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ANTI-DIABETIC AND ANTI-OXIDANT ACTIVITIES OF EXTRACTS OF *Triplochiton scleroxylon* IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

In the objective to ascertain the anti-diabetic and anti-oxidative activities of aqueous and 50% ethanol extracts of *Triplochiton scleroxylon* (K. Schum), plasma catalase, glutathione peroxidase and superoxide dismutase activities were determined in streptozotocin-induced diabetic rats. Plasma glucose concentration was also determined periodically. The rats used were of Wistar strain and weighed 130 to 186 g. Test rats received 200 mg extracts per kilogramme body weight twice daily (p. o) with the aid of the gavage. Standard methods requiring spectrophotometry were used to analyse all plasma samples. Results obtained show that aqueous and 50% ethanol extracts decreased plasma glucose concentration significantly ($P<0.05$) when compared to diabetic control. Activities of all anti-oxidant enzymes investigated decreased in diabetes. However, treatment with aqueous and ethanol extracts of *T. scleroxylon* for 28 days resulted in significant increase ($P<0.05$) in plasma catalase, glutathione peroxidase and superoxide dismutase activities when compared to diabetic control. Streptozotocin-induced diabetes caused tubular necrosis and infiltration of inflammatory cells in the kidneys and fatty liver. However, aqueous extract did not cause any adverse histological changes in the organs investigated. Vacuolation of tubular epithelium and necrosis were observed in the kidneys of diabetic rats treated with 50% ethanol extract.

Aqueous extract of *T. scleroxylon* would be safer in treating diabetes mellitus *al beit* 50% ethanol extract has also demonstrated anti-diabetic potentials. Extracts possess ability to

stimulate anti-oxidant activities and protect cells from oxidative stress and its associated complications.

Keywords: Anti-oxidant Enzymes, Diabetic Rats, Aqueous and Ethanol Extracts, *Triplochiton scleroxylon*, Plasma Glucose

INTRODUCTION

Free radicals usually arise from normal cellular metabolism and diabetes. They are responsible for a wide range of cellular damages and complication, some of which have resulted to terminal consequences. However, their harmful effects are frequently controlled by the scavenging systems some of which include, superoxide dismutase, catalase and glutathione peroxidase known to protect the cells against oxidative stress under normal cellular metabolism **[1]**. Most of the natural products used in traditional medical systems for treating diabetes contain a wide scale of antioxidants with a potent scavenging activity for reactive oxygen species (ROS) **[2, 3]**. Aqueous bark extract of *Triplochiton scleroxylon* is *commonly* used to treat diabetes mellitus in the rural communities and amongst most impoverished urban dwellers in the southern and western parts of Nigeria **[4, 5, 6]**. *T. scleroxylon* belongs to the family of tropical medicinal plants **[7]** whose active ingredients are believed to be at the stem bark. *T. scleroxylon* in the kingdom: plantae, division: magnoliophyta, class: magnoliopsida, order: malvales, family: sterculiaceae (APG: Malvaceae), genus: *triplochiton* and species: *T. scleroxylon*, K. Schum, is found in the humid ever green semi-deciduous forest along water ways in the tropical West Africa **[7, 8]**.

This study presents data on the effect of treatment of streptozotocin-induced diabetes mellitus in rats with aqueous and 50% ethanol extracts of *T. scleroxylon* on some of the plasma anti-oxidant enzymes activities. This is to ascertain any possible health risks or advantages of using these extracts in the treatment of diabetes mellitus in some parts of Nigeria.

MATERIALS AND METHODS

Experimental protocols were according to our Institutional Animal Ethics Committee guidelines as well as internationally accepted practices for use and care of laboratory animals as contained in US guidelines **[9]**.

Experimental Animals

Male albino rats used in this study (Wistar strain) were obtained from the animal house of the College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria. The rats which weighed between 130 and 186 g, were housed in clean cages under standard laboratory conditions of temperature, humidity and light. All the albino rats were allowed access to standard laboratory diet supplied by Ewu feeds Ltd. Ewu, Edo State, Nigeria and distilled water *ad libitum* for a period of 2 weeks to acclimatize to the new environment. All animals were handled with humane care **[10]**.

Chemicals/Reagents

All reagents/chemicals used were of analytical grades.

Medicinal Plant

Fresh stem barks of *Triplochiton scleroxylon* were obtained from the forest of Uokha, Owan - East local government area, Edo State, Nigeria. After identification by experts in the Department of Botany, University of Ibadan, Ibadan, Oyo State, Nigeria, as *Triplochiton scleroxylon* K. Schum, with voucher specimen number, UIH – 22329, they were accepted as experimental samples **[10]**.

Extraction and Preparation of Plant Extracts

The barks of *T. scleroxylon* were washed with clean water, dried and cut into tiny pieces. They were pulverized into powder and 1000 g of powdered bark of this plant was then extracted separately in 7000 ml of aqueous (distilled water) and 50 % ethanol in cold percolation by maceration technique under room temperature. This was followed by periodic stirring. The macerated samples were filtered with sintered glass funnel under suction to eliminate particles after 72 hours. The filtrates collected were then concentrated on a reduced pressure using the rotary evaporator to yield thick brown viscous pastes which were further dried under vacuum in a freeze dryer **[11]**. The freeze dried samples were then kept in the freezer at -21^{o}C until used. The yields were 14.22 and 12.81 % (w/w) for aqueous and 50% ethanol extracts, respectively **[10]**.

Blood Collection

The tail of the restrained rats was cleansed with a ball of cotton wool soaked in methylated spirit. A little vaseline was then smeared on the tail to reduce friction while massaging to redness. Gentle massage towards the tip of the tail continued until blood accumulated at the tip of the tail of the rat. The red tip of the tail was then slightly incised with new and sterilized blade and further massaged gently as the blood tickled into immobilized sample tubes containing lithium heparin (for enzyme assays) and fluoride oxalate (for glucose assay). Cotton wool soaked in methylated spirit was again used to cleanse the incised area of the tail. Blood samples collected were subjected to centrifugation for 10 minutes at 3,000 G to obtain the plasma for all biochemical analyses. Analyses were carried out immediately after centrifugation **[10]**.

Streptozotocin Injection

Streptozotocin (100 mg), dissolved in commercial saline (5 ml) was administered to overnight fasted rats (65 mg/kg body weight) by intra-peritoneal route. Rats with blood glucose level two or three times the basal values, seven days after injection were selected for the experimental study **[12-15]**.

Experimental Procedure

Male albino rats (Wistar strain) after acclimatization for a period of two weeks were fasted overnight and randomly divided into four groups of four rats each. Rats in groups 2, 3 and 4 were injected streptozotocin (i.p.) at 65 mg/kg body weight and having being certified diabetic seven days after this injection were treated simultaneously with rats in group 1 as follows:

Group 1: Served as normal control and received distilled water *ad libitum*.

Group 2: Served as diabetic control and received distilled water *ad libitum*

Group 3: Served as diabetic rats and treated with 200 mg of aqueous extract per kilogramme body weight.

Group 4: Served as diabetic rats and treated with 200 mg of 50% ethanol extract per kilogramme body weight.

Administration of Extracts

Extracts (aqueous and 50% ethanol) of *T. scleroxylon* were administered to experimental rats orally (p. o.) with the aid of the gavage.

Biochemical Assays

Determination of plasma glucose concentration.

Plasma glucose was determined with the aid of glucose – oxidase kit by the method described by Randox Laboratories, United Kingdom.

Estimation of superoxide dismutase (SOD) activity by a modified spectrophotometric method **[16, 17]**.

To 0.2 ml plasma sample was added 2.5 ml of 0.05M carbonate buffer (pH 10.2) followed by equilibration at room temperature. 0.3 ml of 0.3M adrenaline solution was then added and mixed thoroughly. The reference tube which contained 0.2 ml distilled water, 2.5 ml of 0.05M carbonate buffer (pH 10.2) and 0.3 ml of 0.3M adrenaline solution was also thoroughly mixed. 3 ml of distilled water served as the blank. The absorbance values were read at 420nm. 1 unit of superoxide dismutase activity was taken as the amount of SOD required to cause 50% inhibition of the auto – oxidation of adrenaline to adrenochrome per minute. Protein in the plasma was determined by the method of Lowry *et al*., **[18]** using bovine serum albumin (BSA) as standard, at 660 nm. Enzyme concentration was then calculated as: Unit/mg protein = %Inhibition/50 x mg protein.

Estimation of catalase activity by a modified method **[19]**

0.5 ml of the sample was added to the iced test tubes while the blank contains 0.5 ml of distilled water. The reaction was initiated by the sequential addition of 5 ml of cold 30 mM H_2O_2 at fixed intervals. It was mixed thoroughly by inversion and after 3 min the reactions were stopped sequentially by rapid addition of 1 ml of 6M H_2SO_4 at the same fixed intervals. The solution was mixed thoroughly by inversion once again. 1 ml of $KMnO_4$ (0.01M) was added to the test samples and the blank and then mixed thoroughly. The standard was prepared by adding 1 ml of 0.01M $KMnO_4$ to a mixture of 5.5 ml of 0.05M phosphate buffer and 1 ml of 6M H_2SO_4. The absorbance was read at 480nm within a minute. The result was expressed in units/mg protein where 1 mole of H_2O_2 consumed per minute was 1 unit of enzyme activity. Plasma protein was estimated by the method of Lowry *et al.*, **[18]**.

Estimation of glutathione peroxidase activity by a modified version of the method of *Faraji et al.*, **[20]**.

All reaction mixtures were dissolved in 20 mM sodium phosphate buffer containing 6 mM EDTA (pH 7.0). The reaction mixture contained 98.8 µL of phosphate buffer, 700 µL of 2.86 mM reduced glutathione, 100 µL of 1 mM sodium azide, 100 µL of 1 mM NADPH and 4.2 µL of glutathione reductase (0.5 units). About 10 µL of plasma sample was added to the reaction mixture and incubated at room temperature for 10 minutes. Afterward, 10 µL of 30 mM t – butyl hydroperoxide (dissolved in distilled water) was added to the reaction mixture and measured at 340 nm for 7 minutes in the spectrophotometer. A molar extinction coefficient of 6.22×10^3 M cm^{-1} was used to determine glutathione peroxidase activity. The enzyme activity was expressed as units/mg protein. Plasma protein was estimated by the method of Lowry *et al.*, **[18]**. International units represent µmole of hydroperoxides transformed per min/ml of enzyme.

Statistical Analysis

Data were expressed as mean ± S. E. M. of three separate determinations. The statistical significance was evaluated by one-way ANOVA using SPSS (statistical package for social sciences) version 16.0, followed by post –hoc LSD and Turkey tests for individual comparisons. Values lower than 0.05 probabilities were accepted as statistically significant.

RESULTS

Results have been presented in **Tables 1-4** and **Figures 1-4**. Photomicrographs of histological examination of the hearts, kidneys and livers of normal and diabetic rats treated with extracts (200 mg/kg body weight) of *T. scleroxylon* for 28 days were also presented **(Plates 1 – 12)**. Both extracts caused a significant decrease (P<0.05) in plasma glucose concentration **(Table 1 and Figure 1)**. However, significant increase (P<0.05) in plasma catalase **(Table 2 and Figure 2)**, glutathione peroxidase **(Table 3 and Figure 3)** and superoxide dismutase **(Table 4 and Figure 4)** activities were obtained when compared to diabetic control. Aqueous extract did not precipitate adverse histological changes in organs investigated **(Plates 7, 8 and 9)** unlike 50% ethanol extract which resulted in necrosis and vacuolation of tubular epithelium of the rat kidneys in treated streptozotocin-induced diabetic rats **(Plate 11)**.

Table 1: Mean Plasma Glucose Concentrations (mg/dl) of Controls and Treated Streptozotocin-Induced Diabetic Rats

S. No.	Groups	0 Days	1 Days	6 Days	12 Days	18 Days	24 Days	28 Days
1.	NC	88.92 ± 1.52^a	89.95 ± 1.88^a	91.94 ± 1.35^a	93.28 ± 1.13^a	93.44 ± 1.18^a	92.38 ± 2.25^a	93.24 ± 1.28^a
2.	DC	81.00 ± 1.58^a	206.94 ± 1.73^b	206.93 ± 1.94^b	208.36 ± 0.97^b	210.00 ± 2.21^b	214.43 ± 8.05^b	203.93 ± 2.11^b
3.	ATD	77.25 ± 2.29^a	211.38 ± 3.59^b	186.18 ± 4.12^c	140.92 ± 4.04^c	126.57 ± 2.36^c	107.39 ± 2.80^c	96.39 ± 2.02^a
4.	ETD	81.00 ± 2.04^a	212.77 ± 2.00^b	199.89 ± 0.64^b	168.23 ± 2.41^c	146.17 ± 2.36^d	113.72 ± 2.69^c	108.29 ± 1.97^c

NOTE: Data are Mean ± S.E.M. (n = 4); Mean in the Same Column with Different Superscript Letters are Significantly Different; P<0.05(one way ANOVA Followed by Post-hoc LSD) when Compared to Diabetic Control; Mean Significantly Different (P<0.05) on the 6th, 12th, 18th, 24th and 28th Days when Compared to Diabetic Control; NC: Normal Control; DC: Diabetic Control; ATD: Aqueous Treated Diabetes; ETD: Ethanol Treated Diabetes

Table 2: Mean Plasma Catalase Activities (unit/mg Protein) of Controls and Treated Streptozotocin-Induced Diabetic Rats

S. No.	Groups	0 Days	1 Days	6 Days	12 Days	18 Days	24 Days	28 Days
1.	NC	0.15 ± 0.01^a	0.16 ± 0.01^a	0.16 ± 0.01^a	0.16 ± 0.01^a	0.17 ± 0.00^a	0.18 ± 0.00^a	0.18 ± 0.00^a
2.	DC	0.16 ± 0.01^a	0.11 ± 0.00^b	0.10 ± 0.00^b	0.08 ± 0.00^b	0.06 ± 0.01^b	0.04 ± 0.00^b	0.03 ± 0.01^b
3.	ATD	0.16 ± 0.01^a	0.13 ± 0.01^b	0.15 ± 0.01^a	0.17 ± 0.00^a	0.20 ± 0.01^c	0.24 ± 0.01^c	0.27 ± 0.01^c
4.	ETD	0.15 ± 0.01^a	0.11 ± 0.00^b	0.12 ± 0.00^c	0.15 ± 0.00^a	0.17 ± 0.01^a	0.20 ± 0.01^a	0.22 ± 0.01^d

NOTE: Data are Mean ± S.E.M. (n = 4); Mean in the Same Column with Different Superscript Letters are Significantly Different; P<0.05(one way ANOVA Followed by Post-hoc LSD) when Compared to Diabetic Control; Mean Significantly Different (P<0.05) on the 6th, 12th, 18th, 24th and 28th Days when Compared to Diabetic Control; NC: Normal Control; DC: Diabetic Control; ATD: Aqueous Treated Diabetes; ETD: Ethanol Treated Diabetes

Table 3: Mean Plasma Glutathione Peroxidase Activities (unit/mg Protein) of Controls and Treated Streptozotocin-Induced Diabetic Rats

S. No.	Groups	0 Days	1 Days	6 Days	12 Days	18 Days	24 Days	28 Days
1.	NC	0.40±0.00[a]	0.41±0.01[a]	0.42±0.01[a]	0.46±0.01[a]	0.44±0.02[a]	0.45±0.01[a]	0.49±0.01[a]
2.	DC	0.41±0.01[a]	0.31±0.01[b]	0.27±0.00[b]	0.23±0.01[b]	0.20±0.00[b]	0.12±0.02[b]	0.07±0.01[b]
3.	ATD	0.42±0.02[a]	0.29±0.01[b]	0.30±0.01[c]	0.32±0.01[c]	0.34±0.01[c]	0.36±0.01[c]	0.36±0.01[c]
4.	ETD	0.44±0.01[a]	0.31±0.01[b]	0.31±0.01[c]	0.32±0.01[c]	0.33±0.01[c]	0.33±0.01[c]	0.36±0.01[c]

NOTE: Data are Mean ± S.E.M. (n = 4); Mean in the Same Column with Different Superscript Letters are Significantly Different; P<0.05(one way ANOVA Followed by Post-hoc LSD) when Compared to Diabetic Control; Mean Significantly Different (P<0.05) on the 6th, 12th, 18th, 24th and 28th Days when Compared to Diabetic Control; NC: Normal Control; DC: Diabetic Control; ATD: Aqueous Treated Diabetes; ETD: Ethanol Treated Diabetes

Table 4: Mean Plasma Superoxide Dismutase Activities (unit/mg Protein) of Controls and Treated Streptozotocin-Induced Diabetic Rats

S. No.	Groups	0 Days	1 Days	6 Days	12 Days	18 Days	24 Days	28 Days
1.	NC	0.29±0.02[a]	0.28±0.01[a]	0.28±0.01[a]	0.30±0.01[a]	0.30±0.01[a]	0.30±0.01[a]	0.33±0.01[a]
2.	DC	0.31±0.01[a]	0.25±0.02[a]	.21±0.01[b]	0.18±0.01[b]	0.17±0.01[b]	0.13±0.01[b]	0.11±0.01[b]
3.	ATD	0.33±0.01[a]	0.25±0.01[a]	0.26±0.01[a]	0.28±0.01[a]	0.31±0.01[a]	0.33±0.01[a]	0.35±0.01[a]
4.	ETD	0.32±0.02[a]	0.23±0.01[a]	0.24±0.02[a]	0.28±0.01[a]	0.31±0.01[a]	0.32±0.01[a]	0.36±0.02[a]

NOTE: Data are Mean ± S.E.M. (n = 4); Mean in the Same Column with Different Superscript Letters are Significantly Different; P<0.05(one way ANOVA Followed by Post-hoc LSD) when Compared to Diabetic Control; Mean Significantly Different (P<0.05) on the 6th, 12th, 18th, 24th and 28th Days when Compared to Diabetic Control; NC: Normal Control; DC: Diabetic Control; ATD: Aqueous Treated Diabetes; ETD: Ethanol Treated Diabetes

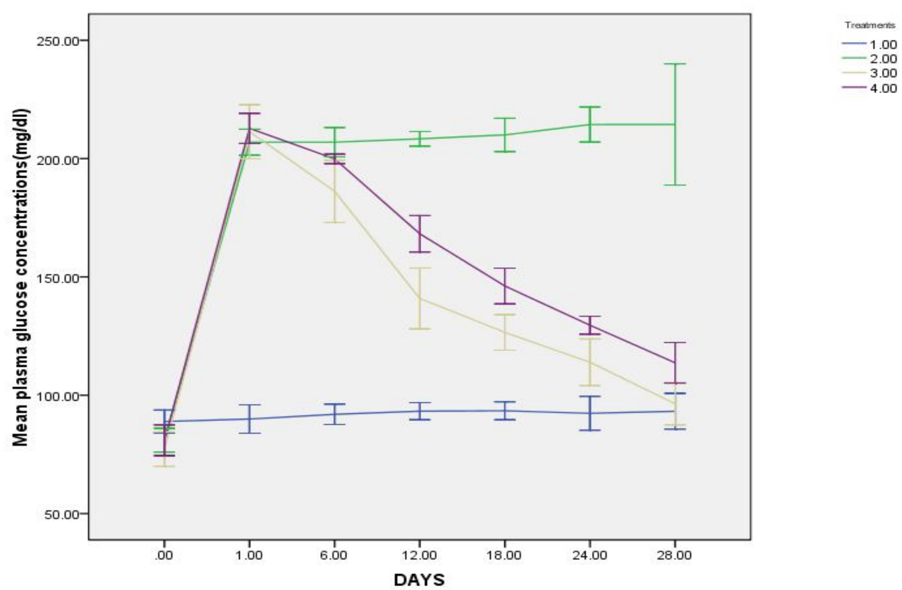

Fig.1:Mean plasma glucose concentrations(mg/dl) of controls and treated streptozotocin-induced diabetic rats.1.00:Normal Control; 2.00:Diabetic Control;3.00:Aqueous extract;4.00:50% Ethanol extract.

Error bars: 95% CI

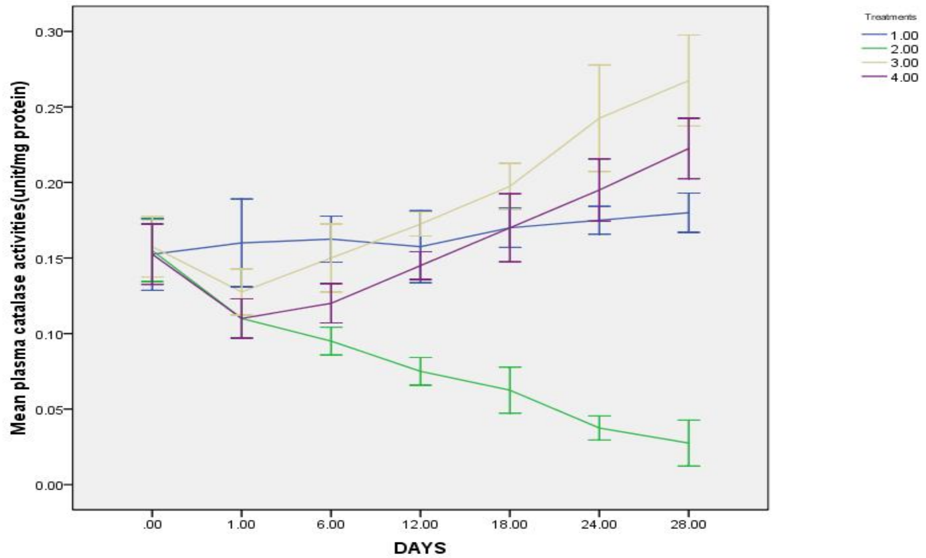

Fig.2:Mean plasma catalase activities(unit/mg protein) of controls and treated streptozotocin-induced diabetic rats.1.00:Normal Control;2.00:Diabetic Control;3.00:Aqueous extract;4.00:50% Ethanol extract.

Error bars: 95% CI

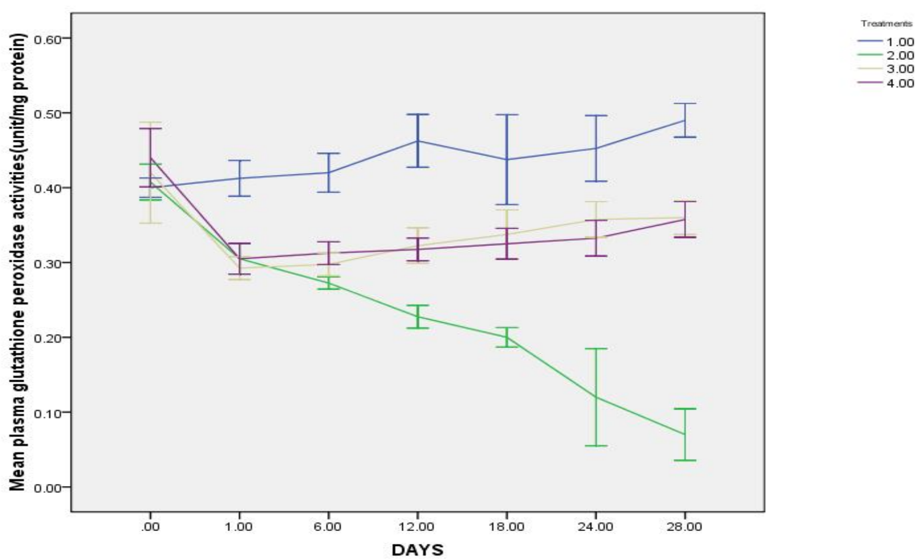

Fig.3:Mean plasma glutathione peroxidase activities(unit/mg protein) of controls and treated streptozotocin-induced diabetic rats. 1.00:Normal Control;2.00:Diabetic Control;3.00:Aqueous extract;4.00:50% Ethanol extract.

Error bars: 95% CI

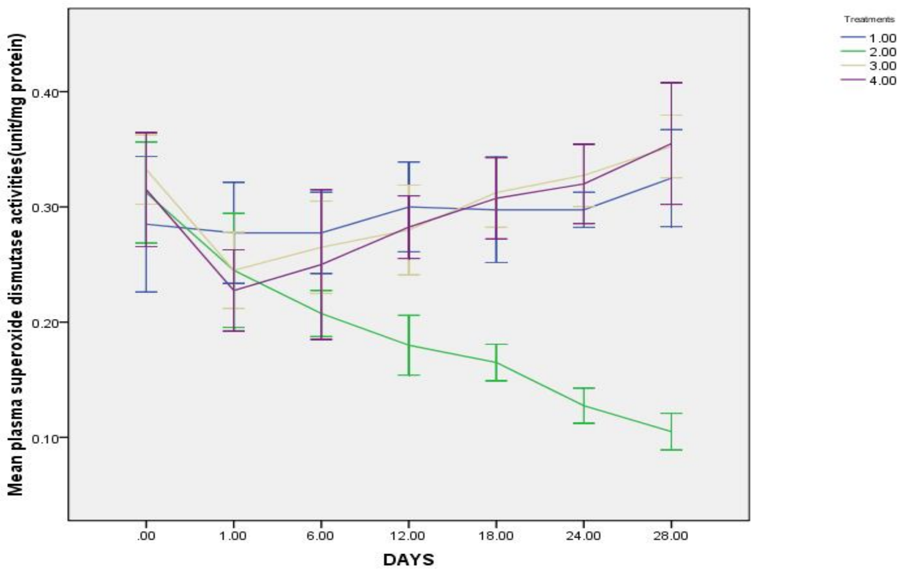

Fig.4:Mean plasma superoxide dismutase activities(unit/mg protein) of controls and treated streptozotocin-induced diabetic rats. 1.00:Normal Control;2.00:Diabetic Control;3.00:Aqueous extract;4.00:50% Ethanol extract.

Error bars: 95% CI

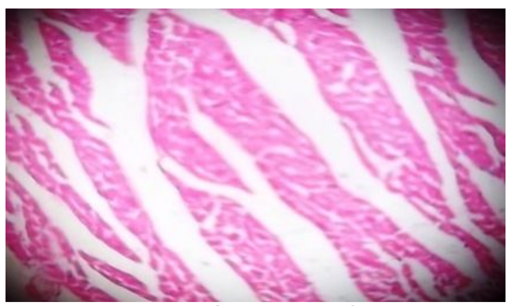

Plate 1: A Photomicrograph Showing a Section of the Heart of a Normal Rat Given Distilled Water for 28 Days; Section Shows Normal Histology; (Control Group), H & E. X 100

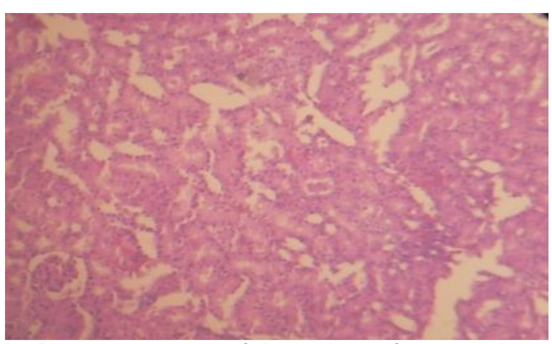

Plate 2: A Photomicrograph Showing a Section of the Kidney of a Normal Rat Given Distilled Water for 28 Days. Section Shows Normal Histology; (Control group); H & E Stain; X 100

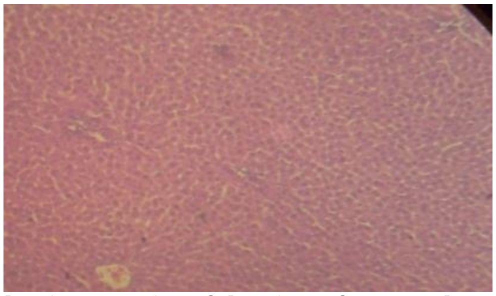

Plate 3: A photomicrograph Showing a Section of the Liver of a Normal Rat Given Distilled Water for 28 days; Section Shows Normal Histology; (Control Group); H & E Stain; X 100

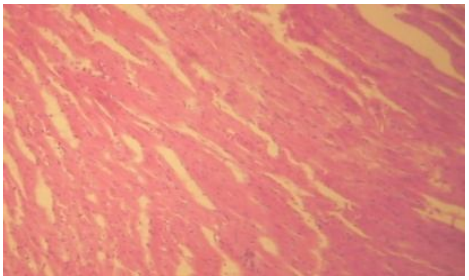

Plate 4: A Photomicrograph Showing a Section of the Heart of Streptozotocin Induced (65 mg/kg body weight) *i. p.* Diabetic Rat Given Distilled Water for 28 days. Section Shows Normal Histology; (Diabetic Control); H & E; X 100

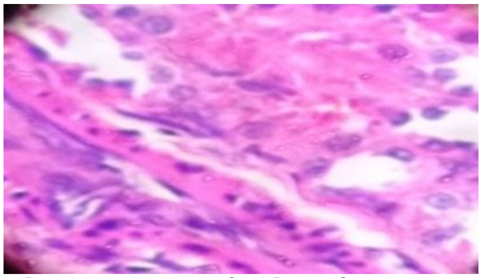

Plate 5: A Photomicrograph Showing a Section of Kidney of Streptozotocin Induced (65 mg/kg body weight) *i. p.* Diabetic Rat Given Distilled Water for 28 days; Section Shows Tubular Necrosis With Infiltrations of Inflammatory Cells; (Diabetic Control); H & E; X 100

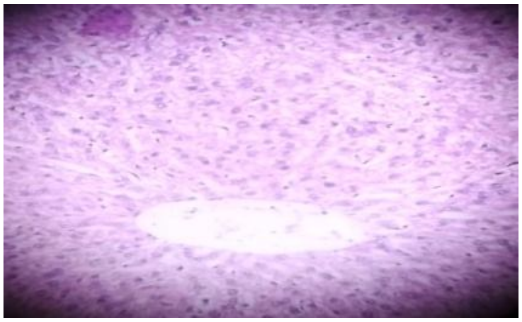

Plate 6: A Photomicrograph Showing a Section of Liver of Streptozotocin Induced (65 mg/kg body weight) *i. p.* diabetic rat given distilled water for 28 days; Section Shows Fatty Changes (Fatty Liver). (Diabetic Control); H & E; X 100

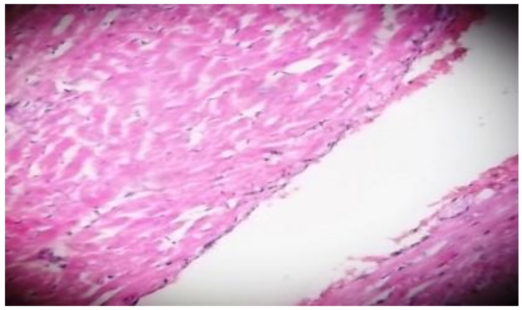

Plate 7: A photomicrograph Showing a Section of the Heart of Streptozotocin Induced (65 mg/kg body weight) *i. p.* Diabetic Rat Treated With Aqueous Extract of *Triplochiton scleroxylon* (200 mg/kg Body Weight) *p. o.* for 28 days; Section Shows Normal Histology. (Aqueous Extract Treated Diabetes); H & E; X 100

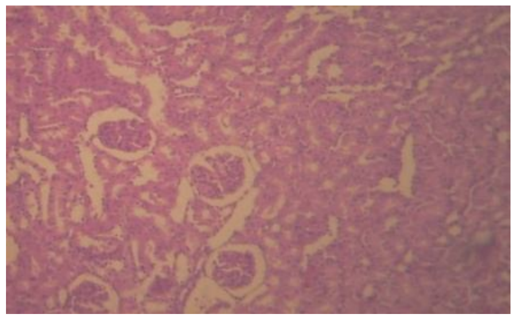

Plate 8: A Photomicrograph Showing a Section of the Kidney of Streptozotocin Induced (65 mg/kg body weight) *i. p.* Diabetic Rat Treated With Aqueous Extract of *Triplochiton scleroxylon* (200 mg/kg body weight) *p. o.* for 28 days; Section Shows Normal Glomerular Architecture with Mild Infiltrations; (Aqueous Extract Treated Diabetes); H & E; X 100

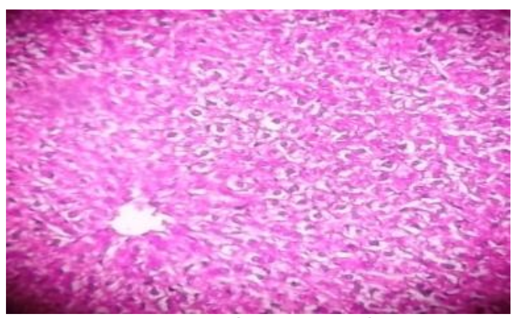

Plate 9: A Photomicrograph Showing a Section of the Liver of Streptozotocin Induced (65 mg/kg body weight) *i. p.* Diabetic Rat Treated With Aqueous Extract of *Triplochiton scleroxylon* (200 mg/kg body weight) *p. o.* for 28 Days; Section shows normal histology; (Aqueous extract treated diabetes); H & E; X 100

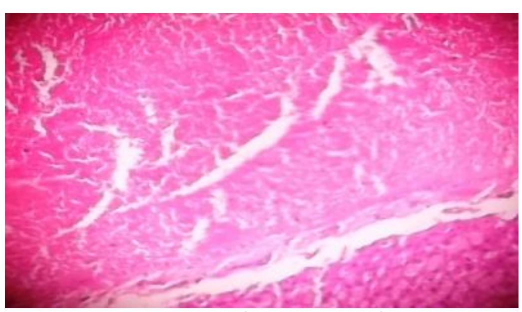

Plate 10: A Photomicrograph Showing a Section of the Heart of Streptozotocin Induced (65 mg/kg Body Weight) *i. p.* Diabetic Rat Treated With Ethanol Extract of *Triplochiton scleroxylon* (200 mg/kg Body Weight) *p. o.* for 28 Days; Section Shows Normal Histology; (Ethanol Extract Treated Diabetes); H & E; X 100

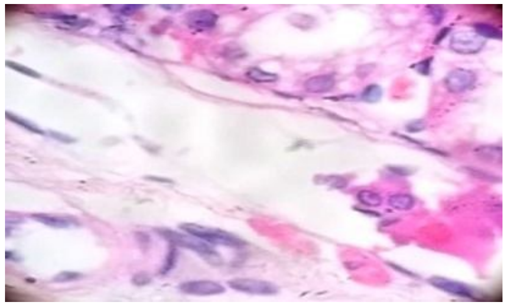

Plate 11: A Photomicrograph Showing a Section of the Kidney of Streptozotocin Induced (65 mg/kg Body Weight) *i. p*. Diabetic Rat Treated With Ethanol Extract of *Triplochiton scleroxylon* (200 mg/kg Body Weight) *p. o*. for 28 Days. Section Shows Vacuolation of Tubular Epithelium With Area of Necrosis; (Ethanol Extract Treated Diabetes); H & E; X 100

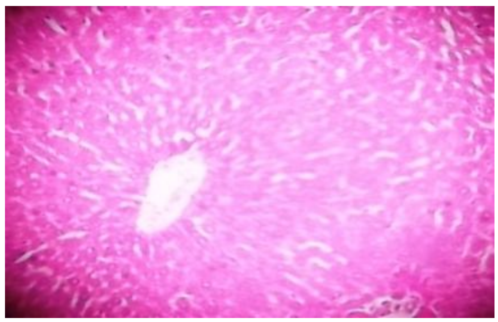

Plate 12: A Photomicrograph Showing a Section of the Liver of Streptozotocin Induced (65 mg/kg Body Weight) *i. p*. Diabetic Rat Treated With Ethanol Extract of *Triplochiton scleroxylon* (200 mg/kg Body Weight) *p. o*. for 28 Days; Section Shows Normal Histology; (Ethanol Extract Treated Diabetes); H & E; X 100

DISCUSSION

Anti-oxidant enzymes are nature's protector against the harmful effects of reactive oxygen species (ROS) generated during normal metabolic processes. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) enzymes are very important scavengers of ROS. They prevent generation of very destructive hydroxyl radical and protect the cellular constituents from oxidative stress or damage **[21]**. In diabetes mellitus, the late diabetic pathological complications are mostly due to excessive elevated production of ROS over the capacity of their removal by internal enzymatic and non –enzymatic mechanisms **[22]**. Additional dietary artificial or natural antioxidants may be of great importance in such a case **[23]**.

Streptozotocin-induced diabetes mellitus in rats resulted in increase in blood glucose concentration **(Table 1 and Figure 1)**. The values of blood glucose level in alloxan-induced diabetic rats **[24]** agreed with the experimental values. Bhaskar *et al*., **[25]**, also reported an increase in blood glucose concentration in streptozotocin-diabetic rats. In experimental studies of diabetes mellitus it is possible to produce complete or partial destruction of the ß – cells of the pancreas with the use of appropriate doses of streptozotocin causing an increase in blood glucose level. However, treatment with extracts of *T. scleroxylon* resulted in significant decrease (P<0.05) in plasma glucose when compared to diabetic control **(Table 1 and Figure 1)**. Anti-diabetic and hypoglycaemic properties of aqueous extract of *T. scleroxylon* in streptozotocin-induced diabetic and normal rabbits have been documented **[14, 15]**. Substances with known hypoglycaemic and anti-diabetic properties would be effective in the management of diabetes mellitus **[26]**. The anti-diabetic potentials of aqueous and ethanol (50%) extracts of *T. scleroxylon* could be attributable to the presence of flavonoids and saponins **[27]** which are common in plants with known hypoglycemic effect **[28]**.

Treatment of diabetic rats with aqueous and 50% ethanol extracts resulted in increase in the activities of anti-oxidant enzymes investigated **(Tables 2, 3 and 4)** and **(Figure 2, 3 and 4)**. The increased activities of SOD, CAT and GPx may be due to free radicals generated by streptozotocin-induced diabetes, the scavenging activities of these enzymes **[29]** and also by the flavonoid content of extracts of *T. scleroxylon*, known to enhance antioxidant activities **[27]**. Flavonoids are ubiquitous in all parts of green plants and constitute one of the largest groups of naturally occurring phenols **[30]**. Most of the natural products used in traditional medical systems for treating diabetes contain a wide scale of antioxidants with a potent scavenging activity for ROS **[2, 3]**. It is possible that flavonoid stimulates antioxidant activities by activating antioxidant enzymes to enhance their free radical scavenging. It has been reported that rats treated with flavonoids recorded significantly enhanced SOD and CAT activities **[30]**. Pari and Amali, **[21]** have reported increased activities of SOD, CAT and GPx in tetrahydrocurcumin and curcumin administered rats. Supplementation of *Ginkgoselect* phytosome as well as silymarin to rifampicin has also been reported to elevate levels of SOD, CAT and GPx to normal values in experimental animals **[31]**.

Histopathological examination of tissues showed that streptozotocin-induced diabetes mellitus caused tubular necrosis and infiltration of inflammatory cells in the kidneys and fatty liver **(Plates 5 and 6)**. However, treatment with aqueous extract (200 mg/kg body weight) presented with normal histology in the tissues examined **(Plates 7, 8 and 9)**. In contrast, 50% ethanol extract caused vacuolation of tubular epithelium and necrosis in the kidneys of treated diabetic rats **(Plate 11)**. It is possible that the presence of toxic chemical substances in 50% ethanol extract which are absent in aqueous extract could be responsible for the adverse histopathological reports.

CONCLUSION

Aqueous and 50% ethanol extracts of *T. scleroxylon* have demonstrated significant anti-diabetic and anti-oxidant activities important in treating diabetes mellitus in rats and protecting against the harmful effects of free radicals, respectively. However, aqueous extract (200 mg/kg body weight) would be safer and useful in treating diabetes mellitus vis-a-vis 50% ethanol extract which affected the rat kidneys adversely.

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