www.jmscr.igmpublication.org
Impact Factor (SJIF): 6.379
Index Copernicus Value: 79.54
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossrefDOI: https://dx.doi.org/10.18535/jmscr/v6i8.132

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Hepatotoxicity of higher doses of stem bark extracts of *Triplochiton scleroxylon* in normal rats

Authors
Prohp[1] T. P., Inegbedion[2], A., Onoagbe[3] I. O.
[1]Department of Biochemistry, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State. Nigeria
[2]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria
[3]Department of Biochemistry, Faculty of life Sciences, University of Benin, Benin City, Edo State, Nigeria
Corresponding Author
Prof. T. P.Prohp.
Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria
Postal: P.O.Box 1373, Yenagoa, Bayelsa State, Nigeria
GSM: +2347035700176; E-mail: *theprophetprohp@yahoo.com*

Abstract
In this study, stem bark extracts of Triplochiton scleroxylon used as panacea for diabetes mellitus in some parts of Nigeria were examined for hepatotoxic activities.The activities of important liver enzyme markers were investigated periodically in the plasma of experimental rats (Wistar strain) with standard methods involving spectrophotometry while sections of the liver were subjected to appropriate histological examination for tissue structures after 28 days of administration of stem bark extracts. Findings showed that aqueous and ethanolic stem bark extracts of Triplochiton scleroxylon caused significant dose-dependent increases in the activities of plasma alkaline phosphatase (ALP), γ-glutamyl transferase (γ-GT) and alanine aminotransaminase (ALT) relative to normal control ($P<0.05$). Histological examination reported adverse changes in the liver of test rats with increasing doses of aqueous and ethanolic stem bark extracts beyond 200 mg/kg body weight with the former being safer. Like synthetic drugs, abuse of herbal concoction and decoction could stimulate chronic systemic complications with fatal consequences.
Keywords: *Hepatotoxicity, Stem bark extracts, Triplochiton scleroxylon, systemic complication.*

Introduction

Triplochiton scleroxylon is one of the over 30 medicinal plants in Nigeria used in some rural areas in the south and west of the country to treat diabetes mellitus. Some urban dwellers who are diabetic especially from amongst the low income earners also use aqueous extract of *Triplochiton scleroxylon* to manage their conditions. This medicinal plant is a deciduous tree commonly found in semi-deciduous forest along waterways. It belongs to the kingdom: plantae, division: magnoliophyta, class: magnoliopsida, order: malvales, family: sterculiaceae, genus: triplochiton and species: T. scleroxylon (Raju and

Mandala, 2005). It is a large tree of 150 to 180 feet high and boles straight, cylindrical and has a trunk diameter that measures up to 5 feet (Ritchter and Dallwitz, 2000).*Triplochitonscleroxylon* rarely flowers in its native West Africa until at least 15 years old and the flowers are frequently impaired by pests (Leakey *et al.*, 1981). In Nigerian it is called Obeche while in Ghana, Cameroon, Ivory Coast and Germany it is known as wawa, ayous, samba and abachi, respectively. The trade name in Britain is Obeche. As no satisfactory therapy is available to cure diabetes mellitus in modern medicine, *Triplochiton scleroxylon* is one of the several tropical medicinal plants whose hypoglycemic and anti-diabetic activities at the dose of 200 mg/kg body weight have been well studied and reported in literature as safe for and useful in the treatment of diabetes mellitus in rats (Prohp and Onoagbe, 2009, 2011, 2012, 2013, 2014). Drug abuse is very common especially with herbal mixtures and the ignorance displayed by users of herbal extracts often results in severe life-threatening complications. In this study the effect of increasing doses of aqueous and ethanolic extracts of *Triplochitonscleroxylon* on the liver of normal rats after 28 days of administration was investigated.

Materials and Methods

Experimental animals

Male albino rats (Wistar strain) were obtained from the animal house of the College of Health Sciences, Faculty of Basic Medical Sciences, Niger Delta University, Wilberforce Island Bayelsa State, Nigeria. All the experimental rats were allowed free access to standard laboratory food pellets and distilled water for a period of two weeks to acclimatize to the new environment. The experimental protocols were according to our Institutional Animal Ethics Committee (IAEC) guidelines as well as internationally accepted practices for use and care of laboratory animals as contained in US guidelines (NIH, 1992).

Chemicals/Reagents

All reagents/chemicals used were of analytical grades.

Medicinal plant

The fresh stem barks of experimental samples were identified as *Triplochiton scleroxylon* K. Schum (voucher specimen number: UIH – 22329) by experts in the Department of Botany, University of Ibadan, Ibadan, Oyo State, Nigeria.

Extraction and preparation of plant extracts

The barks of *Tripochiton scleroxylon* were washed with clean water, dried and cut into small pieces. They were pulverized into powder and 1000 g of powdered bark of this plant was then extracted separately in 7000 ml of 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 with the aid of a freeze dryer. The freeze dried samples were then kept in the freezer at -21 ^{o} C until used (Prohp and Onoagbe, 2014).

Blood collection

The rat was restrained while the tail was cleansed with a ball of cotton wool soaked in methylated spirit. The tail of rat was gently and repeatedly massaged towards the tip following a vaseline smear. The red tip of the tail was then slightly and carefully incised with a new and sterilized blade and further massaged gently as the blood trickled into immobilized fluoride oxalate sample tubes. Cotton wool soaked in methylated spirit was again used to cleanse the incised area of the tail. Blood samples collected with the lithium heparin sample tubes were subjected to centrifugation for 10 minutes at 3,000 g to obtain the plasma for liver enzymes assays. Analysis was carried out immediately after centrifugation (Prohp and Onoagbe, 2014).

Experimental procedure

A total of forty four male rats (Wistar strain) after acclimatization for a period of two weeks were fasted overnight and randomly divided into eleven groups of four rats each. Control group received distilled water. Test male rats in five groups of four rats each were treated with 200, 500, 1000, 2500 and 5000 mg/kg body weight of aqueous extracts of *Triplochiton scleroxylon* respectively. Another set of male rats in five groups of four rats each received ethanolic extracts at the same doses as above. γ-Glutamyl transferase (EC. 2. 3. 2. 2), alanine amino transaminase (EC. 2. 6. 1. 2) and alkaline phosphatase(EC. 3. 1. 3. 1) activities were monitored periodically at intervals of six days according to an optimized standard colorimetric methods of the Deutsche Gesellschaft fur Klinische Chemie as outlined in Randox Laboratories manual.

Administration of extracts

Aqueous and ethanolic extracts of *Triplochiton scleroxylon* were administered to experimental rats orally (p. o.) twice daily with the aid of the gavage.

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 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 (SPSS, 2007).

Results

The data on plasma alkaline phosphatase, gamma glutamyl transferase and alanine aminotransaminase activities in normal rats after 28 days of administration of aqueous and ethanolic extracts of *Triplochiton scleroxylon* are presented (Tables 1 - 6). Higher doses of both extracts resulted in elevated activities of all liver marker enzymes investigated. Photomicrographs of liver sections are also presented (Plates A to E). Extracts were safest at 200 mg/kg body weight (Plate A) but resulted in adverse histological changes in the liver of test rats with increasing doses (Plates B to E).

Table 1: Mean plasma alkaline phosphatase activities (units of paranitrophenol released/min/mg protein) following treatment with aqueous extract of *Triplochiton scleroxylon* at increasing doses.

S/N.	Treatments	Day 0	Day 1	Day 6	Day 12	Day 18	Day 24	Day 28
1.	Control	40.89±0.30^{a}	39.89±0.22^{a}	40.81±0.20^{a}	41.14±0.24^{a}	41.73±0.25^{a}	41.76±0.35^{a}	44.56±0.26^{a}
2.	200mg/kg bw	39.51±0.28^{a}	40.08±0.27^{a}	42.81±0.38^{a}	45.23±0.14^{a}	46.96±0.42^{a}	49.08±0.27^{a}	58.92±0.31^{a}
3.	500mg/kg bw	39.28±0.17^{a}	40.51±0.16^{a}	69.37±0.25^{b}	77.32±0.35^{b}	89.67±0.44^{b}	121.08±0.30^{b}	157.98±0.29^{b}
4.	1000mg/kg bw	39.98±0.28^{a}	41.08±0.22^{a}	69.85±0.21^{c}	83.00±0.41^{c}	99.11±0.28^{c}	142.05±0.32^{c}	187.05±0.25^{c}
5.	2500mg/kg bw	38.82±0.33^{a}	41.46±0.19^{a}	72.56±0.22^{d}	87.04±0.34^{d}	110.55±0.26^{d}	172.27±0.31^{d}	222.14±0.38^{d}
6.	5000mg/kg bw	40.74±0.29^{a}	41.80±0.34^{a}	78.68±0.27^{e}	92.83±0.65^{e}	120.14±0.15^{e}	193.85±0.23^{e}	261.89±0.33^{e}

$^{a-f}$ Test values (mean ± S.E.M. (n = 4)) in the same column with different superscript letters from the control are significantly different (P<0.05).

Table 2: Mean plasma alkaline phosphatase activities (units of paranitrophenol released/min/mg protein) following treatment with ethanolic extract of *Triplochiton scleroxylon* at increasing doses.

S/N.	Treatments	Day 0	Day 1	Day 6	Day 12	Day 18	Day 24	Day 28
1.	Control	40.89±0.30^{a}	39.89±0.22^{a}	40.81±0.20^{a}	41.14±0.24^{a}	41.73±0.25^{a}	41.76±0.35^{a}	44.56±0.26^{a}
2.	200mg/kg bw	41.35±0.06^{a}	41.70±0.36^{a}	43.14±0.27^{a}	43.79±0.23^{a}	48.87±0.33^{a}	52.68±0.24^{a}	59.09±0.33^{a}
3.	500mg/kg bw	40.20±0.41^{a}	40.52±0.18^{a}	80.85±0.26^{b}	162.67±0.24^{b}	210.86±0.41^{b}	220.80±0.24^{b}	252.97±0.27^{b}
4.	1000mg/kg bw	40.90±0.26^{a}	41.30±0.38^{a}	94.94±0.24^{c}	74.80±0.26^{c}	239.83±0.24^{c}	280.72±0.18^{c}	298.78±0.34^{c}
5.	2500mg/kg bw	39.39±0.19^{a}	41.78±0.30^{a}	97.90±0.23^{d}	187.81±0.24^{d}	261.22±0.38^{d}	319.65±0.28^{d}	348.92±0.24^{d}
6.	5000mg/kg bw	40.12±0.29^{a}	43.10±0.29^{a}	150.84±0.37^{e}	201.08±0.15^{e}	290.69±0.30^{e}	370.24±0.27^{e}	431.12±0.34^{e}

$^{a-f}$Test values (mean ± S.E.M. (n = 4)) in the same column with different superscript letters from the control are significantly different (P<0.05).

Table 3: Mean plasma gamma glutamyltransferase activities (units of paranitroaniline released/min/mg protein) following treatment with aqueous extract of *Triplochiton scleroxylon* at increasing doses.

S/N.	Treatments	Day 0	Day 1	Day 6	Day 12	Day 18	Day 24	Day 28
1.	Control	47.71±0.26^{a}	48.02±0.04^{a}	48.36±0.13^{a}	48.73±0.24^{a}	48.96±0.39^{a}	53.14±0.26^{a}	56.01±0.39^{a}
2.	200mg/kg bw	48.85±0.42^{a}	48.90±0.41^{a}	54.13±0.27^{a}	59.24±0.16^{b}	63.91±0.19^{b}	72.82±0.31^{b}	81.06±0.44^{b}
3.	500mg/kg bw	47.11±0.36^{a}	47.39±0.15^{a}	57.73±0.31^{b}	69.94±0.34^{c}	78.75±0.28^{c}	98.85±0.34^{c}	122.95±0.31^{c}
4.	1000mg/kg bw	46.39±0.29^{a}	46.77±0.06^{a}	81.73±0.27^{c}	99.47±0.19^{d}	119.87±0.30^{d}	145.02±0.21^{d}	174.71±0.24^{d}
5.	2500mg/kg bw	49.68±0.21^{a}	51.02±0.28^{a}	97.80±0.32^{d}	124.65±0.19^{e}	147.80±0.29^{e}	172.89±0.30^{e}	198.36±0.28^{e}
6.	5000mg/kg bw	50.03±0.39^{a}	51.78±0.24^{a}	120.14±0.31^{e}	145.62±0.29^{f}	170.77±0.31^{f}	195.17±0.19^{f}	270.81±0.37^{f}

$^{a-f}$ Test values (mean ± S.E.M. (n = 4) in the same column with different superscript letters from the control are significantly different (P<0.05).

Table 4: Mean plasma gamma glutamyltransferase activities (units of paranitroaniline released/min/mg protein) following treatment withethanolic extract of *Triplochiton scleroxylon* atincreasing doses.

S/N.	Treatments	Day 0	Day 1	Day 6	Day 12	Day 18	Day 24	Day 28
1.	Control	47.71±0.26^{a}	48.02±0.04^{a}	48.36±0.13^{a}	48.73±0.24^{a}	48.96±0.39^{a}	53.14±0.26^{a}	56.01±0.39^{a}
2.	200mg/kg bw	50.81±0.26^{a}	51.18±0.20^{a}	60.89±0.28^{b}	62.62±0.27^{b}	62.91±0.28^{b}	63.22±0.29^{b}	63.51±0.17^{b}
3.	500mg/kg bw	51.56±0.29^{a}	51.57±0.29^{a}	62.70±0.25^{b}	67.05±0.26^{c}	72.74±0.21^{c}	84.88±0.24^{c}	121.94±0.39^{c}
4.	1000mg/kg bw	49.91±0.35^{a}	50.77±0.31^{a}	72.97±0.21^{c}	98.96±0.35^{d}	128.98±0.40^{d}	151.56±0.30^{d}	178.79±0.25^{d}
5.	2500mg/kg bw	52.08±0.23^{a}	52.48±0.08^{a}	79.69±0.26^{d}	125.17±0.10^{e}	153.01±0.40^{e}	169.00±0.37^{e}	197.65±0.28^{e}
6.	5000mg/kg bw	52.67±0.27^{a}	53.11±0.30^{a}	99.10±0.35^{e}	147.02±0.23^{f}	172.16±0.34^{f}	190.70±0.26^{f}	220.96±0.29^{f}

$^{a-f}$ Test values (mean ± S.E.M. (n = 4)) in the same column with different superscript letters from the control are significantly different (P<0.05).

Table 5: Mean plasma alanine aminotransaminase activities (units of pyruvate hydrazone released/min /mg protein) following treatment with aqueous extract of *Triplochiton scleroxylon* at increasing doses.

S/N.	Treatments	Day 0	Day 1	Day 6	Day 12	Day 18	Day 24	Day 28
1.	Control	57.67±0.21^{a}	57.81±0.21^{a}	58.22±0.22^{a}	58.14±0.24^{a}	58.14±0.25^{a}	57.60±0.24^{a}	58.32±0.05^{a}
2.	200mg/kg bw	59.00±0.27^{a}	59.07±0.28^{a}	64.92±0.30^{b}	68.80±0.23^{b}	72.96±0.34^{b}	75.63±0.22^{b}	83.09±0.29^{b}
3.	500mg/kg bw	55.01±0.27^{a}	55.09±0.26^{a}	67.20±0.14^{c}	74.63±0.37^{c}	79.81±0.33^{c}	90.22±0.33^{c}	97.95±0.36^{c}
4.	1000mg/kg bw	56.82±0.34^{a}	56.81±0.31^{a}	69.66±0.13^{d}	78.81±0.24^{d}	84.57±0.28^{d}	95.15±0.34^{d}	104.30±0.27^{d}
5.	2500mg/kg bw	56.41±0.01^{a}	56.63±0.09^{a}	70.08±0.27^{d}	79.86±0.28^{e}	88.79±0.24^{e}	99.52±0.24^{e}	127.02±0.28^{e}
6.	5000mg/kg bw	58.89±0.30^{a}	59.34±0.38^{a}	74.99±0.27^{e}	83.81±0.27^{f}	97.47±0.23^{f}	110.84±0.23^{f}	130.76±0.30^{f}

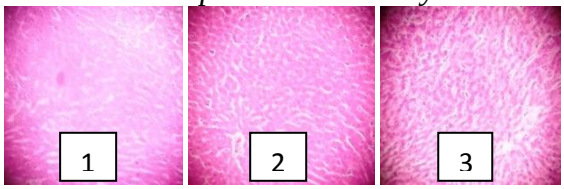
$^{a-f}$ Test values (mean ± S.E.M. (n = 4)) in the same column with different superscript letters from the control are significantly different (P<0.05).

Table 6: Mean plasma alanine aminotransaminase activities (units of pyruvate hydrazone released/min /mg protein) following

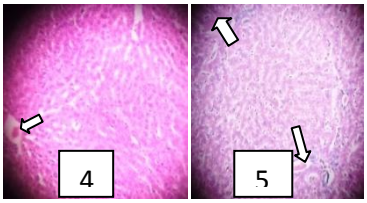
S/N.	Treatments	Day 0	Day 1	Day 6	Day 12	Day 18	Day 24	Day 28
1.	Control	57.67±0.21^{a}	57.81±0.21^{a}	58.22±0.22^{a}	58.14±0.24^{a}	58.14±0.25^{a}	57.60±0.24^{a}	58.32±0.05^{a}
2.	200mg/kg bw	60.58±0.25^{a}	60.64±0.26^{a}	62.62±0.02^{b}	62.49±0.27^{b}	69.04±0.27^{b}	78.77±0.30^{b}	87.95±0.32^{b}
3.	500mg/kg bw	59.96±0.24^{a}	60.03±0.24^{a}	69.77±0.27^{c}	75.34±0.03^{c}	84.37±0.28^{c}	87.52±0.26^{c}	95.00±0.27^{c}
4.	1000mg/kg bw	60.12±0.47^{a}	60.25±0.46^{a}	74.37±0.05^{d}	78.26±0.19^{d}	89.07±0.23^{d}	94.18±0.31^{d}	106.96±0.25^{d}
5.	2500mg/kg bw	59.71±0.40^{a}	59.74±0.40^{a}	82.06±0.26^{e}	88.34±0.03^{e}	95.07±0.23^{e}	104.60±0.23^{e}	118.81±0.25^{e}
6.	5000mg/kg bw	59.97±0.31^{a}	60.08±0.27^{a}	85.70±0.27^{f}	93.18±0.27^{f}	101.50±0.25^{f}	114.90±0.29^{f}	128.83±0.26^{f}

$^{a-f}$ Test values (mean ± S.E.M. (n = 4)) in the same column with different superscript letters from the control are significantly different (P<0.05).

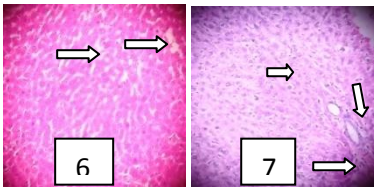
treatment with ethanolic extract of *Triplochiton scleroxylon* at increasing doses.



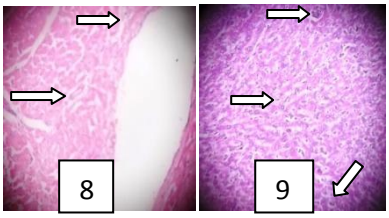
Plates A: Photomicrograph of liver sections showing normal histology after 28 days of oral administration of (1): distilled water (normal control), (2): 200mg/kg body weight of aqueous extract and (3): 200 mg/kg body weight of ethanolic extract of *Triplochiton scleroxylon*. Hematoxylin and eosin stain.X 100.



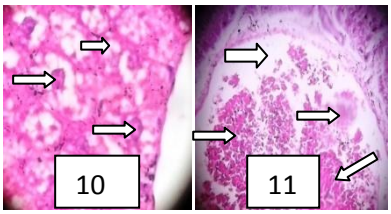
Plates B: Photomicrographs of liver sections showing infiltration of inflammatory cells after 28 days of oral administration of 500mg/kg body weight of aqueous extract(4)and infiltration of inflammatory cells and necrosis at 500 mg/kg body weight of ethanolic extract(5)of *Triplochiton scleroxylon*. Hematoxylin and eosin stain. X 100.



Plates C: Photomicrographs of liver sections showing fatty changes after 28 days of oral administration of1000mg/kg body weight of aqueous extract(6)and fatty changes, vacuolation and congestion of sinusoids at 1000 mg/kg body weight of ethanolic extract (7) of *Triplochiton scleroxylon*. Hematoxylin and eosin stain. X 100.



Plates D: Photomicrographs of liver sections showing fatty changes after 28 days of oral administration of2500mg/kg body weight of aqueous extract(8)and fatty changes with vacuolationat 2500 mg/kg body weight of ethanolic extract (9) of *Triplochiton scleroxylon*. Hematoxylin and eosin stain. X 100.



Plates E: Photomicrographs of liver sections showing apoptosis and fatty changes after 28 days of oral administration of 5000mg/kg body weight of aqueous extract (10) and fatty changes, necrosis and apoptotic bodies at 5000 mg/kg body weight of ethanolic extract (11) of *Triplochiton scleroxylon*. Hematoxylin and eosin stain. X 100.

Discussion

Elevated levels of liver enzyme markers (alkaline phosphatase, γ-glutamyltransferase and alanine amino transaminase) in the plasma are usually indicative of their reduced clearance, increased cell damage or increased rate of enzyme synthesis due to 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). Increase in the activities of alkaline phosphatase (Tables 1 and 2), γ-glutamyltransferase (Tables 3 and 4) and alanine amino transaminase (Tables 5 and 6) obtained in this study must have been due to leakages from

the liver as a result of damage on it caused by higher doses of both aqueous and ethanolic extracts of *Triplochiton scleroxylon*. Elevation in the activities of liver biomarkers in the plasma is often useful as an index of liver damage (Paula *et al*., 2009) and clinically associated with liver diseases (Kaplan *et al*., 1997; Nelson and Cox, 2001). At 200 mg/kg body weight the activities of plasma liver enzyme markers were lowest following the administration of aqueous and ethanolic extracts of *Triplochiton scleroxylon* to normal rats.

Normal histology was observed at 200 mg/kg body weight with both extracts (Plates A). At 500 mg/kg body weight aqueous and ethanolic extracts caused infiltration of inflammatory cells (Plates B). Fatty changes and vacuolation with congestion of sinusoids were observed with aqueous and ethanolic extracts at 1000 mg/kg body weight respectively (Plates C). Fatty changes were very prominent in the livers at 2500 mg/kg body weight (Plates D) while extracts caused apoptosis besides fatty changes at 5000 mg/kg body weight (Plates E).At 200 mg/kg body weight both extracts of *Triplochiton scleroxylon* exhibited little or no adverse histology, thus ascertaining this dose as effective in the treatment of experimental diabetes in rats. Avci*et al.,* (2006) reported 100 mg/kg body weight as effective dose for aqueous and ethanolic extracts of *Agrostemma githago, Potentilla reptans, Thymbra spicata, Urticadioica* and *Viscum album.* Patil*et al.,* (2009), documented 40 mg/kg body weight (subcutaneously) for *Lactuca sativa* (lettuce), *Petroselinum crispum* (parsley) and *Bacopa monniera* (brahmi). While 15 g/kg body weight was reported for aqueous leaf extract of *Rothmannia longiflora* (Ikpi*et al.,* 2009), the findings of Tanko *et al.*,(2007) of 200 mg/kg body weight as an effective dose for ethanol extract of *Cissampelos mucronata* agreed with the result of this study.

Conclusion

At 200 mg/kg body weight aqueous and ethanolic extracts of *Triplochiton scleroxylon* have proven to be effective in decreasing plasma glucose concentration ($P<0.05$) in several reports. However, higher doses of these extracts are not safe as adverse histological effects are enormous and almost life threatening as is common with drug abuse.

References

1. Avci, G., Kupeli, E., Eryavuz, A., Yesilada, E. and Kucukkurt, I. (2006). Antihypercholesterolemic and antioxidant activity assessment of some plants used as remedy in Turkish folk medicine.*Journal of Ethno-Pharmacology*.107: 418 – 423.
2. Ikpi, D. E., Obembe, A. O. &Nku, C. O. (2009). Aqueous leaf extract of *Rothmannia longiflora* improves basal metabolic rate and electrolyte parameters in alloxan-induced diabetic rats. Nigerian Journal of Physiological Sciences. 24 (1), 67 – 71.
3. Kaplan, A., Jack, R., Opheim, K.E., Toivola, B. and Lyon, A.W. (1997). Enzymes. In: *Clinical Chemistry, Interpretation and Techniques.* (4thed.) Williams and Wilkins, Baltimore, USA. 300 - 307.
4. Leakey, R.R.B., Ferguson, N.R. and Longman, K. A. (1981).Precious flowering and reproductive biology of *Triplochiton scleroxylon* K. Schum. Common wealth Forestry Research. 60 (2): 117 – 126.
5. Nelson, D. L. and Cox, M. M. (2001).Assays for tissue damage. In: *Principles of Biochemistry* (3rd ed.). Worth Publishers Inc., New York. 621.
6. NIH (National Institute of Health) (1992). Institutional animal care and use committee guidebook. NIH Publication, Washington D.C. USA. Government Printing Office. 92 – 3415.
7. Patil, R. B., Vora, S. R. and Pillai, M. M. (2009). Antioxidant effect of plant extracts

on phospholipid levels in oxidatively stressed male reproductive organs in mice. *Iranian Journal of Reproductive Medicine*.7 (1): 35 – 39.

8. Paula, H., Pedrosa, M. L., Junior, J. V. R., Haraguchi, F. K., Cardoso dos Santos, R. and Silva, M. E. (2009). Effect of an aqueous extract of annatto (*Bixaorellana*) seeds on lipid profile and biochemical markers of renal and hepatic function in hypercholesterolemic rats. *Brazilian Archives of Biology and Technology*.52 (6): 1373 – 1378.
9. Prohp, T. P.,Onoagbe, I. O. and Uwumarongie, O. H. (2011).Importance of knowledge of chemical composition of stem bark of *Triplochitonscleroxylon* K. Schum. in traditional treatment of diabetes mellitus in Nigeria. African Journal of Plant Science.5(10): 552 - 556.
10. Prohp, T. P., and Onoagbe, I. O. (2009a). Anti – diabetic properties and Toxicologial studies of *Triplochitonscleroxylon* on liver enzymes in normal and streptozotocin – induced diabetic rabbits. *Pakistan Journal of Nutrition*.8 (7): 1018 – 1024.
11. Prohp, T. P., and Onoagbe, I. O. (2009b). Anti – diabetic properties and Toxicologial studies of *Triplochiton scleroxylon* on Heart enzymes in normal and streptozotocin – induced diabetic rabbits. *Pakistan Journal of Nutrition*.8 (7): 1025 – 1029.
12. Prohp, T. Pand Onoagbe I. O. (2011). Anti-diabetic studies of aqueous extract of *Triplochitonscleroxylon* on platelets and associated parameters in alloxan-induced diabetic rabbits. *African Journal of Plant Science*.5(12): 697 – 701.
13. Prohp, T.P. and Onoagbe, I. O. (2012a). Effects of aqueous extract of *Triplochiton scleroxylon* on some haematological parameters and blood glucose concentrations in non-diabetic rabbits. *African Journal of Biotechnology*. 11 (1): 198 – 202. DO1:105897/AJB11.2819.
14. Prohp, T. P. and Onoagbe I. O. (2013a).Anti-diabetic and anti-oxidant activities of extracts of *Triplochiton scleroxylon* K. Schum in diabetic rats. *International Journal of Biology, Pharmacy and Allied Science*.2 (1): 43 – 58.
15. Prohp, T. P. and Onoagbe I. O. (2013b). Effects of extracts of *Triplochiton scleroxylon* K. Schum.on plasma glucose and non-enzyme anti-oxidant concentrations in diabetic rats. *Journal of Global Bioscience*.2 (1): 1 – 9.
16. Prohp T. P., Onoagbe I. O (2014). Plasma electrolyte concentrations in normal and streptozotocin-induced diabetic rats treated with extracts of *Triplochitonscleroxylon* K. Schum. American Journal of Research Communications.2(5): 154 - 174.
17. Raju, S. M. & Mandala, B. (2005).Plasma enzymes in clinical practice. In: Illustrated Medical Biochemistry. (ed.) Jaypee Brothers, Medical Publishers, Ltd. 195 - 197.
18. Richter, H. G. and Dallwitz, M. J. (2000). Commercial timbers: descriptions, illustration, identification and information retrieval in English, French, German and Spanish. Version: 4th May 2000. http://www.biodivesity.uno.edu/delta
19. SPSS (2007).Statistical Package for the Social Sciences for Windows, Release 16.0.1.Standard version.
20. Tanko, Y., Yaro, A. H., Isa, A. I., Yerima, M., Saleh, M. I. A. & Mohammed, A. (2007). Toxicological and hypoglycaemic studies on the leaves of *Cissampelos mucronata (Menispermaceae)* on blood glucose levels of streptozotocin-induced diabetic wistar rats. Journal of Medicinal Plants Research.1 (5), 113 -116.