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Total lipid profile with aqueous fruit extract of *Solanum macrocarpum* Linn. in hypercholesterolaemic albino rats

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Abstract

Studies were undertaken to investigate the effects of the aqueous fruit extract of *Solanum macrocarpum* Linn. on the total lipid profile: total cholesterol, triglycerides, high density lipoprotein-cholesterol (HDL-C) and low density lipoprotein-cholesterol (LDL-C) on hypercholesterolaemic rats. Total serum cholesterol, triglycerides and LDL-C were lowered in the hypercholesterolaemic rats with increasing dose of the extract whilst serum HDL-C increased. These changes however were not significant ($p > 0.05$) when compared to the control. The results show that the aqueous fruit extract of the plant may be capable of reducing circulating lipids in high cholesterol-diet fed rats. However, more work still needs to be done on this as the changes in the serum lipid profile were not significant so as to determine whether the plant either inhibits lipid biosynthesis or stimulates lipid catabolism.

Keywords: *Solanum macrocarpum*; Triglycerides; Cholesterol; LDL-cholesterol; HDL-cholesterol; Aqueous extract

Introduction

About half of the world's medicinal compounds are probably derived or obtained from plants (Akerele, 1988; Ahmadu *et al*., 2006). Important drugs of the past 50 years or so that revolutionised modern medical practice were mostly isolated from plants that have established history from which for one purpose or the other have been employed in ancient civilisation (Schultes, 1986). Systematic screening of plants for bioactive compound is now a routine in many laboratory devoted to biomedical research of particular interest in the search for compounds with hypolipidaemic activities. This has gained importance in recent years because of the alarming side effects and high cost of existing hypolipidaemic drugs. The plant *Solanum macrocarpum* L. (synonym: *S. macrocarpon* L. senso stricto or *S. daysphyllum* Schumach & Thonn) is extensively cultivated in the North East Arid Zone of Nigeria, Sierra Leone, Kenya and Uganda (Grubben and Denton, 2004). Trado-medical uses of the plant include: the young fruits and flowers are laxatives and for clearing the teeth and treatment of cardiac diseases and hyperlipidaemia. The heated leaves are chewed to treat throat troubles whilst the juice of boiled roots is drunk to treat stomach troubles (Grubben and Denton, 2004). The present study investigated the

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hypocholesterolaemic effects of the aqueous fruit extract of the plant on hypercholesterolaemic albino rats.

Experimental

Plant collection and identification. 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 Biological Sciences, University of Maiduguri, Maiduguri, Nigeria. Specimen voucher (No. 548 A) was deposited at the Research Laboratory of the Department of Chemistry.

Extraction. The fruit (40 kg) was air-dried in the laboratory and extracted according to the methods of Mittal *et al*., (1981); Fernando *et al*., (1991); Lin *et al*., (1999). The 2.2 kg of the ground fruit was subjected to Soxhlet extraction in distilled water at 100°C to give the aqueous extract (yield: 15.34%$^{w}/_{w}$). The resultant extract was concentrated *in vacuo* and stored at room temperature until when required.

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

Animals. Twenty-five Wistar rats of both sexes 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, University of Maiduguri. The animals were randomly quarantined 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.

Administration of cholesterol and extract. Twenty-five albino rats were made hypercholesterolaemic by feeding them orally (p.o.) with 1% cholesterol (BDH Biochemical Ltd., Poole, England) and groundnut oil for 3 weeks and given water *ad libitum* (Odetola, *et al*., 2004). The rats were divided into 5 groups of 5 animals each. At the end of the 3 weeks, the rats were administered with graded doses of the plant extract for 1 week. Group I was the control and it was given distilled water only. Groups II, III, IV and V were fed with geometrical doses (25 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg) of the fruit extract intraperitoneally (i.p.) for 7 days. At the end of the experimental periods, i.e. one week after extract administration, the rats in each group were sacrificed by cutting the throat with a clean razor and blood was collected from the vena cava into clean, labelled, centrifuged 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.

Determination of total lipid profile. The total lipid profile estimated from the serum was cholesterol, triglycerides, high density lipoprotein-cholesterol (HDL-C) and low density lipoprotein-cholesterol (LDL-C). Cholesterol was assayed by Tindar's reaction (Evans and Stein, 1986; NIH, 1990) using commercial kits from Fortress Diagnostics Ltd., Antrim. Serum triglycerides level was determined as described by Fossati and Prencipe, (1982); Cole *et al*., (1997) using commercial kit (IVD). HDL-C determination was carried out using commercial kit (Human Cholesterol Liquicolour Test Kit, Germany)

(Grove, 1979). The serum LDL-C level was determined by Friedewald's formula (Friedwald *et al*., 1972; Hardman and Limbird, 2002; Sood, 2006). The calculation for serum LDL-C is given by:

Serum LDL-C = Total cholesterol – Triglycerides ÷ 2.2 – HDL-C

Statistical analysis. Data were expressed as the mean ± S.D. The results obtained were subjected to analysis of variance (ANOVA) using Graph Pad Software (1988).

Results

Phytochemical screening. The result of the phytochemical investigation of the aqueous fruit extract of *Solanum macrocarpum* (Table 1) showed the presence of alkaloids, steroidal glycosides, tannins, flavonoids, saponins, combined reducing sugars, reducing sugars and ketoses.

Effect of extract on body weight of rats. The weights of the rats increased over the period of study, but the increase was not significant ($p > 0.05$) when compared to the control (Table 2).

Effect of extract on total lipid profile of hypercholesterolaemic rats. The effects of the aqueous fruit extract of *Solanum macrocarpum* on lipid profile of hypercholesterolaemic rats are shown in Table 3. Even though there was an increase in HDL-C when compared to the control, with increase in the dose of the extract, it was not statistically significant ($p > 0.05$). At the same time, the decrease in the levels of total cholesterol, triglycerides and LDL-C on extract administration, when compared to the control was also not statistically significant ($p > 0.05$).

Discussion

The increase in mean body weight of the rats before and after extract administration was not statistically significant ($p > 0.05$) probably implying that the aqueous fruit extract of *Solanum macrocarpum* at the doses employed had no appreciable effect on cholesterol metabolism that could be statistically significant in terms of body weight.

The decrease in total cholesterol, triglycerides and LDL-C and the increase in HDL-C on extract administration were not statistically significant ($p > 0.05$). Triglycerides are true fats, esters of glycerol and fatty acids that belong to the organic group of compounds called lipids. Upon hydrolysis, they yield glycol and fatty acids. Low levels of triglycerides imply that there is no risk factor related to atherosclerotic disease that causes thickening of the walls of larger arteries which may lead to a heart attack. Also, an increase in serum triglycerides leads to hyperlipidaemia (Mukherjee, 1988). In the present study, though the decrease recorded in triglycerides was not statistically significant, it may still probably be said that the aqueous fruit extract of *Solanum macrocarpum* lowers hypercholesterolaemia.

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

S/N	Class of chemical component	(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) Borntrager'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) Hydrochloric 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 (Froth 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 Selivanoff's test)	+
	f) Pentoses	–

Key: +++ = Copiously present ++ = Moderately present + = Slightly present – = Absent

Table 2: Effect of *Solanum macrocarpum* fruit extract on mean body weight of hypercholesterolaemic rats

Dose (mg/kg)	Mean Body Weight ± S.D. (g)				
	Week 0	Week 1	Week 2	Week 3	Week 4
Control	$189.62^{a} \pm 53.13$	$199.54^{a} \pm 54.17$	$203.86^{a} \pm 55.32$	$203.86^{a} \pm 55.32$	$226.90^{a} \pm 54.82$
25.00	$155.82^{a} \pm 38.64$	$164.82^{a} \pm 38.22$	$174.52^{a} \pm 40.02$	$176.58^{a} \pm 38.26$	$189.40^{a} \pm 33.14$
50.00	$164.70^{a} \pm 44.86$	$174.45^{a} \pm 44.55$	$183.90^{a} \pm 48.14$	$183.95^{a} \pm 46.30$	$185.03^{a} \pm 44.02$
100.00	$202.10^{a} \pm 14.64$	$211.83^{a} \pm 15.65$	$212.70^{a} \pm 14.22$	$219.80^{a} \pm 19.44$	$221.72^{a} \pm 17.17$
200.00	$152.26^{a} \pm 14.75$	$162.16^{a} \pm 14.91$	$173.00^{a} \pm 21.88$	$174.20^{a} \pm 16.40$	$179.06^{a} \pm 19.60$

$p > 0.05$ Within rows, mean with same superscript are not statistically significant when compared with control.
Week 0 = Immediately before cholesterol administration; Week 3 = Beginning of extract administration;
Week 4 = Last week of extract administration; Control = Not given any extract at all; n = 5 animals

Table 3: Effect of *Solanum macrocarpum* fruit extract on total lipid profile on hypercholesterolaemic rats

Dose (mg/kg)	Total cholesterol (mmol/L)	Triglycerides (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
Control	$2.25^{a} \pm 0.19$	$1.26^{a} \pm 0.20$	$0.98^{a} \pm 0.16$	$0.68^{a} \pm 0.35$
25.00	$2.18^{a} \pm 0.49$	$1.23^{a} \pm 0.10$	$0.98^{a} \pm 0.22$	$0.63^{a} \pm 0.26$
50.00	$2.18^{a} \pm 0.29$	$1.20^{a} \pm 0.28$	$1.00^{a} \pm 0.00$	$0.60^{a} \pm 0.18$
100.00	$2.14^{a} \pm 0.23$	$1.20^{a} \pm 0.14$	$1.03^{a} \pm 0.13$	$0.50^{a} \pm 0.26$
200.00	$1.98^{a} \pm 0.17$	$1.20^{a} \pm 0.14$	$1.05^{a} \pm 0.10$	$0.43^{a} \pm 0.05$

$p > 0.05$ Difference between means within columns, with same superscript are not statistically significant when compared with control; Control = No extract was administered; n = 5 animals

Low levels of triglycerides imply that there is no risk factor related to atherosclerotic disease that causes thickening of the walls of larger arteries which may lead to a heart attack. Also, an increase in serum triglycerides leads to hyperlipidaemia (Mukherjee, 1988). In the present study, though the decrease recorded in triglycerides was not statistically significant, it may still probably be said that the aqueous fruit extract of *Solanum macrocarpum* lowers hypercholesterolaemia.

Cholesterol is the main lipid found in blood, bile and brain tissues (Sood, 2006). Normal serum cholesterol varies with age, diet and from country to country (Odutola, 1992). Cholesterol is classified as a sterol because it is required for formation of steroids and cellular membranes (Sood, 2006). Increased levels of cholesterol lead to coronary artery disease, hyperproteinaemia, diabetes, cirrhosis, haemolytic jaundice, acute infection, malnutrition and hyperthyroidism (Odutola, 1992; Mukherjee, 1988). Increased levels of oestrogens can also lead to hypercholesterolaemia (Odutola, 1992). In the present study, the decrease in serum cholesterol on extract administration, though not significant, is in agreement with the hypercholesterolemia 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 that recorded with the fruit of *Solanum melongena* L. and *Solanum gilo* Raddi in New Zealand rabbits which were fed 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 hypercholesterolaemic rats. At present though, plants products are more important in the dietary aspect of controlling hyperlipidaemia rather than as drug treatment (Williamson *et al*., 1996). Dietary cholesterol has little influence on serum cholesterol in most people. Nonetheless, high levels of cholesterol in serum may be as a result of high dietary intake known as exogenous hyperlipidaemia (Odutola, 1992).

In the present study, the decrease in LDL-C was also not significant ($p > 0.05$). LDL-C are derived from the metabolism of VLDL-C and they have a very low half life ($t_{½}$), 3 to 4 days (Hardman and Limbird, 2001). The results also buttressed the fact that the aqueous fruit extract of *Solanum macrocarpum* could probably lower the hypercholesterolaemia induced in the rats.

The reduction in the LDL-C, though not significant in the present study, ($p > 0.05$) could be caused by the fact that the aqueous fruit extract of *S. macrocarpum* inhibited the enzyme acyl cholesterol acyltransferase (ACAT, also called acyl-Co A) which is responsible for the conversion of the cholesterol to the cholesteryl ester, at micromolar concentration (Dunn and Le-Blanc, 1994). Thus inhibition of ACAT *in vivo* should reduce serum cholesterol levels. This should provide a possible mechanism by which the fruit of *Solanum macrocarpum* and

other vegetables like *Vernonia amygdalina* Del. lower serum cholesterol levels. However, more work still needs to be done on this plausible mechanism of action. Also, the phytochemistry revealed that the fruit of *S. macrocarpum* contains alkaloids. *Solanum macrocarpum* contains plant steroids known as steroidal alkaloids or azosteroids. Reports have shown the *Solanum* alkaloids to be solanidine and solasidine (Olaniyi *et al*., 1998; http://www.chandel.com/products/ solasodine.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 this plant are cholesterol-lowering agents (Cheeke, 1971), thus, the fruit of *S. macrocarpum* can be used in the treatment of hypercholesterolemia as claimed in traditional medicine.

Conclusion

The present study shows that the aqueous fruit extract of *Solanum macrocarpum* may be used in the dietary aspect of controlling hypercholesterolaemia as claimed in traditional medicine as the fruit contains chemical constituents like saponins and steroidal alkaloids that have lipid-lowering activities.

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