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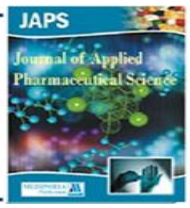

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Biochemical liver function with aqueous fruit extract of *Solanum macrocarpum* linn in albino rats acutely administered triton-x to induce hyperlipidaemia

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ABSTRACT

The effect of the aqueous fruit extract, *Solanum macrocarpum* Linn on some biochemical indices of liver function was studied in triton-induced hyperlipidaemic wistar rats. Thirty rats (160-200g) were used in the study and assigned to 5 groups of 6 rats each. Group I hyperlipidaemic control rats received distilled water only, whereas groups II, III, IV and V, the experimental hyperlipidaemic rats, were administered graded doses of the plant extract (25mg/kg, 50mg/kg, 100mg/kg and 200kg/mg) per body weight intraperitoneally after which blood samples were taken from the rats 24hrs, 48hrs and 72hrs, respectively after extract administration. Serum aspartate amino transferase (AST) dose dependently and significantly decreased ($P<0.05$) at 48hrs and 72hrs. The values of alanine amino transferase (ALT) decreased significantly ($P<0.05$) at 72hrs when compared to the control. The decrease in alkaline phosphatase (ALP) activity was not significant (ALP) activity was not significant ($P>0.05$) when compared to the control. Serum protein and albumin decreased significantly ($P<0.05$) while bilirubin increased significantly ($P<0.05$) at 72hrs of study. In conclusion, *Solanum macrocarpum* probably has hepatoprotective effects.

Key words: Hyperlipidaemic, *Solanum macrocarpum*, liver function, rats.

INTRODUCTION

Natural substances of botanical origin have been used throughout the world for human and animal health care (Enzo, 2006). About half of the world's medicinal compounds are probably derived or obtained from plants (Ahmadu *et al.*, 2006). The use of medicinal plants in West Africa is probably as old as the duration of human settlement (Abdulrahman *et al.*, 2010). *Solanum macrocarpum*("Gorongo" in Kanuri) is one of the agents used for folklore medicinal purposes. Although the unripe fruit of the plant is used by traditional healers for the treatment of various ailments (Grubben and Denton, 2004), information on the hepatotoxicity of the extract in man and animals is not readily available except that by Sodipo *et al.*, 2009 that investigated the effect of the aqueous fruit extract of the plant on the liver function of diet-induced hypercholesterolaemic rats. The present study investigated the effect of the fruit of *S. macrocarpum* on triton-induced hyperlipidaemic rats in an attempt to find an alternative hypolipademic agent that is both therapeutically and cost effective, but with fewer side effects than the existing ones which are expensive and at the same time have numerous side effects (Hardman and Limbird, 2001).

MATERIALS AND METHOD

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, University of Maiduguri.

Extraction

The fruit of *S. macrocarpum* with the calyx removed was air dried and pulverized by grinding using pestle and mortar. The 2.2kg of the ground fruit was subjected to exhaustive Soxhlet-extraction in distilled water at 100^{o}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 at room temperature until when required.

Animals

Thirty male albino rats of Wistar strain weighing 160-200g were used in this study. The animals were obtained from the Animal House Unit of the Department of Veterinary Physiology and Pharmacology, 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 hyperlipidaemic by feeding them orally (p.o) for 1 week with normal feed diet and triton-X (Sigma Chemical Co. St. Louis, M.O. USA) at a dose of 400mg/kg in saline suspension from the stock concentration of 535g/ml. The rats were divided into 5 groups of 6 animals each. After seven (7) days, the rats were administered with graded doses of the fruit extract. Group I was the control and it was given distilled water only. Groups II, III, IV and V were administered with geometrical doses (25mg/kg, 50mg/kg, 100 mg/kg and 200mg/kg) of the fruit extract intraperitoneally (i.p.) from a stock concentration of 200mg/ml. After 24hrs, 48hrs, and 72hrs, respectively of the effect of the extract on the hyperlipidaemic 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 revolutions 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 weight was taken. The weights were taken before and after administration of triton-X for 7 days.

Biochemical liver function tests

The liver function parameters estimated from the serum were protein, albumin, total bilirubin and liver enzymes which included aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP). AST and ALT were assayed using commercial Randox kits (UK) and by Quinica Clinical Applicanda, JA Kits (Moss *et al.*, 1986). The total protein in the serum was estimated using direct Biuret method (Peters *et al.*, 1982; Afonja, 1997). Serum albumin and bilirubin were determined by the dye bromocresol-green method by Doumas *et al.*, (1971); Spencer and Price (1977); Teitz (1994).

Statistical analysis

Data ware expressed as the mean ± SD. The results obtained were subjected to Analysis of Variance (ANOVA) using Graph Pad Software (1998).

RESULTS

Effect of extract on body weight of rats

The effect of triton-X on mean body weight of albino rats is shown in Table 1. The increase in body weight observed in the rats was not statistically significant (P>0.05) when compared with the zero in all the groups except in group 1 where the increase was significant (P<0.05).

Table 1: Change in body weight of male albino rats after being administered Triton-X (400 mg/kg) orally for 7 days.

Group	Mean Body Weight ± S.D. (g)	
	Days of Treatment	
	0	7
One	114.33 ± 11.76^{a}	129.00 ± 9.22^{b}
Two	98.83 ± 11.55^{a}	111.83 ± 11.18^{a}
Three	140.83 ± 37.57^{a}	151.67 ± 36.82^{a}
Four	137.33 ± 30.89^{a}	147.00 ± 30.44^{a}
Five	175.33 ± 31.10^{a}	194.83 ± 37.12^{a}

Within rows, means with the same superscript are not statistically significant (p > 0.05) when compared with day 0 using student t-test.
0 day = Before triton-X administration
7 days = After oral administration of triton-X
n = 6 rats

Effect of extract on liver function

The effect of the aqueous fruit extract of *Solanum macrocarpum* on protein, albumin and total bilirubin are shown in Table 2 whilst those of the liver enzymes are shown in Table 3. Serum protein and albumin decreased significantly (P<0.05) whilst bilirubin increased significantly (P<0.05) at 72hrs of study with increase in extract dose when compared to the control. Aspartate amino transferase (AST) dose-dependently and significantly decreased (P<0.05) at 48hrs and 72hrs. The values of alanine

amino transferase (ALT) decreased significantly (P<0.05) at 72hrs when compared to the control. The decrease in alkaline phosphatase (ALP) activity was not significant (P>0.05) when compared to the the control.

Table 2: Effect of the aqueous fruit extract of *S. macrocarpum* on protein albumin and total bilirubin of hyperlipidaemic rats administered orally with Triton-X for 7 days.

Hours after extract administration	Extract dose mg/kg	Protein (g/L)	Albumin (g/L)	Total Bilirubin (µmol/L)
24	Control	71.00 ± 1.41[a]	39.00 ± 1.41[a]	2.50 ± 0.71[a]
	25.00	65.50 ± 5.00[a]	37.5 ± 0.71[a]	2.50 ± 0.71[a]
	50.00	65.00 ± 4.24[a]	37.00 ± 1.41[a]	3.50 ± 0.71[a]
	100.00	64.50 ± 0.71[a]	36.00 ± 0.00[a]	4.00 ± 0.00[a]
	200.00	64.50 ± 5.00[a]	35.50 ± 0.71[a]	4.00 ± 1.41[a]
48	Control	72.00 ± 2.83[a]	39.00 ± 1.41[a]	3.50 ± 0.71[a]
	25.00	69.50 ± 0.71[a]	37.50 ± 0.71[a]	4.00 ± 0.50[a]
	50.00	68.50 ± 2.12[a]	37.00 ± 1.41[a]	4.00 ± 1.41[a]
	100.00	66.50 ± 2.12[a]	36.50 ± 0.71[a]	4.50 ± 0.71[a]
	200.00	66.00 ± 1.41[a]	35.50 ± 0.71[a]	5.00 ± 0.71[a]
72	Control	67.00 ± 1.41[a]	36.50 ± 0.71[a]	2.50 ± 0.71[a]
	25.00	64.00 ± 2.83[b]	34.00 ± 1.41[b]	3.50 ± 0.71[b]
	50.00	61.50 ± 0.71[b]	33.51 ± 0.71[b]	4.00 ± 1.41[b]
	100.00	60.50 ± 0.71[b]	33.50 ± 0.71[b]	4.50 ± 0.71[b]
	200.00	59.50 ± 0.71[b]	32.50 ± 0.71[b]	6.50 ± 0.71[b]

Means with different superscripts are statistically significant (p < 0.05) among the groups. Control = Distilled water.

Table 3: Effect of the aqueous fruit extract of *S. macrocarpum* on serum enzymes of hyperlipidaemic rats administered orally with Triton-X for 7 days.

Hours after extract administration	Extract dose mg/kg	Serum enzymes (U/L)		
		AST	ALT	ALP
		Mean ± S.D.		
24	Control	89.50 ± 0.71[a]	32.00 ± 9.90[a]	174.00 ± 15.56[a]
	25.00	89.00 ± 0.00[a]	31.50 ± 3.54[a]	171.50 ± 6.36[a]
	50.00	78.00 ± 5.56[a]	27.00 ± 2.83[a]	170.50 ± 6.36[a]
	100.00	59.50 ± 10.61[a]	23.00 ± 2.83[a]	167.50 ± 16.26[a]
	200.00	58.50 ± 0.71[a]	21.00 ± 0.00[a]	131.00 ± 15.56[a]
48	Control	59.00 ± 0.00[a]	36.50 ± 3.54[a]	144.50 ± 0.71[a]
	25.00	52.50 ± 0.71[b]	36.50 ± 3.54[a]	143.00 ± 2.83[a]
	50.00	38.50 ± 3.54[b]	34.00 ± 0.71[a]	141.00 ± 1.41[a]
	100.00	36.00 ± 7.07[b]	25.00 ± 0.00[a]	137.00 ± 7.07[a]
	200.00	31.50 ± 0.71[b]	25.00 ± 0.00[a]	126.50 ± 8.49[a]
72	Control	71.50 ± 6.36[a]	45.50 ± 3.54[a]	110.00 ± 2.83[a]
	25.00	67.00 ± 0.00[b]	43.50 ± 0.71[b]	108.00 ± 2.83[a]
	50.00	52.50 ± 0.71[b]	41.00 ± 2.83[b]	105.00 ± 0.71[a]
	100.00	44.00 ± 4.24[b]	39.00 ± 0.00[b]	104.50 ± 0.71[a]
	200.00	44.00 ± 4.24[b]	31.50 ± 3.64[b]	102.50 ± 0.71[a]

Means with different superscripts are statistically significant (p < 0.05) among the groups. Control = Distilled water

DISCUSSION

The observed decrease in serum protein which was significant at 72hrs (P<0.05) in the present study may be associated with liver damage, nutritional deficiency and renal failure (Mukherjee, 1980; Odutola, 1992; Sood, 2006). Since proteins are constituents of muscle, enzymes, hormones and several other key factors, invariably these factors will be affected. Thus, the effect of the extract on the hyperlipidaemic rats is probably that of liver toxicity or excessive protein catabolism. The reduction in total protein in this study agrees with the reports of Rabo *et al.,* (2003) working on *Butyrospermum paradoxum* extracts in rats; Iweala and Okeke (2005) on the aqueous leaf, flowers and tender stem extract of *Catharanthus roseus* Linn. (Madagascar periwinkle-Apocynaceae) on alloxan induced diabetic rats and Sodipo *et al.*, (2009) on the aqueous fruit extract of diet-induced hypercholesterolaemic rats. This decrease in serum protein was attributed to increased binding of the plant components to serum albumin. The decease in total protein probably portrays hepatocellular damage. The decrease in albumin level which was significant (P<0.05) at 72hrs with increase in extract dose on the hyperlipidaemic rats probably potrays liver damage at this contact time. The observed decrease in albumin levels is in conformity with the results of Uhegbu and Ogbuechi (2004) who reported a decrease in albumin levels. This decrease in albumin in this study contrasts the report by Atangwho *et al.*, (2007) that the more protected the heaptocytes become, the more the boost to their synthetic function.

The increase in bilirubin levels which was significant (P<0.05) at 72hrs, was caused by increasing doses of the extract. Increase in bilirubin values may be cuased by liver damage, excessive haemolytic destruction of erythrocytes, obstruction of the biliary tract (obstructive jaundice) and in drug-induced reactions (Mukherjee, 1988, Odutola, 1992; Sood, 2006). However, if the AST and ALT values are normal, the diagnosis of hepatocellular damage cannot be confirmed (Odutola, 1992). In the present study, the effect of the extract on AST and ALT was that of reduction, significant for AST at 48hrs and 72hrs and for ALT at 72hrs of study respectively, thus confirming the extract's protective ability on the liver cells. Thus, the aqueous fruit extract of *Solanum macrocarpum* under the condition of the present study was probably not toxic just like in the hypercholestolaemic rats (Sodipo *et al.*, 2009).

The result of the liver enzymes showed that the extract had a significant decrease (P<0.05) on AST of the hyperlipidaemic rats at 48hrs and 72hrs and the decrease in ALT was significant (P<0.05) at 72hrs. However, the decrease in ALP for all the times of study was not significant (P>0.05). This observation is in line with the recent findings of Kim *et al.*, (2006); Atangwho *et al.*, (2007) who reported a decrease in elevated liver enzymes upon treatment of alloxan-induced diabetic rats with ethanol leaf extract of *Veronia anygdalina* Del. The value of the liver function tests depends on the specificity for damage as well as their sensitivity (Cutler, 1974; Okonkwo *et al.*, 1997; Sodipo *et al.*, 2009). Although serum levels of both AST and ALT become elevated when disease processes affect the liver integrity, ALT is the more liver specific enzyme and therefore generally more sensitive to changes in activity levels than AST (Kachmar and Moss, 1976; Sodipo *et al.*, 2009).

The results of the present study in which ALT was significantly reduced at 72hrs and AST was significantly reduced

at 48hrs and 72hrs of study respectively, therefore, suggest that the extract had no significant influence on the liver function. Also, AST is highly concentrated in several tissues including the heart, muscle, liver, skeletal muscle and kidney while ALT has its highest concentration in the liver (Kaneko and Cornelius, 1971; Wilkinson, 1976; Okonkwo *et al*., 1997; Nduka, 1997; Mayne, 1998; Atangwho *et al.*, 2007; Sodipo *et al.*, 2009), therefore, measure of ALT in serum is of greater diagnostic specificity in confirming or excluding liver damage. Since the decrease in ALT in the present study was significant at 72hrs, then there is no likelihood of liver damage by the aqueous fruit extract of *Solanum macrocarpum*.

A non-statistically significant (P>0.05) decrease in ALP value as obtained on extract administration in the present study is not of much clinical significance (Atangwho *et al.*,2007, Sodipo *et al*., 2009). Even if there had been an elevation in the ALP upon extract administration, it could still not have confirmed liver damage because according to Odutola (1992), ALP and AST originate from different tissues such as the liver, bones, intestine and placenta.

In the present study, upon extract administration, both the liver enzymes AST and ALT decreased significantly whilst ALP did not show any significant change. All these may show that the effect of the extract on the rats was not that of toxicity. Therefore, the aqueous fruit extract *Solanum macrocarpum* appears to be safe.

CONCLUSION

The present study shows that the aqueous fruit extract of *Solanum macrocarpum* may probably have a hepatoprotective effect. However, the results of the histopathological studies on hepatic architecture are required in order to confirm the findings of the biochemical indices of liver function.

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