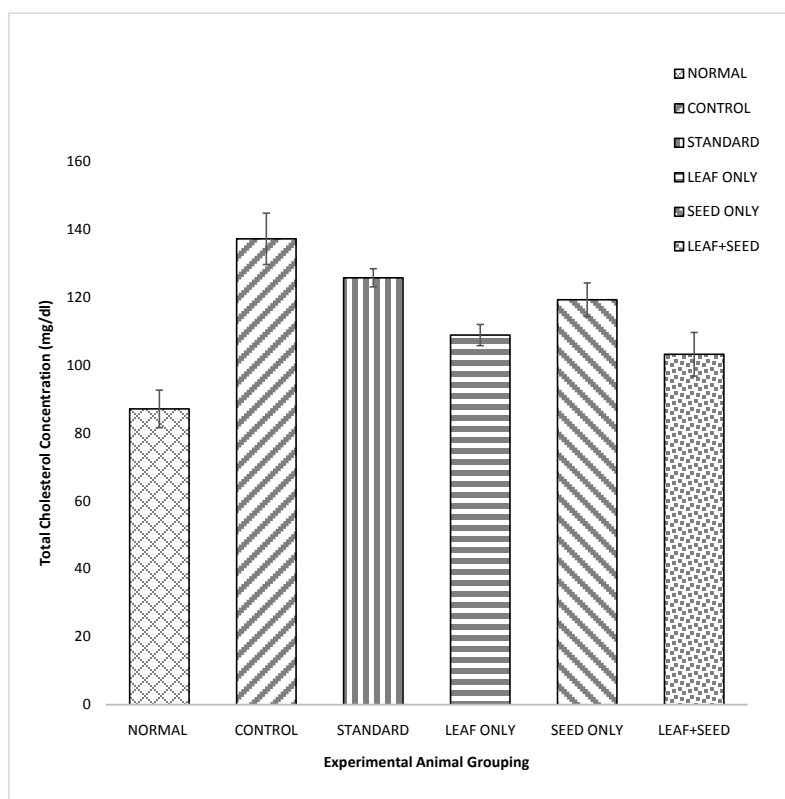


Figure 8. Serum Total Cholesterol Concentrations (mg/dl). Values are expressed as Mean $\pm$ Standard Deviation.

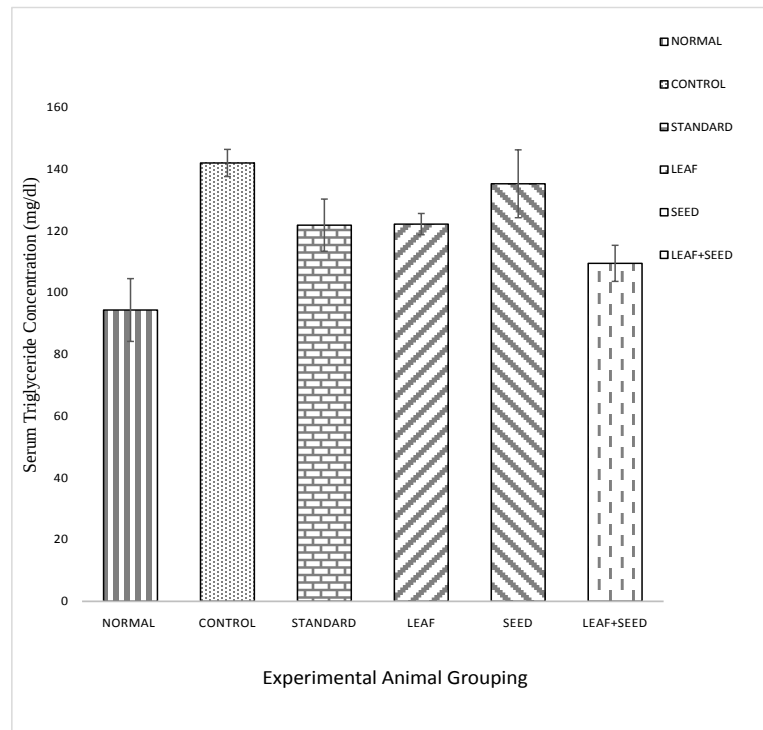


Figure 9. Serum Triglyceride Concentration (mg/dl). Values are expressed as Mean $\pm$ Standard.

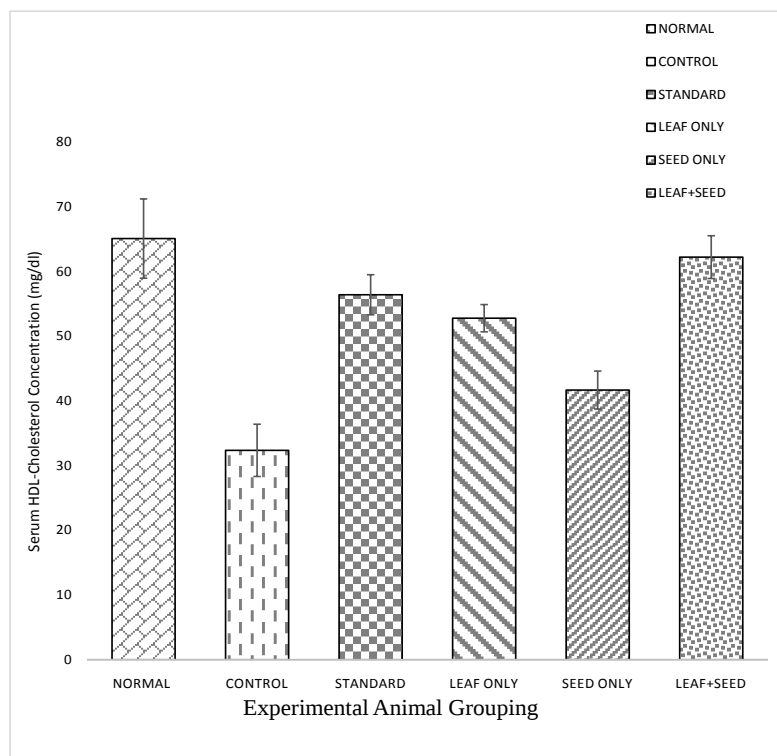


Figure 10. Serum HDL-Cholesterol Concentration (mg/dl). Values are expressed as Mean $\pm$ Standard Deviation.

3.5. Histological Studies

3.5.1. Histological Studies of the Pancreas of Alloxan-Induced Diabetic Rats

The histology of the pancreatic tissue of the “Normal” rat group showed normal pancreatic acini and beta cells (Figure 12). The diabetic control group showed enlarged islets of Langerhan with distortion and loss of beta cells (Figure 13). This is as a result of the overwhelming oxidative stress on the pancreas. The standard drug, metformin-treated group showed normal pancreatic acini with partially diffused nuclei of

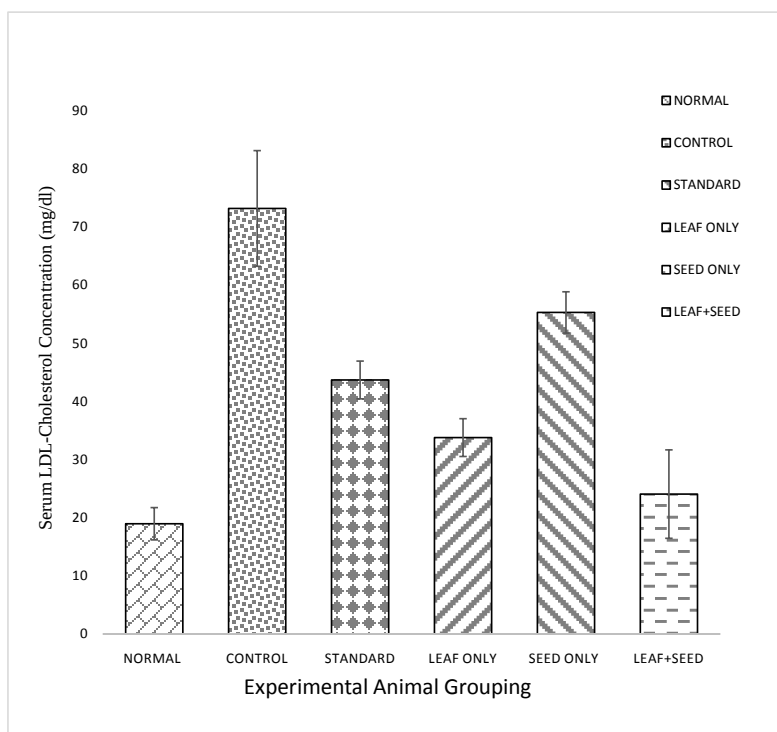


Figure 11. Serum Low-Density Lipoprotein (LDL) Cholesterol (mg/kg). Values are expressed as Mean $\pm$ Standard.

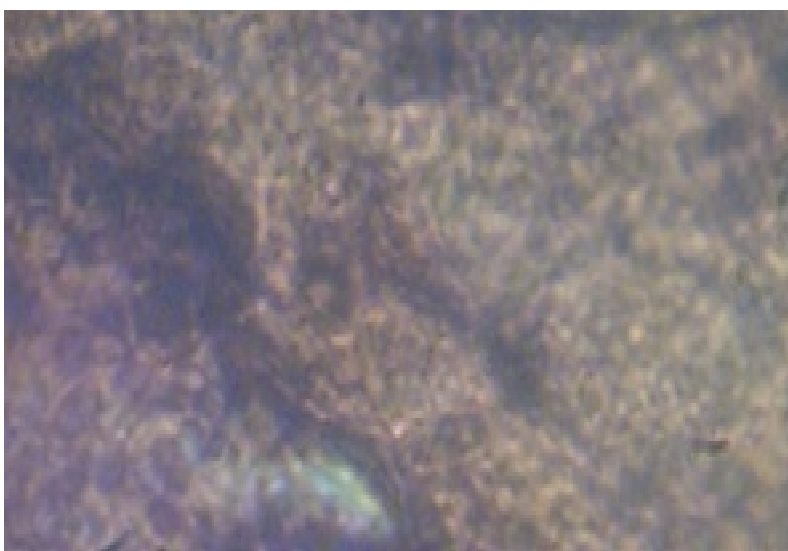


Figure 12. Photomicrographs of Normal Group (400 $\times$) (Pancreas).

beta cells, with an enlarged islet of Langerhans (**Figure 14**). The seed only group showed normal pancreatic acini, but with degenerated islet of Langerhans with diffused beta cells, which indicated a weak ameliorating effect of the seed—only extracts in diabetes mellitus (**Figure 15**). The Leaf only group (**Figure 16**) and Leaf + Seed groups of rats (**Figure 17**) showed pancreatic cells that are normal when compared to the normal non-diabetic group. These results highlight the superiority of amelioration of diabetic-induced pancreatic tissue damage by leaf and seed extracts of TCME over metformin treatment.

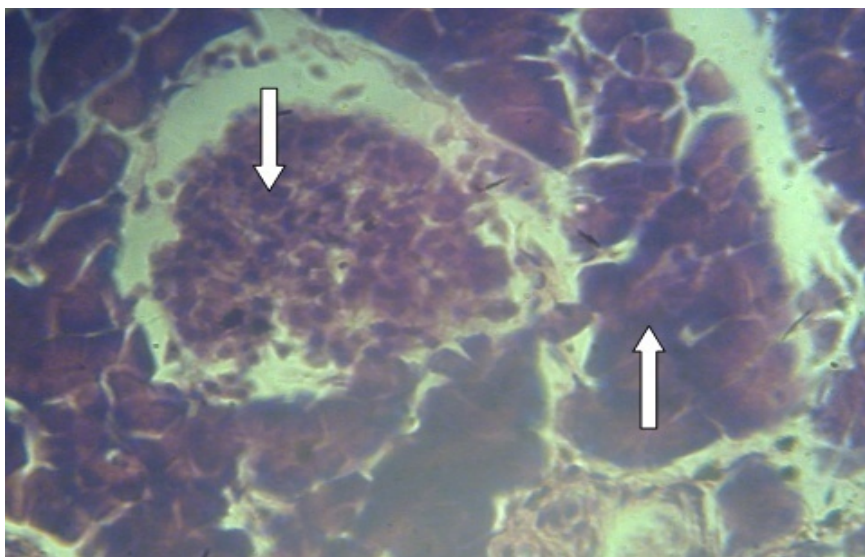


Figure 13. Photomicrographs of Diabetic Control Group (400×) (Pancreas). Down arrow shows enlarged islet of Langerhans, Up Arrow shows distortion and loss of beta cells.

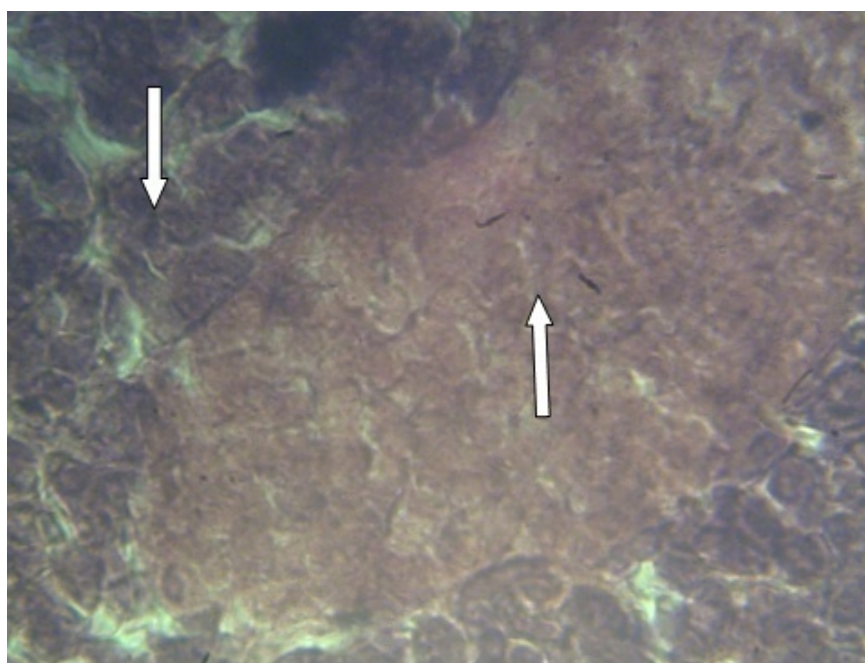


Figure 14. Photomicrographs of Standard Drug Group (400×) (pancreas) Down arrow shows a normal pancreatic acini Up arrow shows partially diffused nuclei of beta cells with an enlarged Islet of Langerhans.

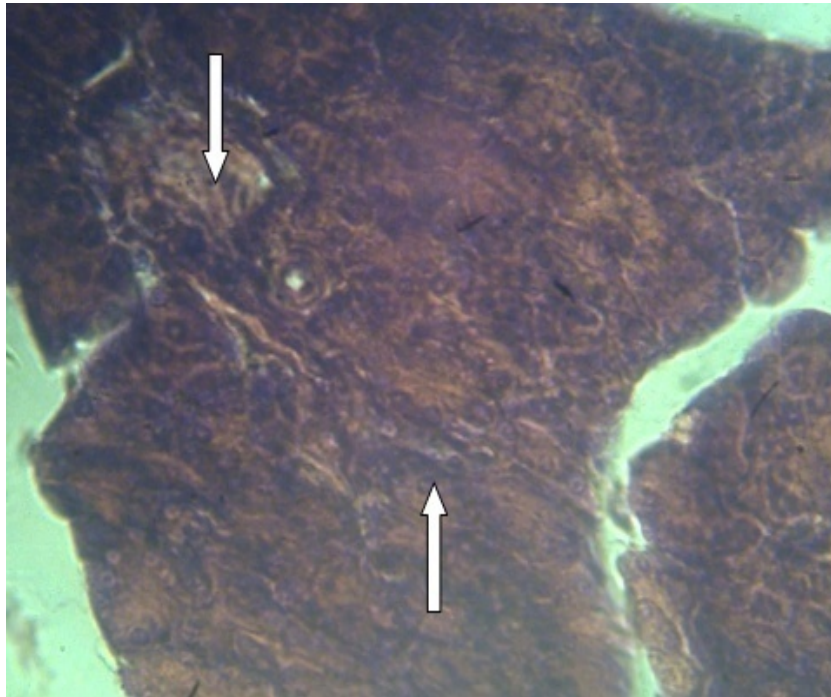


Figure 15. Photomicrographs of Seed Group (400×) (Pancreas) Down arrow shows degenerated islet of Langerhans with diffused beta cells. Up arrow shows a normal pancreatic acini.

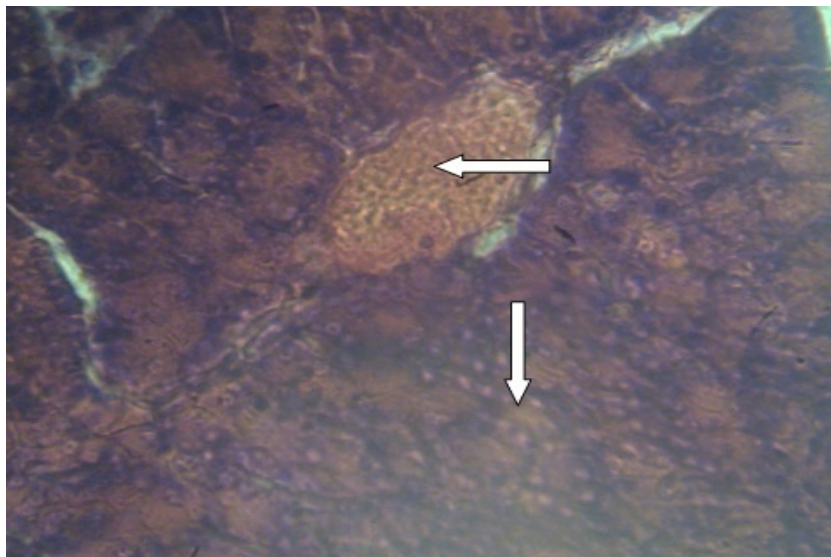


Figure 16. Photomicrographs of Leaf Group (400×) (Pancreas) (Pancreas) • Left arrow shows a normal beta cell. • Down arrow shows a normal pancreatic acini.

3.5.2. H & E Stained Photomicrographs of Liver of Alloxan-Induced Diabetic Rats

The histology of the Liver tissue of the “Normal” rat group shows a normal radial arrangement of hepatocytes with normal nuclei (Figure 18). The diabetic control group (Figure 19) showed distortion of hepatocytes with disrupted radial arrangement, which could have been as a result of the overwhelming oxidative stress on the Liver parenchyma. The standard metformin-treated group (Figure 20) showed enlarged hepatocytes with the presence of Kupffer cells. The seed only group (Figure 21) showed mild radial

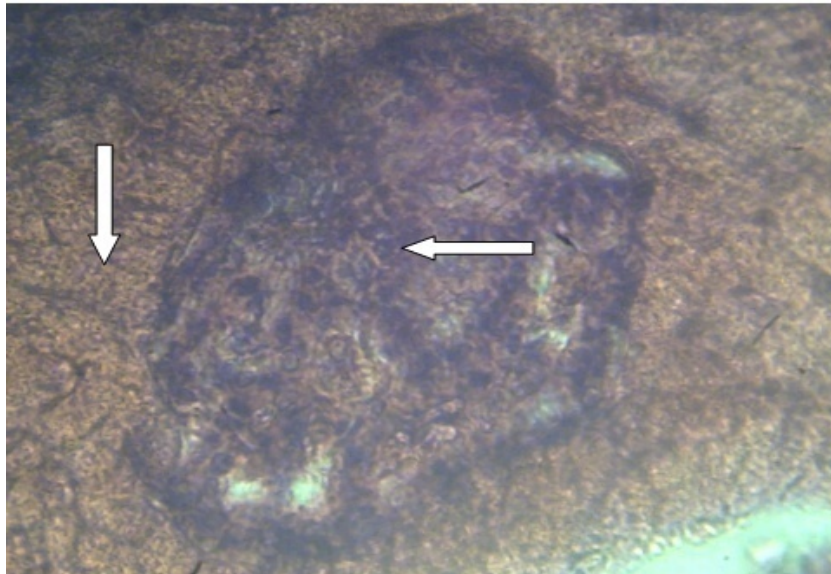


Figure 17. Photomicrographs of Leaf + Seed Group (400×) • Down arrow shows a normal pancreatic acini. • Left arrow shows a normal Beta cell.

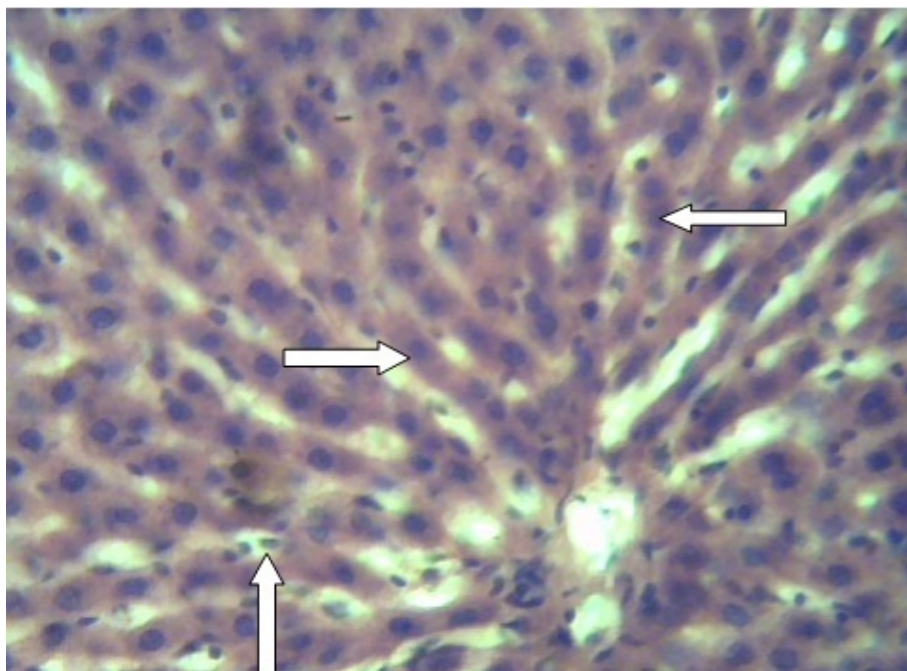


Figure 18. Photomicrograph of a normal group (400×) (Liver) • Right arrow shows a normal radial arrangement of hepatocytes • Up arrow shows kupffer cells within the sinusoid of hepatocytes. • Left arrow shows a normal nuclei.

distortion of hepatocytes and enlargement of nuclei. The Leaf only group (Figure 22) and Leaf + Seed group (Figure 23) of treated rats showed hepatocytes which appeared normal, having normal cellular nuclei comparable to that seen in the normal (non-diabetic) groups.

These studies also showed that TCME extracts treatment can totally reverse diabetes-induced damage of liver tissues, better than metformin.



Figure 19. Photomicrograph of a Diabetic Control Group ($\times 400$) (Liver) • Down arrow shows distortion of hepatocytes; • Left arrow shows disrupted radial arrangement; • Up arrow shows the central vein.

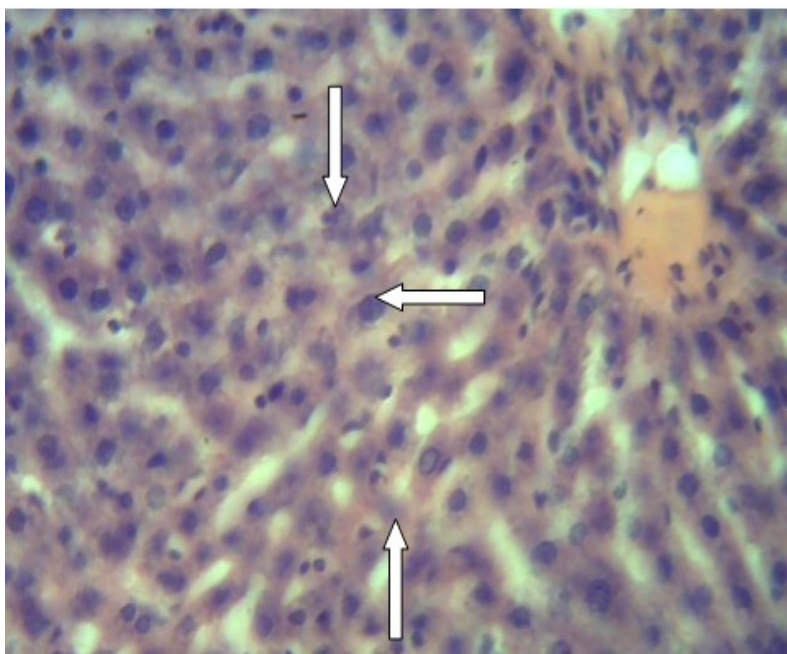


Figure 20. Photomicrograph of Standard Drug Group ($400\times$) (Liver). • Down arrow shows presence of kupffer cell. • Left arrow shows an enlarged nuclei. • Up arrow shows presence of kryotocis.

4. DISCUSSION

The anti-hyperglycaemic effect of methanolic extracts of *T. conophorum* seeds and leaves in aloxan-induced diabetic wistar rats have been shown to be very strong in this study. Seed extracts showed the

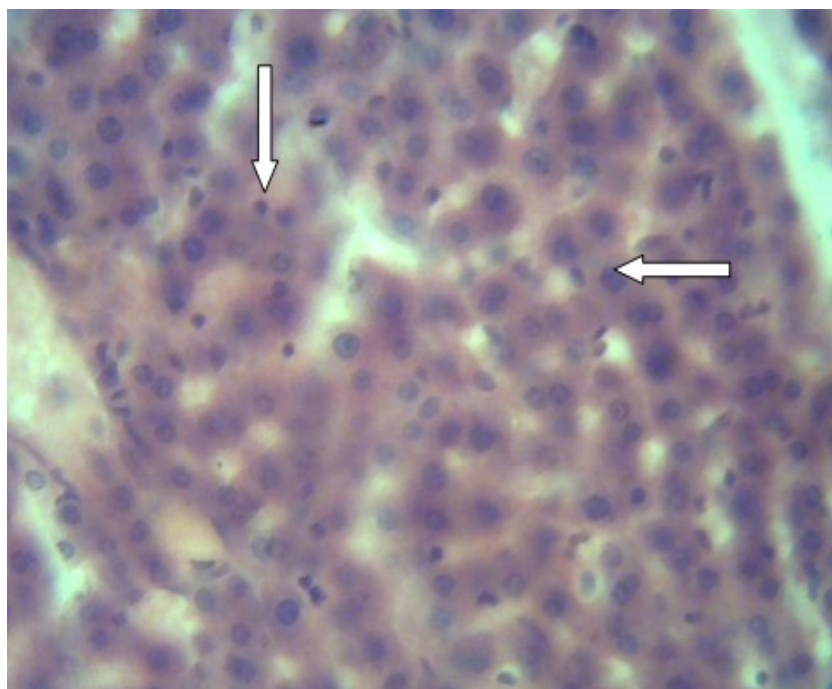


Figure 21. Photomicrographs of Seed Group (400×) (Liver). • Left arrow shows enlargement of nuclei; • Down arrow shows a mild distortion in the radial; arrangement of hepatocytes.

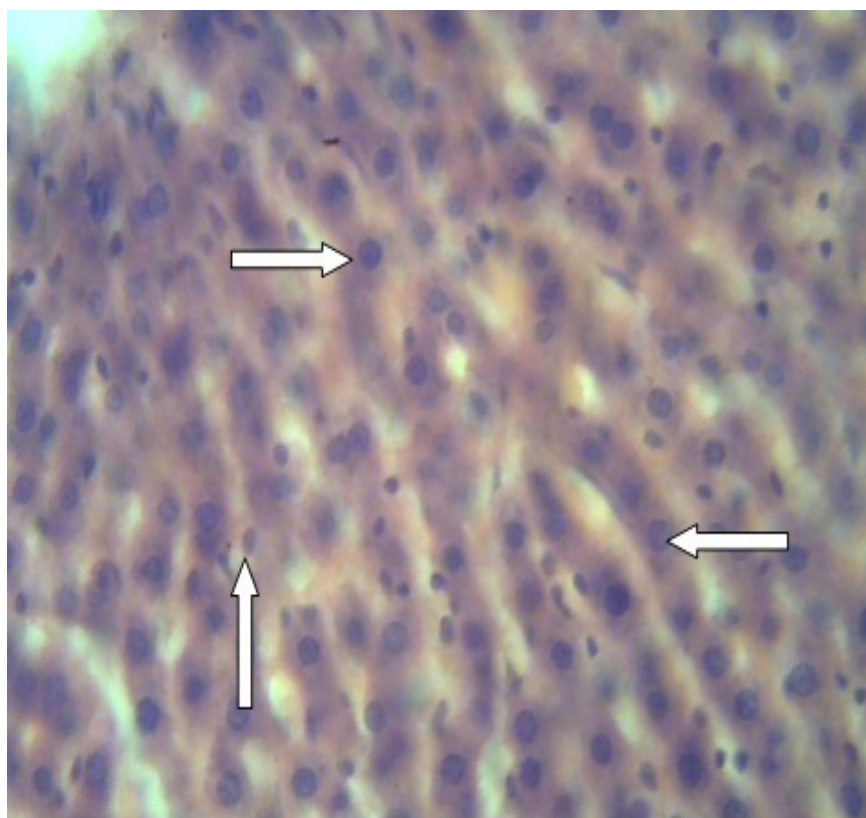


Figure 22. Photomicrographs of Leaf Group (400×) (Liver). • Left arrow shows a normal hepatocyte; • Up arrow shows kupffer cells; • Right arrow shows normal hepatocyte.

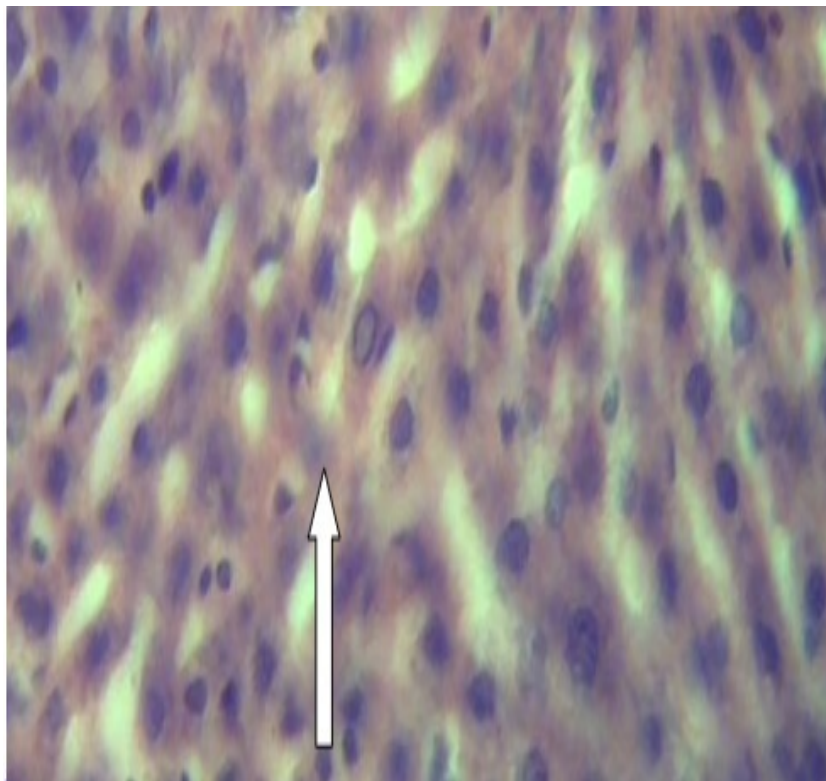


Figure 23. Photomicrographs of Leaf + Seed Group (400×) (Liver) • Up arrow shows a normal radial arrangement.

weakest effects, but compared to diabetic control animal group, seed extract-treated group showed very significant differences in the fasting sugar levels ($t = 16.29$, $p < 0.0001$). This result is consistent with that obtained by Onwuli *et al.* (2014), who treated alloxan-induced diabetic rats with walnut rations (21.3 g, 42.6 g and 85.2 g and obtained fasting blood glucose levels at 3rd, 7th, and 10th days, which were significantly lower than the negative control group. They concluded that walnut has anti-hyperglycemic effects in diabetic rats. In this study, the leaf + seed-treated group of diabetic rats showed the greatest anti-hyperglycaemic effect which was significantly greater than in seed extract treatment ($t = 10.17$, $p < 0.0001$). The anti hyperglycaemic effects demonstrated by treatment of the diabetic rats with leaf extract only was not as effective as the leaf + seed treatment, suggesting that the presence of seed components introduced some synergistic effect on the efficacy of leaf extracts in lowering blood sugar. This synergistic effect of seed components present in Leaf + seed extracts prevailed in all other assays in this study, including enzyme modulation, effective antioxidant activities and serum lipid control, and also in the capacity of the extracts to ameliorate tissue damage caused by oxidative stress.

High serum metabolic enzyme levels are indicators of compromised liver functions due to oxidative stress in diabetic rats. Serum levels of metabolic enzymes (AST, ALT, ALP) were drastically reduced to near normal levels by Leaf + seed extracts these reversal of diabetes induced tissue damage process were confirmed by the photomicrographs of liver and kidney tissues of leaf, as well as leaf+ seed-treated rats, compared to the untreated diabetic control group. The photomicrographs also revealed that seed extract only partially ameliorated tissue damage, while leaf + seed extract appeared to have completely restore the tissue to normal state.

The anti oxidant activity of *T. conophorum* leaves *in vivo* was clearly demonstrated by the activity of malondialdehyde and GSH in the liver. Treatment with Leaf + seed, as well as leaf extracts of TCME drastically brought down the extremely high levels of MDA of untreated rats to normal levels. This result is in

agreement with the findings of other authors. Akomolafe *et al.* (2015) determined the capacity of *Tetracarpidium conophorum* leaf extract to reduce malondialdehyde level in reproductive organs and accessory glands of male wistar rats and reported that testicular MDA levels was highly significantly different from that of control. In addition, Amaeze *et al.* (2011) evaluated the antioxidant activity of fresh and dried leaves of *T. conophorum* by DPPH free radical, nitric oxide radical inhibition and Ferric reducing antioxidant power assays. The authors recorded antioxidant activities comparable to standard antioxidants, Vitamin C and E.

Our study has shown that the leaf extract of *T. conophorum* can, in fact, raise GSH levels to concentrations above the normal value recorded for untreated-non-diabetic rats, and much higher than the standard diabetic drug, metformin. Kanu *et al.* (2015) have reviewed the chemical composition and medicinal value of *T. conophorum* and concluded that the plant seeds are important for nutritional, health benefits and medicinal use.

Furthermore, this study has revealed that diabetes causes serious abnormalities in the normal concentrations of serum lipids (total cholesterol, Tg, HDL, LDL), which are also corrected by leaf and leaf+seed extracts of TCME in a fashion much more highly effective than metformin. Of particular interest is the HDL-cholesterol, which was restored to normal levels by seed + leaf treatment and also the LDL-cholesterol level which was reduced to near normal levels, much more effectively than metformin.

5. CONCLUSION

This study has revealed that methanolic extracts of *T. conophorum* leaves have great anti-hyperglycaemic and superior antioxidant potentials explorable for the treatment of diabetes-related pathogenesis, which appears to be more effective than the standard drug metformin, and also has the advantage of eliminating the side-effects of the latter, since it is routinely consumed as food. These findings, though obtained in experimental rat diabetes, appear to be applicable to human diabetes because results are compared to metformin, a human drug used to treat human diabetes, in every experiment. However, further work is necessary to actually confirm the effect of these extracts in human diabetes, particularly, in terms of dose and route of application. Human experiments with this plant extracts are feasible because of the edibility of the plant. In addition, purification of the active anti-glycemic and anti-oxidant agents of the plant will be necessary in order to determine the optimal dose for the treatment of human diabetes.

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