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BIOCHEMICAL LIVER FUNCTION WITH AQUEOUS FRUIT EXTRACT OF *SOLANUM MACROCARPUM* LINN. IN ALBINO RATS CHRONICALLY ADMINISTERED TRITON-X TO INDUCE HYPERLIPIDEMIA

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ABSTRACT

The effect of the aqueous fruit extract, *Solanum macrocarpum* Linn. on some biochemical indices of the liver function was studied in chronic triton-induced hyperlipidaemic wistar rats. Thirty six male rats (160-200g) were used in the study and assigned to 6 groups of 6 rats each. Group I negative control rats received distilled water only, whereas groups 3, 4, 5, and 6, the experimental hyperlipidaemic rats, were administered graded does of the plant extract (25mg/kg, 50mg/kg, 100mg/kg and 200kg/mg) per body weight intraperitoneally with exception of group 2, the positive control hyperlipidaemic rats after which blood samples were taken from the rats 24 hrs and 72 hrs, respectively after extract administration. Blood was also collected at the same time interval from the rats in groups and two. Serum aspartate amino transferase (AST) dose dependently and significantly decreased ($P<0.05$) at 48 hrs. The values of alanine amino transferase (ALT) and alkaline phosphatase (ALP) decreased significantly ($p<0.05$) at 72 hrs when compared to the negative control. Serum protein and albumin increased significantly ($P<0.05$) at 24 hrs while bilirubin decreased significantly ($P<0.05$) at 48 hrs of study. In conclusion, *Solanum macrocarpum* probably has hepatoprotective effects on chronic triton-induced hyperlipidaemic rats.

KEYWORDS: Chronic hyperlipidaemic rats, *Solanum macrocarpum,* liver function.

INTRODUCTION

The use of medicinal plants in West Africa is probably as old as the duration of human settlement in the region [1]. *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 [2], information on the hepatotoxicity of the extract in man and animals is not readily available except that by Sodipo *et al.,* [3] that investigated the effect of the aqueous fruit extract of the plant on the liver function of diet-induced hypercholesterolaemic rats and acute triton-induced hyperlipidaemic rats respectively. The present study investigated the effect of the fruit of *S. macrocarpum* on chronic titron-induced hyperlipidaemic rats in an attempt to find an alternative hypolipidaemic 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 [4, 5].

METHODS AND MATERIALS

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 2.2 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 [6, 7, 8]. The resultant solution was concentrated *in vacuous* and it was stored in a specimen bottle 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 and Pharmacology and Biochemistry, University of Maiduguri, Maiduguri. The animals were housed under standard laboratory condition in plastic cages.

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They were fed with 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 [9] 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 whilst the remaining 6 (the negative control) which made up group I were given only normal feed and distilled water for 90 days making a total of 36 rats, (6 groups with 6 animals each). Thirty (30) rats were divided into 5 groups of 6 animals each. After ninety (90) days, the 24 of the 30 rats were administered with graded doses of the fruit extract. Group I was the negative control and it was given distilled water only. Groups 3, 4, 5, and 6 were administered with geometrical doses (25mg/kg, 50mg/kg, 100mg/kg and 200mg/kg) of the fruit extract intraperitoneally (i.p.) from a stock concentration of 200mg/ml with the exception of group 2 which was the positive control. After 24 hrs, 48 hrs, and 72 hrs, respectively of the effect of the extract on 24 of hyeprlipidaemic rats, adapted [10], 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, 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 the 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 30, 60 and 90 days respectively.

Biochemical Liver Function Tests

The liver function parameters estimated from the serum were protein, albumin, total bilirubum 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 [11]. The total protein in the serum was estimated using direct Biuret method [12, 13]. Serum albumin and bilirubin were determined by the dye bromocresol-green method [14-16].

Determination of total cholesterol

Two rats in each group were humanly sacrificed by cutting the throat with a sterile blade. Blood was collected from the vena cava into clean, labeled centrifuge tubes without anticoagulant after the extract had been allowed to act for 24, 48 and 72 hrs respectively. 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. Cholesterol was assayed by Tindar's reaction [17, 18], using commercial kits, from Fortress Diagnostic Ltd, Antrim.

Statistical Analysis

Data were expressed as the mean±S.D. The results obtained were subjected to Analysis of Variance (ANOVA) using Graph Pad Software [19].

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) Days of Treatment				% Increase in Mean Body Weight
	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

Table 2. Effect of the aqueous extract of *S. macrocarpum* on protein, albumin and total bilirubin of hyperlipidaemic rats administered orally with triton-X for 90 days.

Hours after extract administration	Group	Extract dose (mg/kg)	Protein (g/L)	Albumin (g/L)	Total Bilirubin (mol/L)
			Mean ± S.D.		
24	One	-ve control	76.00±2.83[a]	35.00±0.00[a]	4.00±1.41[a]
	Two	+ve control	66.50±2.12[b]	32.00±0.00[b]	5.50±2.12[a]
	Three	25.00	64.50±2.12[b]	35.50±1.41[b]	5.00±0.71[a]
	Four	50.00	66.50±2.12[b]	36.00±1.41[b]	4.50±0.71[a]
	Five	100.00	73.50±0.71[b]	36.50±0.71[b]	4.00±0.71[a]
	Six	200.00	79.50±0.71[b]	37.00±1.41[b]	3.50±0.00[a]
48	One	-ve control	61.00±14.14[a]	31.50±2.12[a]	4.50±0.71[a]
	Two	+ve control	58.50±3.54[a]	30.00±7.01[a]	6.50±0.71[b]
	Three	25.00	60.00±9.90[a]	31.50±4.95[a]	6.00±0.00[b]
	Four	50.00	66.00±2.83[a]	32.00±0.00[a]	5.00±1.41[b]
	Five	100.00	72.50±3.53[a]	32.50±4.95[a]	4.50±0.71[b]
	Six	200.00	73.00±4.24[a]	34.50±0.79[a]	3.00±0.00[b]
72	One	-ve control	62.50±3.54[a]	31.50±2.12[a]	3.00±1.41[a]
	Two	+ve control	56.50±3.54[a]	29.50±2.12[a]	4.00±1.41[a]
	Three	25.00	63.50±3.52[a]	31.00±1.41[a]	3.50±0.71[a]
	Four	50.00	65.00±0.71[a]	31.50±2.12[a]	2.50±0.71[a]
	Five	100.00	66.00±2.83[a]	32.00±2.83[a]	2.00±1.41[a]
	Six	200.00	74.50±3.59[a]	35.50±0.71[a]	1.50±0.71[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

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 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 the 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 of aqueous fruit extract of *Solanum macrocarpum* on some serum enzymes

The results of the effect of the aqueous fruit extract on some serum enzymes are shown in Table 3. The ALT and ALP decreased significantly at 72 hrs ($P<0.05$) whilst the decrease in AST was significant ($P<0.05$) at 48 hrs.

Effect of the aqueous fruit extract on protein, albumin and total bilirubin

The effect of the aqueous fruit extract of *Solanum macrocarpum* on serum protein, albumin and total bilirubin are shown in Table 2. The protein and albumin increased while the total bilirubin decreased with increase in extract dose. The increase in protein and albumin was only significant ($P<0.05$) at 24 hrs, whilst the decrease in total bilirubin was only significant at 48 hrs ($P<0.05$).

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 72 hrs respectively. The oral administration of triton-X resulted in a rise in serum cholesterol of rats in the positive control groups (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 [20]. It probably suggests that the triton-X has induced arthrosclerosis as atherosclerosis takes three to six months to be induced in rats [10]. There was an increase in protein and albumin levels, significant at 24 hrs of study ($P<0.05$) when increasing doses of *S. macrocarpum* were administered to the hyperlipidaemic rats probably supporting the hepatoprotective ability of the aqueous fruit extract of *S. macrocarpum* in this study as earlier described for hypercholesterolaemic [21] and acute hyperlipidaemic rats [3]. The increase in serum protein in the present study probably implies that the liver is not damaged since proteins are constituents of muscle, enzymes, hormones and several other key factors, invariably these factors will not be affected [22, 23, 24, 3]. Thus, the effect of the extract on the chronic hyperlipidaemic rats is probably that of the hepatoprotection.

The increase in albumin levels which was significant ($P<0.05$) at 24 hrs with increase in extract dose on the chronic hyperlipidaemic rats probably portrays live protection at this contact time. This increase in albumin in this study is in agreement with the report of Antanghwo *et al.,* [25] that the more protected the hepatocytes become, the more the boost to their synthetic function. The decrease in bilirubin levels, significant at 48 hrs ($P<0.05$) was caused by increasing doses of the extract on the hyperlipidaemic rats. Increase in bilirubin values may be caused by liver damage, excessive haemolysis/destruction of RBC, obstruction of the biliary tract (obstructive jaundice) and in drug-induced reactions [22, 23, 24]. Thus in the present study, the aqueous fruit extract of *S. macrocarpum* is probably not toxic just like in the hypercholesterolaemic [21] and acute triton-induced hyperlipidaemic rasts [3]. Also if the AST and ALT values are normal, the diagnosis of hepatocellular damage cannot be confirmed [23]. In the present study, the effect of the extract on AST and ALT was that of reduction, significant for AST at 48 hrs and for ALT at 72 hrs of study respectively, thus confirming the extract protective ability on the liver cells just like in the hypercholesterolaemic [21] and acute triton-induced hyperlipidaemic rats [3].

The result of the liver enzymes showed that the extract had significant decrease on AST at 48 hrs, ALT and ALP at 72 hrs. This observation is in line with the findings of [26, 25] who reported a decrease in elevated liver enzymes upon treatment of alloxan-induced diabetic rats with ethanol

leaf extract of *Veronia amygdalina* Del. The results also tally with the recent findings of [21, 3] who reported a decrease in liver enzymes in diet-induced hypercholesterolaemic and acute triton-induced hyperlipidaemic rats administered with aqueous fruit extract of *Solanum macrocarpum* Linn. The value of the liver function tests depends on the specificity for damage as well as their sensitivity [27, 28, 21, 3]. 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 [3, 21, 29]. The results of the present study in which ALT and ALP were significantly reduced at 72 hrs and AST at 48 hrs 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 [30, 31, 28, 32, 33, 25, 21, 3], therefore, measure of ALT in serum is of greater diagnostic specificity in confirming or excluding liver damage. Since the decrease in ALT was maximal at 72 hrs, then there is no likelihood of liver damage by the aqueous fruit extract of *Solanum macrocarpum*. Just like AST, ALP also originates from different tissues such as the liver, bones, intestine and placenta, [3], so a significant decrease, maximal at 72 hrs like ALT, is not specific for diagnosing liver damage. In the present study, upon extract administration, both the protein and albumin levels increased significantly whilst the liver enzymes ALT, AST and ALP decreased significantly. All these may show that the effect of the extract on the chronic triton-induced hyperlipidaemic rats was not that of liver toxicity. Therefore, the effect of the aqueous fruit extract of *Solanum macrocarpum* appears to be that of hepatoprotection.

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

Hours after extract administration	Group	Extract dose (mg/kg)	Serum enzymes (U/L)		
			AST	ALT	ALP
			Mean ± S.D.		
24	One	-ve control	58.50±24.75[a]	31.50±3.54[a]	207.50±21.92[a]
	Two	+ve control	82.50±9.19[a]	43.00±12.73[a]	253.00±14.14[a]
	Three	25.00	74.00±21.21[a]	32.50±4.95[a]	221.00±24.04[a]
	Four	50.00	71.50±6.36[a]	29.50±9.91[a]	215.00±45.26[a]
	Five	100.00	58.50±24.75[a]	27.50±0.71[a]	207.50±21.92[a]
	Six	200.00	43.00±2.83[a]	24.50±0.71[a]	178.00±2.85[a]
48	One	-ve control	67.00±0.00[a]	27.50±0.79[a]	211.50±68.59[a]
	Two	+ve control	89.00±0.00[b]	40.50±16.26[a]	284.80±8.49[a]
	Three	25.00	77.00±0.00[b]	29.50±0.71[a]	232.00±2.88[a]
	Four	50.00	67.00±0.00[b]	27.50±0.79[a]	202.00±12.83[a]
	Five	100.00	63.50±4.95[b]	27.00±0.79[a]	153.00±4.24[a]
	Six	200.00	45.56±6.36[b]	24.50±4.95[a]	130.50±38.89[a]
72	One	-ve control	69.50±14.85[a]	28.00±1.41[a]	216.50±2.12[a]
	Two	+ve control	84.00±7.07[a]	34.50±0.71[b]	232.50±3.54[b]
	Three	25.00	73.00±4.24[a]	34.00±0.00[b]	225.00±7.07[b]
	Four	50.00	62.50±37.48[a]	24.50±6.36[b]	207.50±2.12[b]
	Five	100.00	55.50±4.95[a]	22.50±2.12[b]	190.00±3.54[b]
	Six	200.00	43.50±3.84[a]	20.50±0.71[b]	108.00±2.83[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

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 [20]. It probably suggests that the triton-X has induced arthrosclerosis as atherosclerosis takes three to six months to be induced in rats [10].

There was an increase in protein and albumin levels, significant at 24 hrs of study ($P<0.05$) when increasing doses of *S. macrocarpum* were administered to the hyperlipidaemic rats probably supporting the hepatoprotective ability of the aqueous fruit extract of *S. macrocarpum* in this study as earlier described for hypercholesterolaemic [21] and acute hyperlipidaemic rats [3]. The increase in serum protein in the present study probably implies that the liver is not damaged since proteins are constituents of muscle, enzymes, hormones and several other key factors, invariably these factors will not be affected [22, 23, 24, 3]. Thus, the effect of the extract on the chronic hyperlipidaemic rats is probably that of the hepatoprotection.

Table 4. Effect of the aqueous fruit extract *S. macrocarpum* in total cholesterol of hyperlipidemic rats administered orally with Triton-Z 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	2.35±0.07[a]
	Six	200.00	1.35±0.07[a]
48	One	-ve control	1.70±0.14[a]
	Two	+ve control	2.55±0.07[a]
	Three	25.00	2.50±0.14[a]
	Four	50.00	1.50±0.07[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	2.20±0.42[a]
	Five	100.00	1.70±0.14[a]
	Six	200.00	2.35±0.07[a]

Within columns, mean 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

The increase in albumin levels which was significant ($P<0.05$) at 24 hrs with increase in extract dose on the chronic hyperlipidaemic rats probably portrays live protection at this contact time. This increase in albumin in this study is in agreement with the report of Antanghwo *et al.,* [25] that the more protected the hepatocytes become, the more the boost to their synthetic function.

The decrease in bilirubin levels, significant at 48 hrs ($P<0.05$) was caused by increasing doses of the extract on the hyperlipidaemic rats. Increase in bilirubin values may be caused by liver damage, excessive haemolysis/destruction of RBC, obstruction of the biliary tract (obstructive jaundice) and in drug-induced reactions [22, 23, 24]. Thus in the present study, the aqueous fruit extract of *S. macrocarpum* is probably not toxic just like in the hypercholesterolaemic [21] and acute triton-induced hyperlipidaemic rasts [3]. Also if the AST and ALT values are normal, the diagnosis of hepatocellular damage cannot be confirmed [23]. In the present study, the effect of the extract on AST and ALT was that of reduction, significant for AST at 48 hrs and for ALT at 72 hrs of study respectively, thus confirming the extract protective ability on the liver cells just like in the hypercholesterolaemic [21] and acute triton-induced hyperlipidaemic rats [3].

The result of the liver enzymes showed that the extract had significant decrease on AST at 48 hrs, ALT and ALP at 72 hrs. This observation is in line with the findings of [26, 25] who reported a decrease in elevated liver enzymes upon treatment of alloxan-induced diabetic rats with ethanol leaf extract of *Veronia amygdalina* Del. The results also tally with the recent findings of [21, 3] who reported a decrease in liver enzymes in diet-induced hypercholesterolaemic and acute triton-induced hyperlipidaemic rats administered with aqueous fruit extract of *Solanum macrocarpum* Linn. The value of the liver function tests depends on the specificity for damage as well as their sensitivity [27, 28, 21, 3]. 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 [3, 21, 29].

The results of the present study in which ALT and ALP were significantly reduced at 72 hrs and AST at 48 hrs 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 [30, 31, 28, 32, 33, 25, 21, 3], therefore, measure of ALT in serum is of greater diagnostic specificity in confirming or excluding liver damage. Since the decrease in ALT was maximal at 72 hrs, then there is no likelihood of liver damage by the aqueous fruit extract of *Solanum macrocarpum*. Just like AST, ALP also originates from different tissues such as the liver, bones, intestine and placenta, [3], so a significant decrease, maximal at 72 hrs like ALT, is not specific for diagnosing liver damage.

In the present study, upon extract administration, both the protein and albumin levels increased significantly whilst the liver enzymes ALT, AST and ALP decreased significantly. All these may show that the effect of the extract on the chronic triton-induced hyperlipidaemic rats was not that of liver toxicity. Therefore, the effect of the aqueous fruit extract of *Solanum macrocarpum* appears to be that of hepatoprotection.

CONCLUSION

The present study shows that 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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