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ACUTE TOXICITY AND DOSE RESPONSE STUDIES OF AQUEOUS AND ETHANOL EXTRACTS OF *TRIPLOCHITON SCLEROXYLON* K. SCHUM, (STERCULIACEAE).

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ABSTRACT: The acute toxicity and dose response studies of aqueous and 50% ethanol extracts of stem bark of *Triplochiton scleroxylon* were investigated in albino rats (Wistar strain) of average weight, 137.67g. Male albino rats were administered (p.o) extracts at the dose of 200, 500, 1000, 3000 and 5000 mg/kg body weight for 28 days respectively and activities of liver enzyme markers (alkaline phosphatase (ALP), alanine amino transaminase (ALT) and γ-glutamyl transferase (γ-GT) determined using spectrophotometry. Kidneys, hearts and livers of experimental albino rats were subjected to histological examination after 28 days of administration of plant extracts. The lethal dose (LD_{50}) was beyond 5000 mg/kg body weight for both extracts investigated as no signs of toxicity were observed even at larger doses. Dose response studies show a dose dependent increase ($P<0.05$) in the activities of liver enzyme markers in the plasma of rats and a dose dependent decrease in plasma glucose concentrations ($P<0.05$) on the 6^{th}, 12^{th}, 18^{th} and 28^{th} days of administration of extracts to test rats when compared to control. 200 mg/kg body weight was the safest dose for both extracts as no adverse histological changes were observed on the tissues examined. Substances that have hypoglycaemic properties with little or no side effects would be useful for and safe in the treatment of diabetes mellitus.

Key words: Acute toxicity, Dose response, Stem bark extracts, *Triplochiton scleroxylon*.

INTRODUCTION

Triplochiton scleroxylon is one of the over 30 medicinal plants used in the rural areas and also by some impoverished urban dwellers in the western and southern parts of Nigeria to treat diabetes mellitus (Prohp and Onoagbe, 2009a, b). It belongs to the family of tropical medicinal plants (Russel et al., 1997).The active ingredients are believed to be at the bark of this plant whose aqueous extract is commonly used as an anti – diabetic preparations. Several preliminary studies have proved valid the claims of its hypoglycaemia and anti-diabetic properties in normal, alloxan and streptozotocin-induced diabetic rabbits (Prohp et al., 2007; Prohp and Onoagbe, 2009a, b). *Triplochiton scleroxylon* is found in the humid ever green semi-deciduous forest along water ways in the tropical West Africa (Russel et al., 1997). This plant in the kingdom: plantae, division: magnoliophyta, class: magnoliopsida, order: malvales, family: sterculiaceae (APG: Malvaceae), genus: triplochiton and species: *T. scleroxylon*, is a tropical tree of Africa known also as Abachi under the Nigerian name Obeche whilst in Ghana, Cameroon and Ivory Coast it is called wawa, ayous and samba respectively. The trade name in Britain is Obeche (Russel et al., 1997). Investigation of the acute toxicity is the first step in the toxicological investigations of an unknown substance. The index of the acute toxicity is the LD_{50} (Lorke, 1983). Scientific investigation of previously unknown and known plants is necessary not only because of the need to discover new drugs but to assess the toxicity faced by the users. Besides, it is important that traditionally claimed therapeutic properties of plants be confirmed and its toxicity limit determined.

Acute toxicity studies are essential to prevent any overdose of drug which may interfere with general physiological and biochemical mileu and precipitate adverse health risks. It is therefore the interest of this research to determine the toxicity profile of aqueous and 50% ethanol extracts of *Triplochiton scleroxylon*, $LD_{50,}$ and the effective dose to reduce to the barest minimum possible risks users might face.

MATERIALS AND METHODS

All the experimental protocols were in compliance with our Institutional Animal Ethics Committee guidelines as well as internationally accepted practices for use and care of laboratory animals as contained in US guidelines (National Institute of Health, 1992).

Experimental Animals : Albino rats (Wistar strain) with an average weight of 137.67 g were used in this study. They were obtained from the animal house of the College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria. The animals were housed in clean cages under standard laboratory conditions of temperature, humidity and light. They were allowed free 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.

Chemicals/Reagents : All reagents/chemicals used were of analytical grades.

Medicinal plant : The barks of *T. scleroxylon* were obtained from the forest of Uokha, Owan - East local government area, Edo State, Nigeria. They were then identified by experts in the Department of Botany, University of Ibadan, Ibadan, Oyo State, Nigeria, as *T. scleroxylon* K. Schum where a voucher specimen (UIH – 22329) had been deposited.

Extraction and Preparation of Plant Extracts

The barks of *T. scleroxylon* were washed with clean water, dried and cut into tiny strands. They were then 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 (Somchit et al., 2003). Dry concentrates obtained were kept in the desiccators until used. The yield was 13.36% (w/w) and 10.94% (w/w) for aqueous and 50% ethanol dried concentrates, respectively.

Plasma glucose determination: Glucose was determined according to procedure described by Randox Laboratories Ltd., UK.

Liver enzyme assays : Activities of liver marker enzymes (alkaline phosphatase (ALP), alanine amino transaminase (ALT) and γ –glutamyl transferase (γ-GT) were determined in the rat plasma by the method outlined by Randox Laboratories Ltd., UK.

Collection of Blood : The tail of the restrained rat was cleansed with a ball of cotton wool soaked in methylated spirit and vaseline was applied on the tail to reduce friction while massaging to redness. Gentle massage towards the tip of the tail continued until the tip became red; sign of blood accumulation. The red tip of the tail was then slightly incised and further massaged gently as the blood tickled into EDTA (for γ - GT assay), lithium heparin (for ALP and ALT assays) and fluoride oxalate (for glucose assay) sample tubes. The incised section of the tail was then cleansed with a ball of cotton wool soaked in methylated spirit to avoid any form of infection. Blood samples collected were subjected to centrifugation for 10 minutes at 3000 rpm to obtain the plasma for analyses. All analyses were carried out immediately after centrifugation.

Administration of extracts : Aqueous and 50% ethanol extracts of T. scleroxylon were administered to experimental rats orally (p.o) with the aid of the gavage.

Acute toxicity studies : The lethal doses (LD_{50}) of the aqueous and ethanol (50%) stem bark extracts of *T. scleroxylon* were determined by Lorke, (1983) method, using 13 rats of Wistar strain after two weeks of acclimatization to the standard animal cage conditions followed by an overnight fast. In phase one, male rats were randomly placed into three groups of three rats each and administered orally (p.o) aqueous bark extract of *T. scleroxylon* at the following doses: 10, 100 and 1000 mg/kg body weight (b.w), respectively. Another set of male rats in three groups of three rats each group, was also administered orally (p.o) ethanol (50%) bark extract of this plant in a similar manner as above. Female rats in separate groups as above, also received oral administration (p.o) of both extracts respectively at the same doses for phase one male rats. All the rats were observed for 24 hrs for signs of toxicity. In the second phase, four male rats in four different groups of one rat each, received oral administration (p.o) of aqueous bark extract at the doses of 1000, 1600, 2900 and 5000 mg/kg body weight. Ethanol (50%) extract was also administered at the same doses to another set of four male rats. Four female rats were also similarly treated. Again the rats were observed for 24 hrs for any signs of toxicity. The median lethal dose (LD_{50}) was calculated using the second phase.

Experimental procedure – Dose response studies

Male rats (Wistar strain) after acclimatization for a period of two weeks, were fasted overnight and randomly divided into eleven groups of three rats each and treated as follows:

Group 1: served as normal control and received distilled water.
Group 2A: served as test rats and received 200mg/kg b. w. of aqueous extract.
Group 2B: served as test rats and received 200mg/kg b. w. of 50% ethanol extract.
Group 3A: served as test rats and received 500mg/kg b. w. of aqueous extract.
Group 3B: served as test rats and received 500mg/kg b. w. of 50% ethanol extract.
Group 4A: served as test rats and received 1000mg/kg b. w. of aqueous extract.
Group 4B: served as test rats and received 1000mg/kg b. w. of 50% ethanol extract.
Group 5A: served as test rats and received 3000mg/kg b. w. of aqueous extract.
Group 5B: served as test rats and received 3000mg/kg b. w. of 50% ethanol extract.
Group 6A: served as test rats and received 5000mg/kg b. w. of aqueous extract.
Group 6B: served as test rats and received 5000mg/kg b. w. of 50% ethanol extract.

Histological studies : The rats were sacrificed on the 28th day and the kidneys, livers and hearts collected in 10% formalin for proper fixation. The tissues were processed and embedded in paraffin wax. Sections of 5-6µm in thickness were cut and stained with hematoxylin and eosin dyes for general tissue structures as reported by Pari and Amali (2005).

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 probability level were accepted as statistically significant.

RESULTS

Results have been presented in Tables 1 - 8. LD_{50} for both extracts (aqueous and 50% ethanol) of *T. scleroxylon* was beyond 5000 mg/kg body weight as the rats tolerated the extracts even at larger doses without any signs of acute toxicity. Tables 1 and 2 show dose dependent decrease ($p<0.05$) in plasma glucose concentration on the 6th, 12th, 18th and 28th days while Tables 3 to 8 elaborate dose dependent increase ($p<0.05$) in the activities of liver marker enzymes caused by administration (p. o.) of aqueous and 50% ethanol extracts of *T. scleroxylon* to the test rats for 28 days. Both extracts were safest at the dose of 200 mg/kg body weight as histological examination of tissues did not show adverse consequences (Slides 1 – 9).

Table 1: Mean plasma glucose concentrations (mg/dl) of normal rats administered increasing doses (mg/kgBW) of **aqueous** extract.

S/N	mg/kgBW	0days	1days	6days	12days	18days	28days
1.	Control	91.00±1.24^{a}	80.00±4.32^{a}	94.00±1.56^{a}	75.00±1.37^{a}	76.25±4.86^{a}	9.50±7.87^{a}
2.	200	88.51±0.01^{a}	76.13±1.12^{a}	54.92±3.39^{b}	42.63±4.70^{b}	14.96±0.54^{b}	18.28±0.96^{b}
3.	500	78.62±1.04^{a}	72.25±1.88^{a}	50.55±0.28^{b}	46.97±0.13^{b}	17.99±0.17^{c}	16.61±0.22^{b}
4.	1000	82.64±1.88^{a}	78.17±1.18^{a}	41.14±0.97^{c}	40.60±1.10^{b}	10.70±0.47^{b}	7.82±0.50^{c}
5.	3000	86.38±0.03^{a}	60.56±0.72^{a}	35.15±0.49^{c}	30.30±0.99^{c}	19.83±0.18^{c}	7.82±0.46^{c}
6.	5000	79.96±0.27^{a}	51.82±0.35^{a}	36.54±0.39^{c}	27.88±0.89^{c}	17.82±0.49^{c}	8.21±0.05^{c}

Data are mean ± S.E.M. (n = 3). Mean in the same column with different superscript letters are significantly different, p<0.05 (One –way ANOVA followed by post –hoc LSD). BW: body weight.

Table 2: Mean plasma glucose concentrations (mg/dl) of normal rats administered increasing doses (mg/kgBW) of **ethanol** extract.

S/N	mg/kgBW	0days	1days	6days	12days	18days	28days
1.	Control	91.00±1.24^{a}	80.00±4.32^{a}	94.00±1.56^{a}	75.00±1.37^{a}	76.25±4.86^{a}	99.50±7.87^{a}
2.	200	101.79±13.00^{a}	97.41±4.60^{a}	89.42±0.08^{a}	62.91±0.63^{a}	45.12±1.16^{b}	13.02±1.26^{b}
3.	500	106.52±9.37^{a}	104.52±5.41^{a}	73.40±1.42^{a}	70.98±0.25^{a}	51.25±1.60^{c}	9.63±0.08^{b}
4.	1000	98.26±7.59^{a}	88.69±0.71^{a}	78.87±0.14^{a}	49.09±1.76^{b}	23.35±1.06^{b}	12.24±0.46^{b}
5.	3000	99.13±0.78^{a}	82.74±3.04^{a}	69.99±0.66^{b}	45.07±2.87^{b}	12.92±0.16^{b}	11.46±0.28^{b}
6.	5000	94.78±2.05^{a}	84.78±2.77^{a}	71.25±0.81^{b}	62.51±1.45^{a}	21.30±0.84^{b}	14.06±1.57^{b}

Data are mean ± S.E.M. (n = 3). Mean in the same column with different superscript letters are significantly different, p<0.05 (One –way ANOVA followed by post –hoc LSD). BW: body weight.

Table 3: Mean plasma alkaline phosphatase activities (IU/l) of normal rats administered increasing doses (mg/kgBW) of **aqueous** extract.

S/N	mg/kg BW	0days	1days	6days	12days	18days	28days
1.	Control	35.12±0.57^{a}	37.87±2.00^{a}	31.39±1.11^{a}	36.49±0.98^{a}	36.01±8.27^{a}	43.27±2.84^{a}
2.	200	26.07±2.28^{a}	32.48±2.62^{a}	33.01±3.64^{a}	38.76±0.20^{a}	45.09±3.04^{a}	57.96±4.04^{a}
3.	500	22.08±0.09^{a}	44.40±2.74^{a}	73.17±2.28^{b}	83.57±4.40^{b}	90.93±3.51^{b}	168.36±10.92^{b}
4.	1000	47.48±3.68^{a}	50.59±3.93^{a}	73.61±3.76^{b}	84.15±1.14^{b}	105.52±3.35^{b}	177.87±10.93^{b}
5.	3000	36.00±1.42^{a}	56.92±2.48^{a}	63.48±4.48^{a}	77.48±6.72^{b}	95.22±1.27^{b}	213.90±6.21^{c}
6.	5000	32.44±5.09^{a}	59.31±7.62^{a}	133.86±7.37^{c}	85.52±3.62^{b}	154.56±3.86^{c}	250.42±0.56^{c}

Data are mean ± S.E.M. (n = 3). Mean in the same column with different superscript letters are significantly different, p<0.05 (One –way ANOVA followed by post –hoc LSD). BW: body weight.

Table 4: Mean plasma alkaline phosphatase activities of normal rats administered increasing doses (mg/kgBW) of **ethanol** extract.

S/N	mg/kg BW	0days	1days	6days	12days	18days	28days
1.	Control	35.12±0.57^{a}	37.87±2.00^{a}	31.39±1.11^{a}	36.49±0.98^{a}	36.01±8.27^{a}	43.27±2.84^{a}
2.	200	55.56±3.28^{a}	56.07±9.25^{a}	78.39±2.19^{a}	79.40±5.51^{a}	80.04±1.22^{a}	106.72±9.52^{b}
3.	500	62.84±12.71^{a}	62.99±4.13^{a}	143.59±6.63^{b}	224.97±3.69^{b}	206.81±3.06^{b}	250.52±11.32^{c}
4.	1000	62.84±9.64^{a}	65.48±0.38^{a}	136.48±4.99^{b}	158.72±23.52^{c}	303.93±5.47^{c}	320.33±2.06^{d}
5.	3000	40.48±4.26^{a}	55.53±7.17^{a}	89.94±0.12^{c}	193.13±17.40^{c}	228.44±1.16^{b}	385.53±3.27^{e}
6.	5000	52.04±1.13^{a}	62.55±5.04^{a}	132.40±18.45^{b}	208.84±5.14^{b}	282.80±0.95^{c}	391.92±4.54^{e}

Data are mean ± S.E.M. (n = 3). Mean in the same column with different superscript letters are significantly different, $p<0.05$ (One –way ANOVA followed by post –hoc LSD). BW: body weight.

Table 5: Mean plasma gamma glutamyl transferase activities (IU/l) of normal rats administered increasing doses (mg/kgBW) of **aqueous** extract.

S/N	mg/kg BW	0days	1days	6days	12days	18days	28days
1.	Control	45.23±2.71^{a}	45.78±0.79^{a}	46.29±0.34^{a}	44.28±2.82^{a}	47.77±4.81^{a}	59.72±7.19
2.	200	42.92±0.08^{a}	42.49±2.04^{a}	50.09±1.24^{a}	55.60±2.47^{a}	57.39±1.73^{a}	78.06±2.18^{a}
3.	500	53.94±2.07^{a}	54.30±3.90^{a}	57.64±0.29^{a}	67.96±8.75^{a}	75.90±6.54^{a}	114.74±3.12^{b}
4.	1000	74.85±3.06^{a}	72.05±3.41^{a}	109.17±3.97^{b}	112.90±12.85^{b}	131.47±21.27^{b}	161.37±3.32^{c}
5.	3000	75.10±2.97^{a}	78.90±7.99^{a}	108.53±1.54^{b}	126.21±21.81^{b}	154.01±25.41^{b}	193.45±7.90^{d}
6.	5000	76.98±0.27^{a}	79.74±3.05^{a}	166.59±22.83^{c}	179.37±9.38^{c}	186.97±11.70^{b}	194.45±1.52^{d}

Data are mean ± S.E.M. (n = 3). Mean in the same column with different superscript letters are significantly different, $p<0.05$ (One –way ANOVA followed by post –hoc LSD). BW: body weight.

Table 6: Mean plasma gamma glutamyl transferase activities (IU/l) of normal rats administered increasing doses (mg/kgBW) of **ethanol** extract.

S/N	mg/kg BW	0days	1days	6days	12days	18days	28days
1.	Control	45.23±2.71^{a}	45.78±0.79^{a}	46.29±0.34^{a}	44.28±2.82^{a}	47.77±4.81^{a}	59.72±7.19^{a}
2.	200	78.39±0.07^{a}	70.04±5.25^{a}	75.72±1.51^{a}	76.18±2.14^{a}	75.29±2.34^{a}	71.48±15.59^{a}
3.	500	78.51±6.71^{a}	75.83±2.08^{a}	84.59±3.27^{b}	87.24±5.75^{b}	89.58±1.37^{b}	16.92±22.42^{b}
4.	1000	62.70±7.67^{a}	77.21±0.60^{a}	86.48±7.79^{b}	109.65±1.37^{c}	143.64±9.32^{c}	153.27±0.46^{c}
5.	3000	66.60±7.80^{a}	79.08±0.45^{a}	89.94±0.12^{b}	100.75±7.73^{c}	157.32±1.66^{c}	186.85±2.82^{d}
6.	5000	47.48±2.77^{a}	80.70±3.17^{a}	132.40±4.48^{c}	94.57±2.81^{c}	166.01±21.11^{c}	190.27±2.40^{d}

Data are mean ± S.E.M. (n = 3). Mean in the same column with different superscript letters are significantly different, $p<0.05$ (One –way ANOVA followed by post –hoc LSD). BW: body weight.

Table 7: Mean plasma alanine amino transaminase activities (IU/l) of normal rats administered increasing doses (mg/kgBW) of **aqueous** extract.

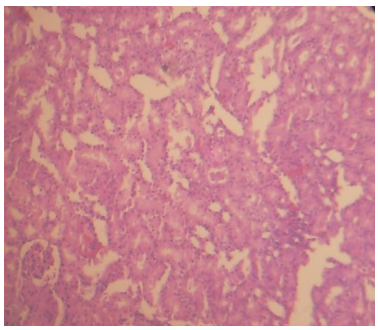
S/N	mg/kg BW	0days	1days	6days	12days	18days	28days
1.	Control	56.79±2.41^{a}	65.82±3.80^{a}	59.28±1.22^{a}	51.97±2.99^{a}	53.44±2.39^{a}	60.68±0.98^{a}
2.	200	68.67±0.89^{a}	67.05±2.29^{a}	60.17±2.97^{a}	60.43±1.57^{a}	62.94±2.70^{a}	66.42±6.71^{a}
3.	500	53.50±1.04^{a}	54.12±3.10^{a}	60.83±1.73^{a}	67.80±2.90^{a}	74.00±1.24^{b}	87.33±4.99^{b}
4.	1000	53.30±3.50^{a}	53.34±3.21^{a}	63.93±3.58^{a}	72.45±2.82^{b}	76.00±8.85^{b}	94.17±2.51^{b}
5.	3000	58.03±2.04^{a}	68.03±1.70^{a}	67.67±0.45^{a}	70.97±2.42^{b}	89.25±2.24^{c}	101.92±8.48^{c}
6.	5000	72.83±0.92^{a}	72.82±2.35^{a}	80.10±0.60^{b}	82.73±1.45^{b}	89.99±1.81^{c}	107.83±4.62^{c}

Data are mean ± S.E.M. (n = 3). Mean in the same column with different superscript letters are significantly different, $p<0.05$ (One –way ANOVA followed by post –hoc LSD). BW: body weight.

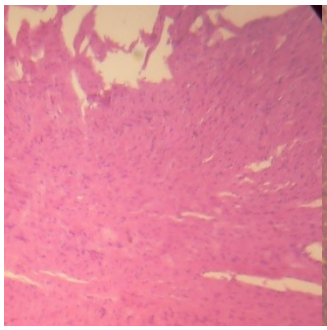
Table 8: Mean plasma alanine amino transaminase activities (IU/I) of normal rats administered increasing doses (mg/kgBW) of **ethanol** extract.

S/N	mg/kg BW	0days	1days	6days	12days	18days	28days
1.	Control	56.79±2.41^{a}	65.82±3.80^{a}	59.28±1.22^{a}	51.97±2.99^{a}	53.44±2.39^{a}	60.68±0.98^{a}
2.	200	74.50±4.19^{a}	75.17±2.96^{a}	76.58±2.01^{b}	77.33±1.67^{b}	80.07±4.46^{b}	82.62±2.77^{b}
3.	500	66.83±2.54^{a}	66.27±2.29^{a}	77.70±3.16^{b}	83.63±6.65^{c}	86.67±7.34^{b}	90.40±4.19^{b}
4.	1000	66.67±0.84^{a}	68.85±0.83^{a}	72.13±3.46^{b}	72.07±0.37^{b}	74.50±2.15^{c}	96.71±2.53^{b}
5.	3000	76.80±3.76^{a}	77.37±4.64^{a}	79.13±2.69^{b}	80.17±7.19^{c}	83.53±0.77^{b}	126.67±2.78^{c}
6.	5000	78.83±0.41^{a}	80.51±0.84^{a}	81.20±4.99^{c}	86.45±6.77^{c}	101.93±0.69^{d}	169.33±15.01^{c}

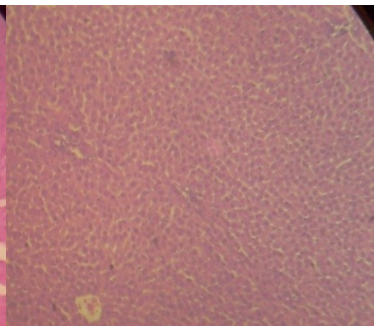
Data are mean ± S.E.M. (n = 3). Mean in the same column with different superscript letters are significantly different, $p<0.05$ (One –way ANOVA followed by post –hoc LSD). BW: body weight.



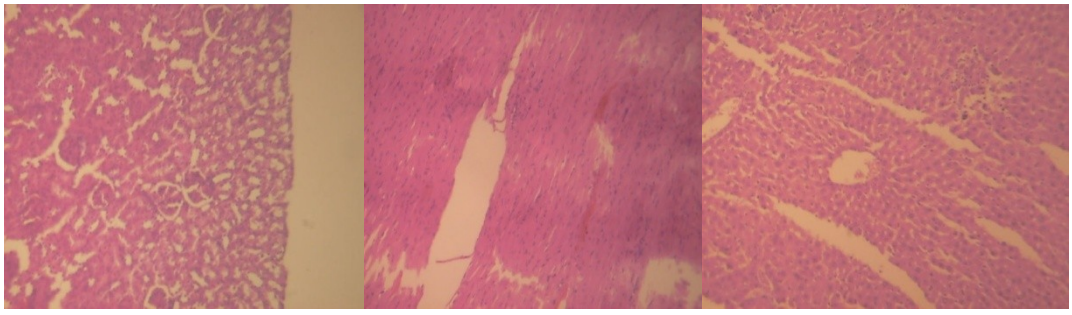
Slide 1: Rat Kidney: Control (x160)



Slide 2: Rat Heart: Control (x160)



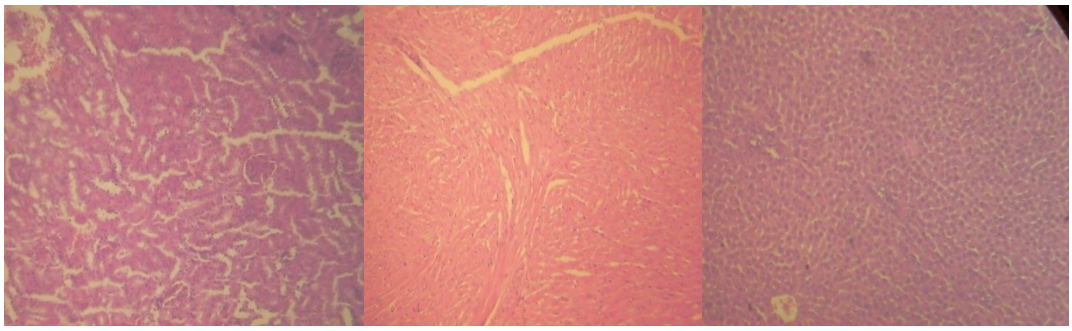
Slide 3: Rat Liver: Control (x160)



Slide 4: Rat Kidney: 200mg/kg b. w. extract of TS (X160)

Slide 5: Rat Heart: 200mg/kg b. w. aqueous extract of TS (x160) (Normal)

Slide 6: Rat Liver: 200mg/kg b.w aqueous aqueous extract of TS (x160) (Normal) (Normal)



Slide 7:Rat Kidney: 200mg/kg b. w. ethanol(50%) extract of TS (x160) Normal

Slide 8: Rat Heart: 200mg/kg b. w. ethanol(50%) extract of TS (x160) Normal

Slide 9: Rat Liver: 200mg/kg b. w. ethanol(50%) extract of TS (x160) Normal

DISCUSSION

Acute toxicity studies are essential to prevent any overdose of drug which may interfere with results of experiment. These studies are also useful in understanding toxicity profiles of plant extracts (Ozbek et al., 2004). The result of acute oral toxicity study in rats (Wistar strain) recorded the LD_{50} of both aqueous and ethanol extracts of *Triplochiton scleroxylon* to be beyond 5000 mg/kg body weight (bw), as the experimental rats tolerated the extracts without any symptoms of acute toxicity (no mortality, skin changes, aggressiveness, diarrhoea, restiveness, seizures, dizziness, weakness, or withdrawal from either food or water) even at larger doses of extracts administered. The lower the LD_{50}, the more toxic the extract tested. LD_{50} beyond 5000 mg/kg bw is of no experimental significance (Lorke, 1983). Tanko et al., (2007), reported LD_{50} of beyond 5000 mg/kg bw for the leaves of *Cissampelos mucronata (Menispermaceae)* in rats (Wistar strain). However, LD_{50} of 4000 mg/kg bw (i.p.) has been reported for the methanolic extract of the leaves of *Salvia officinalis;* sage, in streptozotocin-induced diabetic rats (Eidi et al., 2005) while Azu et al., (2010), reported LD_{50} of 3,981.07 mg/kg bw in methanolic fruit extract of *Kigelia africana*. However, Agbaje et al., (2009), reported LD_{50} for both intraperitoneal and oral routes of *Syzigium aromaticum* (L.) in rodents as 263 and 2500 mg/kg bw respectively.

Tables 1 and 2 show the results of effects of increasing doses (200, 500, 1000, 3000 and 5000 mg/kg bw) of aqueous and 50% ethanol extracts of *T. scleroxylon* on plasma glucose concentration. Increasing doses of *T. scleroxylon* resulted in significant decreases ($p<0.05$) in plasma glucose on the 6^{th}, 12^{th}, 18^{th}, and 28^{th} days when compared to control for both aqueous and 50% ethanol extracts. Some plant extracts reported to have hypoglycemic properties include *Morinda lucida* and *Tetracera alnifolia* (Onoagbe et al., 1999a), *Uvaria chamae* (Onoagbe and Esekheigbe, 1999), *Irvingia grandifolia* (Onoagbe et al., 1999b), *Cissampelos mucronata* (Tanko et al., 2007), *Parinari excels* (Ndiaye et al., 2008), *Leandra lacunosa* (Cunha et al., 2008), *Mucuna pruriens* (Bhasker et al., 2008),

Tephrosia purpurea (Pavana et al., 2009), and *Ageratum conyzoides L* (Nyunai et al., 2009). Medicinal plant extracts that possess hypoglycaemic properties would be useful in the management of diabetes mellitus (Prohp and Onoagbe, 2009a, b). Aqueous and 50% ethanol extracts of *T. scleroxylon* may have caused plasma glucose lowering by stimulating the release of insulin from the β-cell of the pancreas or by influencing rapid mobilization of plasma glucose across the membrane bound insulin receptors.

Tables 3 – 8 show significant increases ($p<0.05$) on the 6^{th}, 12^{th}, 18^{th}, and 28^{th} days in the activities of alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) and alanine amino transaminase (ALT) with the highest increases obtained at higher doses for both extracts. These enzymes are common ones used in clinical diagnosis as liver marker enzymes (http://www.gpnotebook.co.uk). Elevated levels of these enzymes in plasma are reflections of either of the following: their reduced clearance or increased proliferation of cells, increased rate of cell turnover, and/or increased cell damage or increased rate of enzyme synthesis (induction of microsomal enzymes by certain drugs). Decreased levels of enzyme activities in plasma are very rare and where they occur it could be either due to reduced synthesis, congenital deficiency or due to the presence of inherited variants of relatively low biological activity like cholinesterase variants (Raju and Mandala, 2005). Increases in the activities of these enzymes obtained must have been due to leakages from the liver as a result of damage on it caused by increasing doses of aqueous and 50% ethanol extracts of *T. scleroxylon*. Increased levels of ALP, ALT and γ-GT in the plasma are mainly associated with liver diseases. Doses of extracts of *T. scleroxylon* beyond 500 mg/kg bw, could constitute a health risk and can lead to liver damage (Tables 3 – 8). The result of the dose response studies, however, showed that at 200 mg/kg body weight the liver enzyme markers investigated recorded the least activities in the plasma of experimental rats for both extracts (Tables 3 – 8). This observation corroborates with the result of histological examination of tissues after 28 days of administration of extracts where no adverse histological changes were observed at the dose of 200 mg/kg body weight when compared with control slides (Slides 1 – 9). Avci et al., (2006), reported 100 mg/kg bw as effective dose for aqueous and ethanol extracts of *Agrostemma githago*, *Potentilla reptans*, *Thymbra spicata*, *Urtica dioica*, and *Viscum album*. Patil et al., (2009), documented 40 mg/kg bw (subcutaneously) for *Lactuca sativa* (lettuce), *Petroselinum crispum* (parsley) and *Bacopa monniera* (brahmi). However, Ikpi et al., (2009) reported 15 g/kg body weight for aqueous leaf extract of *Rothmannia longiflora*. Tanko et al., (2007), Nadro (2010) and Omonkhua (2010) also reported 200 mg/kg body weight as an effective dose for an ethanol extract of *Cissampelos mucronata*, leaf extracts of *Cassia italica*. and aqueous bark, root and leaf of *Irvingia gabonensis*, *Urena lobata and Carica papaya,* respectively.

CONCLUSION

Aqueous and 50% ethanol extracts of *Triplochiton scleroxylon* were well tolerated by the experimental animals even at larger doses. 200mg/kg body weight was the safest dose that resulted in blood glucose lowering (hypoglycaemia) and will be effective in the treatment of diabetes mellitus.

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