Effect of aqueous and ethanolic extracts of *Triplochiton scleroxylon* K. Schum. on serum cholesterol, triglyceride and phospholipid levels in normal and streptozotocin-induced diabetic rats

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Abstract

Some serum lipids were determined in normal and streptozotocin-induced diabetic rats following oral administration of extracts of *Triplochiton scleroxylon* for 28 days. Extracts of *Triplochiton scleroxylon* were administered twice daily to the test rats (Wistar strain) of average weight, 138 g (p. o) at the dose of 200 mg/kg body weight. Spectrophotometric methods were used in all analyses. Aqueous and ethanolic extracts of *Triplochiton scleroxylon* demonstrated significant (P<0.05) lowering of serum cholesterol, triglyceride and phospholipid concentrations in treated normal and diabetic rats when compared to normal and diabetic controls respectively. Extracts did not cause adverse histological changes in the rat livers following treatment. However, aqueous extract was safer and more effective in reducing the sternness of necrosis in the kidneys than ethanol extract. And its greater hypolipidemic properties could be useful in preventing the onset of coronary heart diseases.

Keywords: Triplochiton scleroxylon, diabetes, cholesterol, triglyceride, phospholipid, serum.

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1.0 Introduction

Hypercholesterolaemia is a risk factor for the development of cardiovascular diseases including atherosclerosis, myocardial infarction and cerebral paralysis. Death due to coronary heart disease caused by atherosclerosis has been identified as a common cause of mortality in affluent nations of the world (Johnston *et al.*, 2003; Johnston and Waxman, 2008). Elevated levels of both blood cholesterol and triglycerides have been widely documented as major worries if cardiovascular diseases and associated conditions are to be controlled (Mansurah 2011). Diabetes which is characterised by hyperglycaemia and lipoprotein abnormalities (Scoppola *et al.*, 2001) has been associated with an increased risk for developing premature atherosclerosis partly contributed by increase in triglycerides and cholesterol amongst others.

Many traditional remedies have been identified to be potent against hypercholesterolaemia and hypertriglyceridaemia. Therefore it is of absolute importance to evaluate the potential of herbal remedies for the purpose of obtaining a lead for the discovery of antidotes for most diseases now fully documented as incurable by orthodox medicine. Triplochiton scleroxylon whose active ingredients are believed to be at the stem bark is commonly used in some western and southern parts of Nigeria to treat diabetes mellitus (Prohp and Onoagbe, 2009 a, b). The hypoglycemic and anti-diabetic properties of extracts of Triplochiton scleroxylon are well documented (Prohp et al., 2012; Prohp and Onoagbe, 2012a, 2013a). This plant is a deciduous forest tree of kingdom: plantae, division: magnoliophyta, class: magnoliopsida, order: malvales, family: sterculiaceae, genus: triplochiton and species: T. scleroxylon (Raju and Mandala, 2005; Prohp and Onoagbe, 2013a, b). Toxicological studies have shown that aqueous and ethanolic extracts of Triplochiton scleroxylon caused significant lowering of total cholesterol and triglycerides in the plasma of non-diabetic rats (Prohp and Onoagbe, 2012b). We have also reported the same significant lowering of total cholesterol and triglycerides in the plasma of streptozotocin-induced diabetic rats treated with extracts of Triplochiton scleroxylon (Prohp et al., 2012). In this study, serum samples of treated diabetic rats were investigated for total cholesterol, trigycerides and phospholipid concentrations over a period of 28 days.

The aim of this study is to understand if the observations in the plasma as reported (Prohp and Onoagbe, 2012b; Prohp *et al.*, 2012) could have been influenced by other plasma component extraneous to serum.

2.0 Materials and Methods

2.1 Ethics on the use of animals in experimental studies

All the 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 (NIH, 1992).

2.2 Experimental animals

Male albino rats of Wistar strain were obtained from the animal house of the College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria. The rats 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 (Prohp and Onoagbe, 2012a, b).

2.3 Chemicals/Reagents

Cholesterol and triglyceride kits obtained from Randox Laboratories United Kingdom were used. Glassdistilled water was used. Other reagents and chemicals for phospholipid determinations were of analytical grade.

2.4 Medicinal plant

The barks of *Triplochiton 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 *Triplochiton scleroxylon* K. Schum where a voucher specimen (UIH – 22329) had been deposited (Prohp and Onoagbe, 2012a, b; 2013a).

2.5 Extraction and preparation of plant extracts

The barks of *Tripochiton scleroxylon* were washed with clean water, dried and cut into tiny strands. 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 °C until used (Prohp and Onoagbe, 2012a, b).

2.6 Blood collection

The tail of the restrained rat 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 the tip became red; sign of blood accumulation. 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 universal sample tubes without anti-coagulant (for serum lipid assays). Cotton wool soaked in methylated spirit was again used to cleanse the incised area of the tail. After 5 minutes serum was decanted from the universal sample tubes kept on the side bench under room temperature (28 °C) and used for all biochemical analyses carried out immediately (Prohp and Onoagbe, 2012a, b).

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2.7 Streptozotocin injection

100 mg of streptozotocin dissolved in commercial saline was administered to overnight fasted rats at the dose of 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 (Ballester *et al.*, 2005; Tanko *et al.*, 2007; Prohp and Onoagbe, 2009a, b).

2.8 Experimental procedure

Toxicological studies

A total of twelve male albino rats (Wistar strain) after acclimatization for a period of two weeks, were fasted overnight and randomly divided into three groups of four rats each. Group 1 served as the normal control and received distilled water while the test groups received 200 mg/kg body weight of aqueous and ethanolic extracts of *Triplochiton scleroxylon* respectively. Serum total Cholesterol triglyceride and phospholipid concentrations were monitored periodically at intervals of six days for 28 days by the respective methods of Roeschlaw *et al.*, (1974) and Tietz, (1990) as outlined in Randox Laboratories manual and Fiske and Subarrow, (1925).

Diabetic studies

A total of sixteen 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. Diabetes was induced in rats in groups 2, 3 and 4 with the aid of streptozotocin injection at the dose of 65 mg/kg body weight, (i. p.). Groups 1 and 2 served as the normal and diabetic controls respectively and received distilled water while treated diabetic groups (3 and 4) received 200 mg/kg body weight of aqueous and ethanolic extracts of *Triplochiton scleroxylon* respectively. Plasma total Cholesterol, triglyceride and phospholipid concentrations were monitored periodically at intervals of six days for 28 days by the respective methods of Roeschlaw *et al.*, (1974) and Tietz, (1990) as outlined in Randox Laboratories manual and Fiske and Subarrow, (1925).

2.9 Administration of extracts

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

2.10 Biochemical assays

Serum cholesterol and triglyceride concentrations were estimated by enzymatic end-point and colorimetric methods respectively, of Roeschlaw *et al.*, (1974) and Tietz, (1990) as outlined in Randox Laboratories manual.

Total serum phospholipid concentration was determined by the method of Fiske and Subarrow (1925). Phospholipids in lipid extracts or in chromatographic fractions were estimated by phosphorus

determination through an acidic digestion. Consequently, inorganic phosphate released react with ammonium molybdate to form an intense blue colour complex whose absorbance value was a function of phospholipid concentration.

2.11 Statistical analysis

Data were expressed as mean \pm S. E. M. of three separate determinations. The statistical significance was evaluated by one-way ANOVA using SPSS (statistical package for social sciences) version 17.0, followed by post –hoc LSD and Turkey tests for individual comparisons. Values lower than 0.05 probabilities were accepted as statistically significant (SPSS, 2007).

3.0 **Results**

Results on the analyses of serum samples for cholesterol, triglyceride and phospholipid concentrations in normal and streptozotocin-induced diabetic rats treated with aqueous and ethanolic extracts of *Triplochiton scleroxylon* are presented in tables (Tables 1 - 6). Both extracts caused significant decrease (P<0.05) in serum cholesterol, triglyceride and phospholipid concentrations when compared to normal and diabetic control rats respectively. Plates 1 to 4 are photomicrographs of tissue sections investigated. Aqueous extract was more effective in resolving adverse effects in the livers and kidneys of diabetic rats than ethanolic extract of *Triplochiton scleroxylon*.

Table 1: Mean serum total cholesterol concentrations (mg/dl) of normal and treated normal rats

S/N	I. Treat	tments Day 0	Day 1	Day 6	Day 12	Day 18	Day 24	Day 28	
1.	NC	129.62±0.38 ^a	129.45±0.42ª	130.10±0.63 ^a	130.72±0.26 ^a	129.95±0.24 ^a	129.59±0.53ª 129.	52±0.17 ^a	
2.	TA	129.88±0.32 ^a	130.05±0.44 ^a	130.18±0.18 ^a	129.60±0.	.50° 124.75±0.2	25 ^b 124.40±0.25 ^b	122.75±0.25 ^b	
3.	TE	129.60±0.25 ^a	129.50±0.65 ^a	127.25±048 ^b	126.00±0	0.41 ^b 124.78±0.	26 ^b 123.10±0.33 ^b	109.65±0.28°	

Data are mean \pm 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). NC =Normal control; TA = Treated with aqueous extract; TE = Treated with ethanolic extract.

Table 2: Mean serum total cholesterol concentrations (mg/dl) of controls and treated streptozotocin-induced diabetic rats

S/N	I. Treatm	ents Day 0	Day 1	Day 6	Day 12	Day	18 Day	24 Day	28
1.	NC	173.98±1.19 ^a	173.76±1.19 ^a	172.06±0.88ª	172.33±1.51ª	174.58±1.43 ^a	175.77±1.29 ^a	176.05±2.00 ^a	
2.	DC	177.47±1.86 ^a	198.30±0.61 ^b	209.41±2.61 ^b	216.48±2.25 ^b	226.42±1.82 ^b	232.88±4.05 ^b	248.30±3.37 ^b	
3.	ATD	181.45±1.95 ^a	198.08±0.94 ^b	181.77±2.94 ^c	176.83±2.51 ^a	151.00±1.76 ^c	125.12±2.38°	109.92±3.66 ^c	
4.	ETD	175.98±1.67 ^a	196.17±2.58 ^b	190.11±0.59 ^c	179.46±5.12 ^a	160.91±4.93°	138.03±2.71 ^d	125.04±2.39 ^d	

Data are mean \pm 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. NC: Normal control; DC: Diabetic control; ATD: Aqueous extract treated diabetic rats; ETD: Ethanolic extract treated diabetic rats.

Table 3: Mean serum triglyceride concentrations (mg/dl) of normal and treated normal rats

S/N. Treatments Day 0			ts Day 0	Day 1	Day 6	Day 12	Day 18	Day 24	Day 28	_
	1	NC	101.54±0.43 ^a	101 88+0 36 ^a	101 15+0 33 ^a	100.92 ± 0.40^{a}	101 26+0 58 ^a	101.40±0.76 ^a	102 30+0 37 ^a	
						90.83±0.33 ^b			75.65±0.33 ^b	
	3.	TE	101.78±0.49 ^a	103.45±0.49 ^a	100.80±0.39 ^a	93.15±0.66 ^c	90.05±0.25 ^b	86.80±0.36 ^c	79.92±0.30 ^c	

Data are mean \pm 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). NC =Normal control; TA = Treated with aqueous extract; TE = Treated with ethanolic extract.

Table 4: Mean serum triglyceride concentrations (mg/dl) of controls and treated streptozotocininduced diabetic rats.

S /	N. Tro	eatments	s Day 0	Day 1	Day 6	Day 12	Day 1	.8 Da	y 24	Day 28
	1.	NC	170.21±0.41 ^a	$170.92{\pm}1.34^{a}$	173.77±1.13ª	174.90±0.83ª	176.63±2.75 ^a	178.77±1.23 ^a	180.92±0.7	5 ^a
	2.	DC	170.67±0.59 ^a	193.35±0.52 ^b	209.83±0.79 ^b	219.12±0.91 ^b	244.88±4.91 ^b	271.98±4.89 ^b	286.64±5.90	b
	3.	ATD	171.82±1.35 ^a	188.10±2.53 ^b	170.93±0.57 ^a	159.30±0.44°	135.96±3.01°	126.40±2.13 ^c	114.27±2.52	2°
	4.	ETD	173.13±1.24 ^a	189.03±5.84 ^b	176.80±2.87 ^a	171.96±3.00 ^a	166.35±2.82ª	147.76±1.32 ^d	129.22±3.75	5°

Data are mean \pm 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. NC: Normal control; DC: Diabetic control; ATD: Aqueous extract treated diabetic rats; ETD: Ethanolic extract treated diabetic rats.

S/N. Treatments Day 0			Day 1	Day 6	Day 12	Day 18	Day 24	Day 28	_
1.	NC	12.05±0.21ª	11.73±0.16 ^a	11.40±0.16 ^a	11.45±0.26ª	11.43±0.13ª	11.20±0.12 ^a	11.54±0.19 ^a	_
2.	TA	12.07±0.21 ^a	11.45±0.21 ^a	11.20±0.15 ^a	10.35±0.29 ^a	9.63±0.24 ^b	9.50±0.29 ^b	9.18±0.12 ^b	
3.	TE	12.31±0.25ª	11.79±0.05ª	11.29±0.19ª	10.33±0.20 ^a	9.75±0.14 ^b	9.70±0.24 ^b	9.13±0.13 ^b	-

Table 5: Mean serum phospholipid concentrations (mg/dl) of normal and treated normal rats

Data are mean \pm 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). NC =Normal control; TA = Treated with aqueous extract; TE = Treated with ethanolic extract.

Table 6: Mean serum phospholipid concentrations (mg/dl) of controls and treated streptozotocininduced diabetic rats

S /I	N. Tre	eatments	Day 0	Day 1	Day 6	Day 12	Day 18	Day 24	Day 28
_									
	1.	NC	14.38 ± 0.28^{a}	14.25±0.23 ^a	14.07±0.08 ^a	11.82±0.21 ^a	12.61±0.28 ^a	13.28±0.19 ^a	14.98±0.29 ^a
_	2.	DC	13.94±0.23 ^a	17.95±0.30 ^b	19.80±0.24 ^b	$20.84{\pm}0.12^{\text{b}}$	$21.62{\pm}0.40^{b}$	20.66±0.25 ^b	20.02±0.16 ^b
	3.	ATD	$14.08{\pm}0.08^{a}$	16.10±0.24 ^b	14.75±0.19 ^a	14.03±0.09 ^c	16.83±0.12 ^c	17.63±0.17 ^c	17.11±0.15 ^c
_	4.	ETD	14.23±0.20 ^a	16.91±0.47 ^b	15.11±0.08 ^c	15.48±0.28 ^d	16.23±0.19 ^c	$16.82{\pm}0.05^{d}$	15.22±0.17 ^a

Data are mean \pm 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. NC: Normal control; DC: Diabetic control; ATD: Aqueous extract treated diabetic rats; ETD: Ethanolic extract treated diabetic rats.

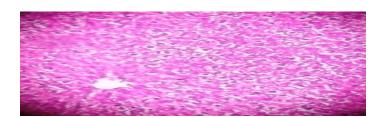


Plate 1: Photomicrograph of liver section of streptozotocin-induced diabetic rat treated with aqueous extract of *Triplochiton scleroxylon* at the dose of 200 mg/kg bw. Section shows normal histology. H & E Stain. X 100.



Plate 2: Photomicrograph of liver section of streptozotocin-induced diabetic rat treated with ethanolic extract of *Triplochiton scleroxylon* at the dose of 200 mg/kg bw. Section shows normal histology. H & E Stain. X 100.



Plate 3: Photomicrograph of kidney section of streptozotocin-induced diabetic rats treated with aqueous extract of *Triplochiton scleroxylon* at the dose of 200 mg/kg bw. Section shows vacuolation of tubular epithelium with mild necrosis. H & E Stain. X 100.



Plate 4: Photomicrograph of kidney section of streptozotocin-induced diabetic rats treated with ethanolic extract of *Triplochiton scleroxylon* at the dose of 200 mg/kg bw. Section shows moderate tubular necrosis and infiltrations of inflammatory cells. H & E Stain. X 100

4.0 Discussion

Streptozotocin-induced diabetes mellitus in rats resulted in increases in the serum triglyceride and cholesterol concentrations (Tables 1 - 4). These agree with the report that lipid profile is altered in the serum of diabetic patients (Orchard, 1990; Betteridge, 1994) resulting in an increase in both triglyceride and total cholesterol levels. These increases may be a significant factor in the development of premature

atherosclerosis (Ugochukwu *et al.*, 2003). However, aqueous and ethanolic extracts caused significant decreases (P<0.05) in the serum concentrations of triglyceride and cholesterol in normal and streptozotocin-induced diabetic rats when compared to normal and diabetic controls respectively. Elevated blood triglyceride is one of the diabetic indicators and a significant decrease in serum triglyceride obtained (Tables 3 and 4) was indicative of the anti –diabetic properties of extracts of *Triplochiton scleroxylon*. Reduction of blood cholesterol protects against the risk of cardiovascular diseases. These reductions of both cholesterol and triglyceride concentrations obtained in this study (Tables 1 - 4) could be beneficial in preventing diabetic complications and improving lipid metabolism in diabetics (Cho *et al.*, 2002).

Diabetes mellitus in rats caused by streptozotocin injection resulted in increase in serum phospholipid concentrations. However, treatment with aqueous and ethanolic extracts caused a significant decrease ((P<0.05) in serum phospholipid levels on the 12^{th} to 28^{th} days when compared to diabetic control (Table 6). In treated normal rats however, the decrease in serum phospholipid concentrations obtained was not significant (P>0.05) when compared to normal control (Table 5). Micro-viscosity of a membrane increases with increasing cholesterol to phospholipid ratio and this leads to cellular rigidity (McConnell and Hubbell, 1971), altering membrane structure and function (Ojo *et al.*, 2006). Alteration of bio-membrane lipid profile disturbs membrane fluidity, permeability, activity of associated enzymes and transport system (Cooper *et al.*, 1977). Decreased cholesterol and phospholipid concentrations in this study in normal and streptozotocin-induced diabetic rats following treatment with aqueous and ethanolic extracts of *Triplochiton scleroxylon* are suggestive of protection (by extracts) against alteration of bio-membrane lipid profile which favours the activity of membranes.

In streptozotocin-induced diabetic rats, treatment with extracts of *Triplochiton scleroxylon* did not have adverse effects in the rat livers (Plates 1 and 2). Aqueous extract however, was found to be more effective in stemming the severity of necrosis in the kidneys than ethanolic extract of *Triplochiton scleroxylon* (Plates 3 - 4).

5.0 Conclusion

The safety of use of aqueous extract of *Triplochiton scleroxylon* exhibited in this study could be a justification for its use in some areas in the southern part of Nigeria as a traditional panacea for the treatment of diabetes mellitus.

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