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Effects of the Aqueous Fruit Extract of *Solanum macrocarpum* Linn. on Some Haematological Indices in Albino Rats Fed With Cholesterol-Rich Diet

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ABSTRACT

The LD_{50} and the effect of the aqueous fruit extract of *Solanum macrocarpum* Linn. on haematological parameters in albino rats fed with cholesterol-rich diet was studied. The intraperitoneal (i.p.) LD_{50} with 95% confidence interval of the aqueous fruit extract was estimated to be 1,280 mg/kg, indicating that the extract is not toxic. Graded doses (25, 50, 100, 200 mg/kg body weight) of the extract were administered i.p. to different groups of hypercholesterolaemic rats for 7 days. Significant dose-dependent increases ($p < 0.05$) in the levels of haemoglobin (Hb), erythrocyte count (RBC), packed cell volume (PCV), mean cell haemoglobin concentration (MCHC) and white blood cell count (WBC) were observed. However, the changes in the mean cell volume (MCV) were not statistically significant ($p > 0.05$). The decrease in mean cell haemoglobin (MCH) was significant ($p < 0.05$). Serum total cholesterol was lowered with increasing dose of the extract though not significantly ($p > 0.05$). The fruit of *Solanum macrocarpum* contains some important chemical components which have therapeutic values. The aqueous fruit extract of *Solanum macrocarpum* appears to have some beneficial effect on haematological parameters of hypercholesterolaemic rats (RBC, Hb, PCV and MCHC) suggesting that it could be used as an anti-anaemic agent.

Key words: *Solanum macrocarpum*, median lethal dose (LD_{50}), haematological indices, medicinal, total cholesterol

INTRODUCTION

Herbal medicines play positive roles in the lives of Nigerians and peoples throughout the world (Abdu-Aguye, 1997, Abdulrahman and Onyeyili, 2001; Okpako *et al*., 2002). About 60% of medicines sold in the pharmacies still come directly from natural sources (Attiso, 1983). Also, about 25% of prescription drugs dispensed in the United States of America contain at least one active ingredient derived from plant materials. Some are made from plant extracts, others are synthesised to mimic a natural compound (http://www.holistic..., 2006). Traditional medicine has been practiced to some degree in all cultures. The traditional medical systems are based on experiments in using plant products in eradication of common ailments (Gupta, 1994). Thus, the World health Organisation (WHO) has encouraged research on herbal medicines with a view to developing and incorporating the effective locally available substitutes/herbs/ remedies into the health care system (Abdulrahman *et al*., 2005).

Solanum macrocarpum ("Gorongo" in Kanuri, garden egg in English) is a nutraceutical herb/shrub cultivated in the North East Arid Zone of Nigeria (Bokhari and Ahmed, 1980). It is a member of the *Solanaceae* family. Although parts of the plant (fruit, leaves, flowers and roots) are used for the treatment of various ailments such as hypercholesterolaemia (Grubben and Denton, 2004), information on the toxicity of the plant extract in man and animal is lacking. Acute toxicity study is important since science requires the validation of drugs by medical practitioners and drug regulatory authorities demand that all potential drugs should pass through a rigorous series of study and scrutiny (Iwu, 1996; Abdulrahman, 2004). Also in traditional medicine, there is no proper dosage or quantity of the material given to recipients and the nature and type of active ingredient in the mixture may not be known. In such practices, the probability of administering toxic doses is high (Musa *et al*., 2005). In view of the

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numerous uses of the fruit of this plant, especially as a laxative and in lowering hyperlipidaemia in traditional medicine, the present study investigated the effect of the aqueous fruit extract of *S. macrocarpum* on haematological indices in hypercholesterolaemic albino rats fed with cholesterol-rich diet.

MATERIALS AND METHODS

Plant material

The plant material used in this study was obtained from Alau in Konduga Local Government Area, 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. 548A) was deposited at the Research Laboratory of the Department of Chemistry, University of Maiduguri.

Preparation of extract

The fruit with the calyx removed was air-dried in the laboratory and ground to a coarse powder. A 2.2 kg of the powdered sample was Soxhlet-extracted with distilled water at 100°C to give an aqueous extract with yield of 15.34%$^{w}/_{w}$ which was coded "CAE" using standard methods (Mittal *et al*., 1981; Fernando *et al*., 1991; Lin *et al*., 1999). The resultant extract was concentrated *in vacuo* and stored in a specimen bottle at room temperature until used.

Phytochemical analysis

The phytochemical analysis of the extract was performed by testing for alkaloids, terpenoids, tannins, saponins, flavonoids, cardiac glycosides, anthracenes, carbohydrates, anthraquinones and polyuronides using standard procedures (Clark, 1975; Odebiyi and Sofowora, 1978; Sofowora, 1984; Awe and Sodipo, 2001; Evans, 2002).

Animals

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

Acute toxicity test

A pilot study using 6 rats was conducted to determine the lowest dose of the fruit extract that did not cause death and the highest dose that caused death in the animals (Aliu and Nwude, 1982; Musa *et al*., 2005). The concentrations of the aqueous fruit extract administered were 200 mg/ml, 300 mg/ml and 500 mg/ml at a dose of 1,600 mg/kg (2 rats per concentration) and the animals were observed for 24 h for death. The pilot study for the acute toxicity test showed that no death was recorded at 200 mg/ml stock concentration of the aqueous extract. So the stock concentration of the extract that was used for LD_{50} determination was 200 mg/ml.

Twenty-five rats were randomly distributed into 5 groups of 5 each. The control group (Group I) received distilled water via the intraperitoneal route while the rats in groups II, III, IV and V were administered 200, 400, 800 and 1,600 mg/kg of the aqueous fruit extract respectively, via the same route. The animals were observed for clinical signs and death over a period of 24 h. Postmortem examinations were also carried out. The LD_{50} with 95% confidence limit was determined using the arithmetic method of Karber, (1931) as modified by Aliu and Nwude (1982).

Administration of cholesterol and extract

The remaining rats (25 in number) were divided into five groups of five each. Before the 1% cholesterol (BDH Biomedical, Poole, England), mixed with 1% groundnut oil (and added to the feed) was administered *ad libitum* (Odetola *et al*., 2004) orally, the weights of the rats in each group were taken. The weights were subsequently taken every week for 3 weeks after cholesterol administration and at 4 weeks after extract had been administered for 7 days. Each group of rats was administered respectively 25 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg of extract per body weight i.p., except the control group which was given distilled water only.

Haematological analysis

The estimation of the various haematological indices were carried out on week 4, i.e. one week after extract administration. At the end of the experimental period, blood samples were collected from the tail of each rat by making a cut right through, at a region of 2.0 cm from the tip. The haematological indices determined included red

blood cell count (RBC), packed cell volume (PCV), haemoglobin concentration (Hb) and total white blood cell count (WBC). The Wintrobe indices, mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were also determined. These haematological parameters were determined using standard procedures (Cole, 1974; Schalm *et al*., 1976, Brown, 1976; Dacie and Lewis, 1984).

Determination of cholesterol

The rats in 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 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. Cholesterol was assayed by Tindar's reaction (Evans and Stein, 1986; NIH, 1990) using commercial kits from Fortress Diagnostics 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

Phytochemical analysis

Phytochemical screening of the aqueous fruit extract revealed the presence of alkaloids, steroidal glycosides, tannins, saponins, flavonoids, reducing sugar, combined sugar and ketoses (Table 1).

Acute toxicity study

The i.p. LD_{50} with 95% confidence limit was 1280 mg/kg. The pilot study for the acute toxicity test showed death at both 300 mg/ml and 500 mg/ml stock concentration of the aqueous extract of *Solanum macrocarpum* at a dose of 1,600 mg/kg. No death was recorded at 200 mg/ml stock concentration of the aqueous extract.
Clinical signs and symptoms of toxicity observed in the animals included weakness and anorexia (which appeared immediately). These were followed by respiratory distress, difficulty in breathing, gasping to death, coma and finally death.

The gross pathology (postmortem) findings revealed clots and haemorrhages in the kidney, liver and hyperemia in the lungs. The spleen was dark whilst the heart was dark and bloody.

Effect of extract on haematological indices

The results of the various doses of aqueous fruit extract of *S. macrocarpum* on haematological indices of hypercholesterolaemic rats are shown in Table 2. The RBC count, Hb, PCV and WBC count increased significantly ($p<0.05$) with increase in extract dose when compared to the control. The MCH however significantly decreased with increase in extract dose ($p<0.05$). For the MCHC, there was a significant increase in the value from 25 mg/kg to 50 mg/kg extract ($p<0.05$) after which further increase in extract dose led to a decrease in the MCHC value. The MCV values however did not follow any specific pattern and the changes were not significant ($p>0.05$).

The effect of extract on total cholesterol

The effect of the aqueous fruit extract of *Solanum macrocarpum* on serum cholesterol of rats fed with cholesterol-rich diet is shown in Table 3. The decrease in the levels of total cholesterol on extract administration when compared to the control was not statistically significant ($p>0.05$).

DISCUSSION

From the pilot study of the acute toxicity study, it was only 200 mg/ml of the stock concentration of the extract that did not cause death. Thus, it is this concentration that should be used in LD_{50} determination and all other experiments involving the aqueous extract of *Solanum macrocarpum*.

The aqueous fruit extract had an i.p. LD_{50} of 1,280 mg/kg. Some workers (Clark and Clark, 1977; Muyibi *et al*., 2000; Abdulrahman, 2004; Biu, 2007) have reported that plant toxicity could be due to the presence of various organic chemical substances which include alkaloids, steroidal glycosides, cardenolides (which could be cardioactive or cardiotoxic glycosides and aglycones), polypeptides and amines, saponins, triterpenoids, resinoids, minerals and cyanides; some of which are seen in *Solanum macrocarpum*. Also the toxicity and degenerative changes observed agree with observations of Rabo, (1998) and Biu, (2007). Any substance whose intraperitoneal LD_{50} in rats is greater than 1,000 mg/kg is not toxic (Clark and Clark, 1977). Therefore, the i.p. LD_{50} of *S. macrocarpum* fruit of 1,280 mg/kg may be regarded as non-toxic.

The aqueous fruit extract of *Solanum macrocarpum* administered at the various doses appear to have some beneficial effects on the haematological indices given the observed increases in the values of RBC, Hb and PCV.

Table 1. Phytochemistry of the crude aqueous extract of petroleum ether (CAE) of the fruit of *Solanum macrocarpum*

S/No.	Class of chemical compound	Distilled water (CAE)
1.	Alkaloids	
	(a) General: Dragendorff's test	+
	Mayer's test	−
	Wagner's test	+
	(b) Tropane alkaloid test	−
	(c) Quinoline alkaloid test	−
	(d) Isoquinoline alkaloid test	−
	(e) Indole alkaloid test	−
2.	Cardiac glycosides	
	(a) Lieberman-Burchard's test (for steroids and triterpenes)	−
	(b) Salkowiski's test (for steroid ring)	−
	(c) Keller-Killani test (for steroid ring)	+
3.	Anthraquinones	
	(a) Bontrigger's test (for free anthraquinones)	−
	(b) Free and/or combined anthraquinones	−
	(c) C-glycoside anthraquinone test	−
4.	Tannins	
	(a) Ferric-chloride test	+
	(b) Lead subacetate test	+
5.	Phlobatannins	
	(a) Hydrchloric acid test	−
	(b) Lime water test	−
6.	Flavonoids	
	(a) Lead acetate test	+
	(b) Sodium hydroxide test	+
	(c) Ferric chloride test	+
	(d) Pew test	+
	(e) Flavone glycoside (flavonoside test)	−
7.	Anthracenes	−
8.	Saponins (Frost test)	+
9.	Polyuronides (mucilages)	−
10.	Carbohydrates	
	(a) General test (Molisch's test)	+
	(b) Monosaccharides (Barfoed's test)	−
	(c) Reducing sugar (Fehling's test)	−
	(d) Combined sugar	−
	(e) Ketoses (resorcinol or Sclivanoff's test)	+
	(f) Pentoses	−

+ = present; − = absent

The improvement in the haematological parameters may be an indication of the possible usefulness of this extract as a haematinic and blood enhancer like the root bark of *Vitex doniana* (Verbanaceae) (Abdulrahman, 2004), root bark extract of *Terminalia avicennoides* (Abdulrahman *et al*., 2005) and the aqueous leaf extract of *Spondias mombin* L. (Adeyemi and Gbolade, 2006). According to Brown, (1976), antianaemic agents tend to stimulate production of RBC and improve the values of Hb and PCV. Furthermore, there was a report that the fruit of *Solanum macrocarpum* contained moderately high iron content, 532.45 ± 7.38 µg/g (Sodipo *et al*., 2008). The presence of iron therefore could have contributed to the observed improvement of haematological parameters. The increase in the haematological parameters in this study may also be due to the activities of the chemical constituents of the plant. Saponins as found in the extract are known to hydrolyse and produce sapogenins which may be steroid or triterpene (Eghianruwa, 2002). Shapiro and Greenfield (1987) reported the stimulatory effects of steroid on bone marrow resulting in increased erythropoiesis. Initial phytochemical studies revealed the presence of the steroidal

Table 2. Effect of the aqueous extract of the fruit of *Solanum macrocarpum* on mean haematological parameters ± S.D. of hypercholesterolaemic rats

Extract dose (mg/kg)	PCV % (Haematocrit)	Hb (g/100 ml)	RBC (mm^3)	WBC (g/100 ml)	MCV (φ^3)	MCH (pg)	MCHC (g/dl)
Control	43.40 ± 2.30^a	11.52 ± 0.99^a	$5.00 \times 10^6 \pm 0.27^a$	$7,260.00 \pm 2,416.20^a$	8.68 ± 1.23^a	2.30 ± 0.40^a	0.27 ± 0.03^a
25.00	43.60 ± 2.88^b	12.88 ± 1.76^b	$5.65 \times 10^6 \pm 0.57^b$	$10,180.00 \pm 697.85^b$	7.79 ± 1.23^a	2.28 ± 0.39^b	0.30 ± 0.39^b
50.00	44.00 ± 1.41^b	13.58 ± 0.85^b	$6.46 \times 10^6 \pm 0.58^b$	$11,175.00 \pm 1,577.70^b$	6.84 ± 1.09^a	2.10 ± 0.18^b	0.31 ± 0.02^b
100.00	48.25 ± 2.87^b	13.65 ± 11.10^b	$6.74 \times 10^6 \pm 1.08^b$	$11,450.00 \pm 2,420.30^b$	7.16 ± 1.09^a	2.02 ± 0.16^b	0.29 ± 0.03^b
200.00	49.00 ± 2.00^b	13.84 ± 0.36^b	$7.00 \times 10^6 \pm 0.58^b$	$11,675.00 \pm 1,721.20^b$	7.00 ± 3.10^a	1.97 ± 0.11^b	0.28 ± 0.03^b

$p<0.05$ = within columns, mean with different letters are statistically significant when compared with control; control = no extract administration; n = 5 (number of rats per group)

Table 3. Effect of the aqueous extract of the fruit of *Solanum macrocarpum* on total cholesterol ± SD on hypercholesterolaemic rats

Extract dose (mg/kg)	Total cholesterol (mmol/L)
Control	2.25 ± 0.19a
25.00	2.18 ± 0.49a
50.00	2.18 ± 0.29a
100.00	2.14 ± 0.23a
200.00	1.98 ± 0.17a

$p>0.05$ = difference between means within columns with the same superscripts are not statistically significant when comparted with the control; Control = no extract was administered; n = 5 (number of rats per group)

nucleus and saponins in the aqueous fruit extract of *Solanum macrocarpum*.

The administration of graded doses of *S. macrocarpum* extract significantly stimulated increased production of WBC ($p<0.05$) which could be a possible stimulation of the immune defense system (Kashinath, 1990). Furthermore, reports have shown that persistent antigen load in the body results in lymphocytosis (Schalm *et al*., 1975; Biu, 2007). The antigenicity of the extract may in part be due to the presence of tannins (Evans, 2002).
The increases in MCH and MCHC values were statistically significant ($p<0.05$). The increase in MCHC with increase in extract doses therefore buttresses the fact that the aqueous fruit extract of *Solanum macrocarpum* has a haematinic effect on the rats. MCV detects changes in red cell size. In the present study, the MCV values were not significant ($p>0.05$) and it did not follow a specific pattern, so the rats cannot be said to be anaemic. According to Odutola, (1992), decrease in MCV suggests a microcyctic red cell common in iron-deficiency anaemia, whilst an increased MCV indicates a macrocyctic cell, commonly caused by Vitamin B_{12} or folic acid deficiency. In the present study, the MCV change did not follow a specific pattern and it was not significant, thus buttressing the fact that the extract could still be acting as an anti-anaemic agent.

Increased levels of cholesterol lead to coronary artery disease, hypercholesterolaemia, diabetes, cirrhosis, haemolytic jaundice, acute infection, malnutrition and hyperthyroidism (Mukherjee, 1988; Odutola, 1992). In the present study, the decrease in serum cholesterol on extract administration, though not significant, is in agreement with the hypocholesterolaemia recorded with the aqueous stem bark of *Pausinystalia yohimbe* (K. Schum) and *Pausinystalia macroceras* (Pierre ex Beille) in male Wistar albino rats (Jacks, 2004) and as reported with the fruit of *Solanum melongena* L. and *Solanum gilo* Radii in New Zealand rabbits which were fed with diet-rich food (1% cholesterol plus groundnut oil (Odetola *et al.,* 2004). The aqueous fruit extract of *S. macrocarpum* could probably then be said to have a cholesterol-lowering effect on the rats fed with cholesterol-rich diet. The phytochemistry revealed that the fruit of *S. macrocarpum* contains alkaloids. Reports have shown the *Solanum* alkaloids to be solanidine and solasidine (Olaniyi *et al.,* 1998; http://www.chandel.com/products/solasidine.htm, 2007). The steroidal alkaloids are said to be responsible for lowering hyperlipidaemia (http://www.3. interscience.viley.com, 2007). Furthermore, saponins as found in these plants are cholesterol-lowering agents (Cheeke, 1971), thus the fruit of *S. macrocarpum* may probably be used in the treatment of hypercholesterolaemia as claimed in traditional medicine.

CONCLUSION

The aqueous fruit extract of *Solanum macrocarpum* has an i.p. LD_{50} of 1,280 mg, indicating that the fruit is not toxic. The fruit of *Solanum macrocarpum* contains some important chemical components which have therapeutic values. The aqueous fruit extract of *Solanum macrocarpum* increased haematological parameters, thus it appears to have some beneficial effect on haematological parameters of hypercholesterolaemic rats (RBC, Hb, PCV and MCHC) suggesting that it could be used as an anti-anaemic agent. The extract also increased the WBC counts of hypercholesterolaemic rats which may indicate an increase in immunity. The present study also shows that the aqueous fruit extract of *S. macrocarpum* may probably be used in the dietary aspect of controlling hypercholes-terolaemia as claimed in traditional medicine. However, the total lipid profile analysis still needs to be determined to confirm this claim.

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