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Biochemical kidney function with aqueous fruit extract of *Solanum macrocarpum* Linn. in albino rats chronically administered triton-X to induce hyperlipidemia

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Studies were conducted on the effect of the aqueous fruit extract of *Solanum macrocarpum* Linn. in chronic titron-induced hyperlipidaemic rats. The plant was Soxhlet-extracted with distilled water and the extract concentrated *in vacuo* with a yield of 15.34%$^{w}/_{w}$. The extract was stored in a specimen bottle in a desicator at room temperature until when required. The kidney function parameters; serum creatinine, urea and electrolytes (sodium, potassium, calcium, and phosphate) were determined. The results showed that with increase in extract dose, the changes in serum concentration of sodium, potassium and phosphate ions were significant (P>0.05). For calcium ions however, the concentration increase was significant (P<0.05) at 48 and 72hrs. Creatinine decreased significantly (P<0.05) at 72hrs and urea decreased significantly at 48hrs and 72hrs when compared to the negative control. Then non-significant changes in sodium and potassium ions imply that the acid-base balance of the physiological system was not affected. Decreased levels of creatinine and urea observed in this study probably implies the kidney is not damaged.

Keywords: *Solanum macrocarpum* Linn, aqueous extract, triton-X, kidney function, chronic hyperlipidaemic rats.

INTRODUCTION

The kidney parameters on administration of “Gorongo” (Kanuri), *Solanum macrocarpum* in hypercholesterolaemic albino rats pre-fed with 1% of cholesterol and groundnut oil (Sodipo et al., 2009) and acute triton-induced hyperlipidaemic rats (Sodipo, 2009) did not confirm kidney damage as increased levels of creatinine observed in these studies may be due to stimulation of muscle activity or impaired kidney function (Sodipo, 2009). Outcome of histopathological studies can only confirm kidney damage. The fruit of the plant because of its numerous uses, locally especially in the treatment of hyperlipidema (Grubben and Denton, 2004), has attracted scientific investigation as many drugs that have been developed and introduced for the management of hyperlypidemia have many serious side effects like aggravating deterioration in kidney function (Lawrence et al., 1997; Katzing 2004; Sodipo et al., 2011). In addition they are expensive (Sodipo et al., 2011). The present study evaluated the effect of the fruit of *S. macrocarpum* on chronic triton-induced hyperlipidemic rats in an attempt to find an alternative hypolipidemic drug that is therapeutically cost effective but with fewer side effects than the existing ones, even after chronic use by the patient.

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MATERIALS AND METHODS

Plant collection and identification

The plant material *(Solanum macrocarpum* Linn.) used in this study was obtained from Alau in Konduga Local Government, Borno State, Nigeria, between October and November, 2007. The plant was identified and authenticated by Prof. S.S. Sanusi of the Department of Biological Sciences, University of Maiduguri, Maiduguri, Nigeria. Specimen voucher No. 548 was deposited at the Research Laboratory of the Department of Chemistry.

Extraction

The fruit of *S. macrocarpum* with the calyx removed was air-dried and pulverized by grinding using pestle and mortar. The 2.2 kg of the ground fruit was subjected to exhaustive Soxhlet-extraction in distilled water at 100 °C to give the extract yield of 15.3 % $^{w}/_{w}$ (Mittal et al., 1981, Fernando et al., 1991; Lin et al., 1999). The resultant solution was concentrated *in vacuo* and it was stored in a specimen bottle and kept in a desicator at room temperature until when required.

Animals

Thirty six (36) male albino rats of Wistar strain weighing 160-200 g were used in this study. The animals were obtained from the Animal House Unit of the Department of Veterinary Physiology, Pharmacology and Biochemistry, University of Maiduguri. The animals were housed under standard laboratory condition in plastic cages. They were fed commercial growers' mash feed (ECWA, Feeds, Jos, Nigeria) and water was provided *ad libitum*. All the animals were handled according to the international Guiding Principles for Biomedical Research Involving Animals (CIOMS, 1985) as certified by the Animal Ethics Committee of the Faculty of Veterinary Medicine, University of Maiduguri.

Administration of triton and extract

Thirty (30) albino rats were made hyperlipidemic by feeding them orally (p.o) for 90 days with normal feed diet and triton-X (Sigma Chemical Co. St. Louis, M.O. USA) at a dose of 400 mg/kg in saline suspension from the stock concentration of 535g/ml. The thirty six (36) rats for the experiment were divided into 6 groups of 6 animals each. After ninety (90) days, 24 of the 30 rats were administered with graded doses of the fruit extract. Group I was the negative control and it was given normal feed and distilled water only. Group two was the positive control and it was given normal feed and triton-X with distilled water only and normal feed. Groups 3, 4, 5, and 6 were administered with geometrical doses (25, 50, 100 and 200mg/kg) of the fruit extract intraperitoneally (i.p.) from a stock concentration of 200mg/ml. After 24, 48 and 72hrs, respectively of the effect of the extract on the hyperlipidemic rats, (adapted from Williamson et al., 1996), two rats from each group were humanely sacrificed by cutting the throat with a sterile blade and blood was collected from the vena cava into clean, labelled centrifuge tubes without an anticoagulant. The blood was centrifuged at a rate of 12,000 revolution per minute (rpm) for 10 minutes. The clear, yellow serum was then separated from settled cellular elements. Before the rats were fed with triton-X, their weights were taken and after administration of triton-X for 30, 60 and 90 days respectively.

Biochemical kidney function tests

The kidney function parameters estimated from the serum were creatinine, urea and electrolytes which include sodium ion (Na^+), potassium ion (K^+), calcium ion (Ca^+) and phosphate ion (PO_4^{3-}). They were estimated using standard methods (Seaton and Ali, 1984; Chaney and Marbach, 1962; Kaplan and Pesche, 1989; Lorentz, 1982; Tietz, 2006).

Determination of total cholesterol

Two rats in each group were humanely sacrificed by cutting the throat with a sterile blade. Blood was collected from the vena cava into clean, labelled centrifuge tubes without anticoagulant after the extract had been allowed to act for 24, 48, 72hrs respectively. The blood was centrifuged at a rate of 12,000 rotations per minute (rpm) for 10 minutes. The clear, yellow serum was then separated from settled cellular elements. Cholesterol was assayed by Tindar's reaction (Evans and Stein, 1986; NIH, 1990) using commercial kits, from Fortress Diagnostic Ltd, Antrim.

Statistical analysis

Test of significance between control and treatment means were carried out by Analysis of Variance (ANOVA) using Graph Pad Software (1998).

RESULTS

Change in mean body weight of male albino rats (Wistar strain) after being administered orally with Triton-X for 90 days

The effect of triton-X on mean body weight of albino rats

Table 1. Change in mean body weight of male albino rats after being administered orally with Triton-X (400 mg/kg) for 90 days

Group	Mean Body Weight ± S.D. (g)				% Increase in Mean Body Weight
	Days of Treatment				
	0	30	60	90	
One*	110.25 ± 10.50[a]	112.50 ± 20.45[a]	114.00 ± 12.51[a]	117.20 ± 15.07[a]	6.30 ± 4.57[a]
Two	100.20 ± 26.64[a]	135.80 ± 41.26[b]	203.44 ± 52.97[b]	214.20 ± 58.61[b]	113.78 ± 32.37[b]
Three	80.00 ± 17.25[a]	110.20 ± 27.52	163.64 ± 26.93[b]	174.20 ± 15.06[b]	117.75 ± 2.19[b]
Four	99.40 ± 29.19[a]	131.40 ± 41.58[b]	184.80 ± 37.58[b]	216.80 ± 41.05[b]	117.30 ± 11.86[b]
Five	116.60 ± 42.58[a]	129.00 ± 11.92[b]	172.78 ± 17.03[b]	194.80 ± 19.74[b]	67.07 ± 22.84[b]
Six	95.00 ± 20.96[a]	120.40 ± 36.65[b]	192.18 ± 34.03[b]	211.95 ± 33.74[b]	122.11 ± 12.78[b]

Within rows, means with different superscripts are statistically significant ($p < 0.05$) when compared to day zero (0) using one way analysis of variance (ANOVA).
0 day = before triton-X administration
n = 6 rats
Group One* = Rats fed with normal diet and had free access to water throughout the 90 days but were not administered triton-X

fed orally with triton-X is shown in Table 1. The increase in body weight observed in the rats was statistically significant ($p < 0.05$) when compared to day zero in all the groups except in Group one. Group one was not administered with triton-X throughout the period of study. Also, there was a significant percentage weight gain ($p < 0.05$) in the hyperlipidaemic rats (Groups two-six) when compared with Group one which received standard diet and water *ad libitum*.

Effect on serum electrolytes

The results of the effect of the extract on serum electrolytes are shown in Table 3. The serum concentration of Na^+ and K^+ ions did not change ($p > 0.05$) with increase in extract dose throughout the period of study.

With increasing dose of extract on the hyperlipidaemic rats there was a decrease in Ca^{2+} ions and this was only significant at both 48 and 72 hrs ($p < 0.05$) whilst there was no change in phosphate ions ($p > 0.05$) throughout the period of study.

Effect on creatinine and urea

The effect of the aqueous fruit extract of *Solanum macrocarpum* on the hyperlipidaemic rats on creatinine levels decreased slightly at 72 hrs ($p < 0.05$) whilst the decrease in urea levels with increase in extract dose was significant ($p < 0.05$) at both 48 and 72 hrs (Table 2).

Effect of extract on total cholesterol

The effect of the aqueous fruit extract of *Solanum macrocarpum* on total cholesterol of hyperlipidaemic rats administered orally with triton-X for 90 days is shown in Table 4. There was a non-significant ($P>0.05$) increase in total cholesterol when compared to the positive control with increase in extract dose at 24, 48 and 72hrs respectively. The oral administration of triton-X resulted in a rise in serum cholesterol of rats in the positive control group (i.e. those administered only Triton-X).

DISCUSSION

The increase in mean body weight of the rats after triton-X administration for 90 days was significant ($p < 0.05$) (Groups two to six), whilst Group one fed with normal diet was not significant ($p > 0.05$). The percentage weight gain in the hyperlipidaemic rats (Groups two to six) was significantly high ($p < 0.05$) when compared to Group one. Excessive weight gain (obesity) has been implicated in hypertension and ischaemic heart disease (Nwanjo et al., 2006). It probably suggests that the triton-X had induced atherosclerosis as atherosclerosis takes three to six months to be induced in rats (Williamson et al., 1996).

The administration of increasing doses of *Solanum macrocarpum* fruit extract to the hyperlipidaemic rats resulted in no change

Table 2. Effect of the aqueous fruit extract of *S. macrocarpum* on kidney function indices of hyperlipidaemic rats administered orally with triton-X for 90 days

Hours after extract Administration	Group	Extract dose (mg/kg)	Creatinine (µmol)	Urea (µmol)
			Mean ± S.D.	
24	One	-ve control	64.50±4.24[a]	4.60±0.59[a]
	Two	+ve control	69.00±1.41[a]	6.90±0.99[a]
	Three	25.00	65.50±6.36[a]	6.75±2.19[a]
	Four	50.00	65.00±1.41[a]	6.55±0.21[a]
	Five	100.00	60.00±2.80[a]	6.10±0.14[a]
	Six	200.00	54.50±7.78[a]	5.90±0.42[a]
48	One	-ve control	60.00±1.41[a]	4.15±0.00[a]
	Two	+ve control	67.50±2.12[a]	6.65±0.21[b]
	Three	25.00	65.00±1.41[a]	6.60±0.21[b]
	Four	50.00	62.50±10.61[a]	5.95±0.21[b]
	Five	100.00	57.00±2.83[a]	5.55±0.64[b]
	Six	200.00	56.60±2.12[a]	4.95±0.35[b]
72	One	-ve control	61.50±2.12[a]	5.56±0.14[a]
	Two	+ve control	68.00±1.41[b]	6.80±0.14[b]
	Three	25.00	65.50±2.12[b]	6.35±0.21[b]
	Four	50.00	63.00±2.83[b]	6.30±0.28[b]
	Five	100.00	60.50±0.71[b]	6.15±0.21[b]
	Six	200.00	55.50±3.59[b]	6.05±0.07[b]

Within columns, means with different superscripts are statistically significant ($P < 0.05$) when compared to Group I (-ve control)
-ve control = Rats fed with normal feed diet and had free access to water
+ve control = Rats fed with normal feed diet and given triton-X

($p > 0.05$) in sodium and potassium ions throughout the period of study implying that the acid-base balance of the physiological system is probably not affected (Odutola, 1992).

There was no change in PO_4^{3-} ion but Ca^{2+} ion increased significantly at 48 and 72 hrs ($p < 0.05$). No change in phosphate ions ($P>0.05$) incicates that the bone was not affected since their decreased levels may lead to bone damage (Fraser et al., 1994; Abdulrahman 2004; Sodipo, 2009). Excess use of laxatives can lead to decrease in PO_4^{3-} ion (Sood, 2006) and the extract had already been shown to be a laxative.

Decreased levels of creatinine at 72 hrs ($p < 0.05$) and urea at 48 and 72 hrs ($p < 0.05$) were recorded with increasing doses of aqueous fruit extract of *Solanum macrocarpum* on chronic hyperlipidaemic rats. Since elevated levels of creatinine are found in renal dysfunction or muscle damage and urea is a waste product of protein breakdown (Mukherjee, 1988), it therefore implies that the kidney is probably not damaged.

Shi et al. (2004) has shown that high saponin diet has an inverse relationship with renal stones. Since the phytochemistry revealed the presence of saponins in the aqueous fruit extract of *Solanum macrocarpum* (Sodipo et al., 2008), this further confirms the fact that the fruit does not cause renal damage.

CONCLUSION

The aqueous fruit extract of *Solanum macrocarpum* probably did not lead to impaired kidney function as significant decreased levels of creatinine (72hrs) and urea (48 and 72hrs) were recorded with increasing dose of the extract on the chronic hyperlipidaemic rats. The fruit should however be used with caution pending the outcome of histopathological studies on the kidney to confirm any probable damage to the organ.

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Table 3. Effect of the aqueous fruit extract of *S. macrocarpum* on kidney function indices of hyperlipidaemic rats administered orally with triton-X for 90 days

Hours after extract administration	Group	Extract dose (mg/kg)	Serum electrolytes (moles/L)			
			Na^+	K^+	Ca^+	PO_4^{3-}
			Mean ± S.D.			
24	One	-ve control	121.00±0.00[a]	5.95±1.34[a]	2.55±0.35[a]	0.60±0.14[a]
	Two	+ve control	122.50±3.54[a]	8.00±0.00[a]	2.75±1.06[a]	0.65±0.07[a]
	Three	25.00	125.00±7.07[a]	6.65±1.34[a]	2.75±0.07[a]	0.70±0.28[a]
	Four	50.00	127.00±14.24[a]	6.50±0.71[a]	2.65±0.21[a]	0.80±0.07[a]
	Five	100.00	128.50±12.02[a]	6.00±1.27[a]	2.35±0.35[a]	0.85±0.07[a]
	Six	200.00	136.00±2.83[a]	5.50±0.71[a]	2.15±0.21[a]	1.20±0.28[a]
48	One	-ve control	120.00±0.00[a]	6.50±1.41[a]	2.45±0.21[a]	0.60±0.00[a]
	Two	+ve control	122.50±3.54[a]	8.80±0.71[a]	2.65±0.57[b]	0.65±0.21[a]
	Three	25.00	125.00±7.07[a]	6.70±0.00[a]	2.50±0.00[b]	0.70±0.14[a]
	Four	50.00	127.00±14.24[a]	6.05±0.35[a]	2.40±0.14[b]	0.80±0.14[a]
	Five	100.00	128.50±12.02[a]	5.80±2.21[a]	2.30±0.14[b]	0.95±0.07[a]
	Six	200.00	136.00±2.83[a]	5.70±0.42[a]	2.05±0.07[b]	1.00±0.14[a]
72	One	-ve control	118.00±2.83[a]	6.70±0.42[a]	2.30±0.07[a]	0.50±0.14[a]
	Two	+ve control	121.00±1.41[a]	7.50±0.71[a]	2.75±0.07[b]	0.65±0.21[a]
	Three	25.00	120.00±0.00[a]	6.80±0.29[a]	2.50±0.00[b]	0.65±0.07[a]
	Four	50.00	122.00±2.83[a]	6.53±0.07[a]	2.45±0.21[b]	0.75±0.07[a]
	Five	100.00	127.50±3.54[a]	5.75±1.01[a]	2.35±0.07[b]	0.85±0.50[a]
	Six	200.00	134.80±8.49[a]	5.30±0.71[a]	2.30±0.14[b]	1.00±0.14[a]

Within columns, means with different superscripts are statistically significant ($p < 0.05$) when compared to Group I (-ve control)
-ve control = Rats fed with normal feed diet and had free access to water
+ve control = Rats fed with normal feed diet and given triton-X

Table 4. Effect of the aqueous fruit extract of *S. macrocarpum* on total cholesterol of hyperlipidaemic rats administered orally with triton-X for 90 days

Hours after extract Administration	Group	Extract dose (mg/kg)	Total Cholesterol (mmol/L)
			Mean ± S.D.
24	One	-ve control	1.70±0.28[a]
	Two	+ve control	2.40±0.29[a]
	Three	25.00	2.15±0.64[a]
	Four	50.00	2.10±0.57[a]
	Five	100.00	1.35±0.07[a]
	Six	200.00	1.15±0.07[a]
48	One	-ve control	1.70±0.14[a]
	Two	+ve control	2.55±0.07[a]
	Three	25.00	2.10±1.13[a]
	Four	50.00	1.50±0.14[a]
	Five	100.00	1.45±0.07[a]
	Six	200.00	1.25±0.35[a]
72	One	-ve control	1.90±0.14[a]
	Two	+ve control	2.40±0.50[a]
	Three	25.00	2.20±0.28[a]
	Four	50.00	2.10±0.42[a]
	Five	100.00	1.70±0.14[a]
	Six	200.00	1.35±0.07[a]

Within columns, means with the same superscripts are not statistically significant ($P>0.05$) when compared to Group I (-ve control)
-ve control = Rats fed with normal feed diet and had free access to water
+ve control = Rats fed with normal feed diet and given triton-X

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