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International Journal of Current Research
Vol. 5, Issue, 12, pp.4249-4255, December, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

ANTI-DIABETIC AND TOXICOLOGICAL STUDIES OF THE ALKALOIDS OF *ACANTHUS MONTANUS* (ACANTHACEAE) LEAF

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ARTICLE INFO

Article History:
Received 14th September, 2013
Received in revised form
26th September, 2013
Accepted 17th October, 2013
Published online 25th December, 2013

Key words:
Acanthus montanus,
Acanthaceae,
Anti-diabetic,
Haematology,
Acute toxicity.

ABSTRACT

This study was conducted to determine the anti-diabetic effect and safety profile of alkaloids of *Acanthus montanus* Leaf (AAML). The anti-diabetic study was carried out using alloxan-induced diabetic rats by daily intraperitoneal administration of AAML at doses of 100, 200 and 400 mg/kg for 4 weeks and 8 weeks. The toxicological assessment of AAML was also done by determining the haematological, biochemical and urinary parameters in blood, serum, and urine samples respectively at the end of the tested periods. In the acute toxicity test, mice were administered intraperitoneally with AAML up to 5000 mg/kg. Animals were then observed for behavioural changes; signs of toxicity, and mortality within 24 h. Surviving mice were monitored for 7 days for signs of delayed toxicity. Result of the anti-diabetic study shows significant ($P < 0.05$) dose-dependent reduction (42.68 %) in blood sugar levels of the hyperglycemic rats when compared with glibenclamide (standard drug). In the acute toxicity test, AAML was practically non-toxic showing no mortality and visible signs of delayed toxicity. The AAML at 4 and 8 weeks elicited significant ($P < 0.05$) increases in haematological parameters (Hb, PCV, RBC, WBC and differential count), reductions in biochemical parameters (levels of liver enzymes - AST, ALT, and ALP), total cholesterol, triglycerides, urea and creatinine and urinary parameter (pH). Results obtained in this study suggest that the alkaloids of leaf *Acanthus montanus* leaf is safe when administered intraperitoneally with potential beneficial effects as immunostimulant, hepatoprotective, and hypocholestrolemic agent, when administered over a long period of time.

INTRODUCTION

The use of herbal remedies by all stratums of people is on the increase worldwide. Based on long history of use in traditional medicine, herbal remedies are generally regarded as safe. They are often promoted to the public as being "natural" and completely "safe" alternatives to conventional medicines (Adewunmi and Ojewole, 2004). However, Fennel *et al.* (2004) reported that many African plants used medicinally and widely assumed to be safe are potentially toxic due to the presence of toxic and potentially lethal constituents. Many plants have been screened for anti-diabetic effect (Odoh *et al*., 2003; Farombi, 2003; Bnouham *et al*., 2006; Atangwho *et al*., 2009). 6So much has been done in screening medicinal plants for efficacy based on traditional claims while less emphasis is placed on the issue of safety, as reports of efficacy far outnumber those of toxicity, probably as a result of the greater demands for resources and time such exercise warrant. According to Ibarrola *et al.* (2000) and Mushtaq *et al.* (2003) pharmacological and toxicological evaluations of medicinal plants are essential for drug development. *Acanthus montanus* (Nees) T. Anders. (Family: Acanthaceae) is a small shrub with sparse branches and soft stems, widespread in Africa, the Balkans, Romania, Greece and the Eastern Mediterranean (Burkill, 1985).

The leaf extract of *Acanthus montanus* has shown great promise as a phytotherapeutic agent based on results of previous pharmacological investigations. Previous biological investigations have shown that; the leaves of *A. montanus* display spasmolytic, analgesic, anti-inflammatory and antipyretic activities (Odoh *et al.*, 2010; Okoli *et al.*, 2008; Adeyemi *et al.*, 2004; Asongalem *et al.*, 2004; Adeyemi *et al.*, 1999). In Cameroon the plant is used traditionally to treat various ailments namely; cough, carious teeth, pharyngitis, dysmenorrhoea, gastritis, false labour, epilepsy, and intestinal helminthiasis, in other regions of Africa it is used to alleviate uretheral discharge, chest pain, emesis, constipation, rheumatic pains, diabetes and syphilis (Burkill, 1985; Adjanohoun *et al.*, 1996). The aim of this study is to evaluate the anti-diabetic and toxicological profile of the alkaloid fraction of *Acanthus montanus* leaf in rats, thus helping in establishing its safety profile.

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MATERIALS AND METHODS

Plant material

The fresh plant was collected from Nsukka town, Enugu State Nigeria in May, 2010. The plant was identified andauthenticated by Mr. A. Ozioko of the International Centre for Ethnomedicine and Drug Development (Inter CEDD), Nsukka, Enugu State Nigeria and voucher specimen (UNN/PCOG/010/409) deposited in the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka. The leaves were shade-dried for 4 days and milled to coarse powder with an electric blender and sieved through 40 mesh.

Animals

Albino mice and rats of both sexes (averagely weighing 20 g and 200 g respectively) used in this study were obtained from the Department of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. The animals were maintained under standard environmental conditions (23 – 25 ^{0}C, 12 h/12 h light/dark cycle) and had free access to standard rodent pellet diet. For a week following their arrival, the animals were allowed free access to the standard rat chow diet and tap water *ad libitum* they were acclimating to the environment. Rats were also monitored daily and cages cleaned thrice weekly. At the start of the experiment animals were randomly distributed so that body weights, initial triglycerides (TG), total cholesterol (TC) and other parameters in all the experimental groups were similar (Odoh and Osadebe, 2010)

Extraction

Alkaloids were extracted according to the method of Harborne (Harborne, 2005). A 500 g of powdered materials were extracted using 2.5 litre of 10 % acetic acid in ethanol. The filtrate was then concentrated on a water bath to 1/4th of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle. Collected precipitates were washed with dilute ammonium hydroxide and then filtered. The residue was dried, weighed and used for the experiment.

Acute toxicity test

The acute toxicity and lethality (LD_{50}) of AAML in mice (n = 13) was estimated using the method described by Lorke (1983). The study was carried out in two stages. In stage one, mice (n = 3) received intraperitoneally 10, 100, or 1000 mg/kg of AAML (suspended in 20 % Tween 80) and were observed for 24 h for number of deaths. At the end of 24 h, no death was recorded in treated mice. Consequently, a fresh batch of mice (n = 1) received 600, 1600, 2900 and 5000 mg/kg of AAML in the second stage of the test and were observed for 24 h for deaths. No Death was recorded at the end of the second stage and surviving animals were observed for a further 7 days for any signs of delayed toxicity. The control group received 20 % Tween 80 intraperitoneally.

Experimental Design

The test samples were suspended in 20 % v/v Tween 80. Glibenclamide (2.5 mg/kg) was used as reference control during the study. All the test samples were administered intraperitoneally. Rats were considered diabetic when the blood glucose level was raised beyond 200 mg/dl.

In normoglycemic rats

The animals were fasted for 18 h, but were allowed free access to water before and throughout the duration of experiment. At the end of the fasting period, taken as zero time (0 h), the basal fasting blood glucose (FBG) levels of the rats were determined using One touch® glucometer kit (Lifescan, Johnson and Johnson Company, Milipitas, CA). Animals were randomly divided into five groups (n = 5). Group I received 20 % v/v Tween 80 (5 ml/kg) as normal control, Group II received glibenclamide (2.5 mg/kg) as positive control and Groups III, IV and V received AAML at doses of 100, 200 and 400 mg/kg respectively. The blood glucose level of each animal was measured prior to (pre-treatment) and at 1, 2, 4 and 6 h after AAML administration.

In Hypoglycemic rats

The hypoglycaemic effect AAML was studied in alloxan-induced diabetic rats. The animals were fasted for 24 h but allowed free access to water. At the end of the fasting period, the basal fasting blood glucose (FBG) levels of the rats were determined. Subsequently, diabetes was induced by single intraperitoneal injection of alloxan mono-hydrate (120 mg/kg) and normal feeding maintained thereafter. Five days later, blood was drawn from each rat by tail snipping and the blood glucose level measured to establish diabetes. Animals with blood glucose level 200 mg/dl were considered diabetic and used for the study. The diabetic animals were randomly divided into five groups (n = 5). Group I received 20 % v/v Tween 80 (5 ml/kg) as normal control, Group II received glibenclamide (2.5 mg/kg) as positive control, Groups III, IV and V received AAML at doses of 100, 200 and 400 mg/kg respectively. The blood glucose level of each animal was measured prior to (pre-treatment) and at 1, 2, 4 and 6 h after AAML administration.

The percentage reductions were calculated as follows:

$$\text{Percentage reduction} = \frac{\text{FBG} - \text{Blood glucose value}}{\text{FBG}} \times \frac{100}{1}$$

Subchronic toxicity test

The anti-diabetic effect of AAML was studied by evaluating the effect of its chronic administration on the blood glucose level of alloxan-induced diabetic rats. The basal fasting blood glucose (FBG) of rats was determined and diabetes induced as described above. Thirty diabetic rats randomly divided into six groups (n=5) were used for the study. Rats were fasted for 8 h but allowed free access to water. A mid-term assessment was carried out in a set of animals from each group at 28 days of administration. Rats in different groups were weighed and FGB levels taking. Group I and II were diabetic controls and received 20 % v/v Tween 80 (5 ml/kg) or glibenclamide (2.5 mg/kg) respectively, while Groups III, IV and V received 100, 200 and 400 mg/kg of AAML respectively. Blood glucose level was measured as earlier described before (pretreatment) and on days 28 (4 weeks) and 56 (8 weeks) after commencement of treatment. The treatments were

administered intraperitoneally to the animals once daily for 8 weeks. They were closely observed for behavioural and general morphological changes. The body weight of each animal was also measured on these days. Urine samples were collected from individual rats for 12 h using metabolic cages on the 27th/28th and 55th/56th days. Animals were anaesthetized by administration of 1 % chloralose in 25 % urethane (w/v) (5 ml/kg, i.p.) to facilitate collection of blood samples through cardiac puncture for haematological and biochemical analysis.

Effect on vital organs

At the termination of treatment, 56th day (8 weeks) vital organs (heart, lungs, liver, kidneys and spleen) were harvested from sacrificed rats. These were carefully examined for gross lesions and weighed [sing Mettler-Toledo GmbH digital weighing balance (Type BD202, SNR 06653)] before preservation in 10 % formo-saline. The weight of each organ was standardized to 100 g body weight of each animal.

Haematological assessment

Blood samples collected from rats into EDTA (ethylenediamine-tetraacetate) bottles were analyzed for the determination of Packed Cell Volume (PCV), Red Blood Cell (RBC) count, haemoglobin, platelet count, total and differential White Blood Cell (WBC) count using standard methods (Ghai, 1995).

Biochemical assessment

Serum separated from the blood sample of each rat collected into plain bottles was assayed for Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Alkaline Phosphatase (ALP) activities, using a Jenway 6100 spectrophotometer (Jenway Ltd., Felsted, Essex, UK) set at 37 ^{o}C. Values were read at 546 nm (AST and ALT) and 405 nm (ALP). The assay was carried out using standard methods (Bessey *et al*., 1946; Reitman and Frankel, 1957). All other parameters were determined using commercial test kits: urea (Urease- Berthelot colorimetric method), total cholesterol (Enzymatic endpoint method, RANDOX LABORATORIES, CRUMLIN, UK) and triglycerides (BIOLABO SA, FISMES, FRANCE).

Urine analysis

Fresh urine samples collected from individual rats for 12 h (8:00 pm – 8:00 am) using metabolic cages on the 27th/28th and 55th/56th days were analyzed for specific gravity, pH and protein using commercial urine analysis strips (URICHECK, REF. U003B, VEDA LAB., ALENCON - FRANCE).

Statistical Analysis

The data were expressed as mean ± standard error mean (SEM). The Significance of differences among the group was assessed using one way and multiple way analyses of variance (ANOVA). The test followed by Dunnet's test p values less than 0.05 were considered as significance.

RESULTS

Acute toxicity test

The AAML did not produce any mortality administered intraperitoneally up to 5 g/kg. Monitored for a further 7 days, no visible signs of delayed toxicity and mortality were observed.

Effect of AAML on blood glucose level of normoglycaemic rats

The AAML caused a significant (P <0.05) dose-related reduction in the FBG of normoglycaemic rats (Fig. 1).

Effect of AAML on blood glucose level of hyperglycaemic rats

The AAML significantly (P <0.05) reduced blood glucose level to varying extents in diabetic rats. The hypoglycaemic effect was dose-related (Fig. 2). The effect on blood sugar level on prolonged treatment was shown in Fig. 3.

Body weight

There were significant differences in the weights of rats treated with AAML (100, 200, and 400 mg/kg) compared to the control (Table 1).

Effect on vital organ

AAML after daily administration for 8 weeks, there was a significant ($P < 0.05$) reduction in the weight of the heart, kidney, spleen and liver at all doses investigated (Table 2). However, all organs were macroscopically comparable to the control.

Haematological parameters

AAML at doses of 200 and 400 mg/kg produced significant effect on haematological parameters when administered daily for 4 weeks. There was significant ($P < 0.05$) increase in the creatinine level only at the dose of 400mg/kg. However, in respect of 8 weeks daily administration, AAML produced significant ($P < 0.05$) increases in Hb, PCV, RBC and WBC counts all doses studied. Considering WBC differential, AAML at all doses caused significant ($P < 0.05$) increase in the proportion of lymphocytes and neutrophils. A significant increase in the proportion of eosinophils was observed only at the dose of 400 mg/kg (Tables 3 and 4).

Biochemical parameters

AAML in the mid-term study produced significant effect in ALT, triglyceride and urea at the doses studied. Significant effect was observed for AST, ALP and total cholesterol at the dose of 200 and 400 mg/kg. There was significant reduction in creatinine levels only at the dose of 400 mg/kg. In respect of the end-term study, the AAML at all doses investigated elicited significant reductions in AST, ALT, ALP, urea and total cholesterol levels except for creatinine that significant reductions was observed at the dose of 400 mg/kg (Table 5).

Urine

AAML did not produce any significant effect on the levels of specific gravity, protein and pH assayed in urine of rats in the mid-term and end-term studies, except in respect of pH which was significantly ($P < 0.05$) increased in the end-term study at 400 mg/kg (Table 6).

Table 1. Effect of AAML on body weight of diabetic rats during prolonged treatment

Treatment	Dose (mg/kg)	Blood glucose concentration (mg/dl)			
		Pre-Diabetic	Diabetic Pre-treatment	Diabetic Post-treatment	
				Mid-term study (4 weeks administration)	End-term study (8 weeks administration)
DCNT	-	221.0 ± 5.57	221.0 ± 5.57	213.0 ± 1.69	202.6 ± 0 50
DCT	2.5	274.8 ± 6.97	273.0 ± 7.40	271.0 ± 0.84	268.0 ± 0.38
AAML	100	224.0 ± 8.28	223.6 ± 8.57	228.6 ± 1.51*	233.0 ± 0.34*
	200	226.0 ± 1.87	220.6 ± 5.42	227.7 ± 1.11 *	236.0 ± 0.02*
	400	220.0 ± 5.24	220.0 ± 5.24	235.0 ± 0.50*	251.6. ± 0.27*

Values are mean ± S.E.M, n = 5; *P<0.05 compared to Diabetic control not treated values (significant, Student's *t*-test);
AAML - Alkaloid fraction of *Acanthus montanus* Leaf, DCNT – Diabetic control non-treated was a diabetic control and received the vehicle.
DCT - Diabetic control treatedand received glibenclamide.

Table 2. Effect of AAML on rat organ weights of diabetic rats (per 100 g body weight) during prolonged treatment

Treatment	Dose (mg/kg)	End-term study (8 weeks administration)			
		Liver	Heart	Kidney	Spleen
DCNT	-	3.01 ± 0.34	0.30 ± 0.13	0.61 ± 0.04	0.36 ± 0.35
DCT	2.5	2.84 ± 0.50	0.29 ± 0.05	0.69 ± 0.09	0.41 ± 0.21
AAML	100	3.15 ± 0.15	0.24 ± 0.12*	0.62 ± 0.13	0.37 ± 0.15
	200	3.45 ± 0.25	0.26 ± 0.33*	0.60 ± 0.11	0.36 ± 0.06
	400	3.19 ± 0.05	0.25 ± 0.20*	0.59 ± 0.08	0.38 ± 0.17

Values are mean ± S.E.M, n = 5; *P<0.05 compared to Diabetic Pre-treatment values (significant, Student's *t*-test);
AAML - Alkaloid fraction of *Acanthus montanus* Leaf, DCNT – Diabetic control non-treated was a diabetic control and received the vehicle. DCT - Diabetic control treatedand received glibenclamide.

Table 3. Effect of AAML on haematological parameters in alloxan-induced diabetic rats during prolonged treatment

Parameter	Treatment	Dose (mg/kg)	Concentration			
			Pre-Diabetic	Diabetic Pre-treatment	Diabetic Post-treatment	
					Mid-term study (4 weeks administration)	End-term study (8 weeks administration)
Hb	DCNT	-	16.13 ± 0.05	15.96 ± 0.03	14.80 ± 0.30	14.00 ± 0.23
	DCT	2.5	15.08 ± 0.08	14.58 ± 0.07	13.82 ± 0.15	13.66 ± 0.07
	AAML	100	14.78 ± 0.12	13.06 ± 0.03	13.82 ± 0.14*	14.70 ± 0.13*
		200	16.68 ± 0.06	15.06 ± 0.25	15.55 ± 1.06*	16.07 ± 1.02*
		400	16.20 ± 0.16	14.98 ± 0.19	15.60 ± 0.08*	16.14 ± 0.05*
PCV (%)	DCNT	-	48.32 ± 0.84	42.07 ± 0.34	43.40 ± 0.80	43.02 ± 0.10
	DCT	2.5	51..45 ± 0.14	37.54 ± 0.25	40.60 ± 1.68	46.60 ± 2.13
	AAML	100	49.20 ± 0.32	44.15 ±0.58	44.92 ± 0.28	47.10 ± 0.17*
		200	50.56± 0.54	36.64 ± 0.19	40.48 ± 0.13*	46.02 ± 0.13*
		400	52.80 ± 0.75	38.02± 1.34	42.52 ± 0.49*	49.98 ± 0.49*
RBC (x 10^6) (mm^3)	DCNT		6.84 ± 0.17	6.26 ± 0.31	5.31 ± 0.71	5.08 ± 0.18
	DCT		5.37 ± 0.05	4.66± 0.08	4.59 ± 0.19	4.51 ± 0.06
	AAML	100	6.15± 0.12	5.20 ± 0.60	5.45 ± 1.41	6.02 ± 0.25*
		200	6.39 ± 0.09	4.92 ± 0.18	5.67 ± 0.36*	6.11 ± 0.08*
		400	6.98 ± 0.10	4.37 ± 0.09	5.38 ± 0.05*	6.75 ± 0.08*
WBC (x 10^6) (mm^3)	DCNT	-	7.90 ± 0.20	6.42 ± 0.22	6.39 ± 0.12	6.10 ± 2.30.
	DCT	2.5	8.71 ± 0.28	7.73 ± 0.08	6.30 ± 0.07	8.46 ± 0.06
	AAML	100	8.16 ± 0.16	7.04 ± 1.24	7.80 ± 3.08	8.06 ± 1.15*
		200	7.66 ± 1.09	6.10 ± 0.04	6.53 ± 0.12	7.54 ± 0.07*
		400	9.80 ± 2.16	7.18 ± 0.14	8.95 ± 0.11*	9.62 ± 0.10*

Values are mean ± S.E.M, n = 5; *P<0.05 compared to Diabetic Pre-treatment values (significant, Student's *t*-test); AAML - Alkaloid fraction of *Acanthus montanus* Leaf, DCNT – Diabetic control non-treated was a diabetic control and received the vehicle. DCT - Diabetic control treated and received glibenclamide

Table 4. Effect of AAML on Differential leucocyte count in alloxan-induced diabetic rats during prolonged treatment

Treatment	Dose (mg/kg)	Pre-Diabetic					Diabetic Pre-treatment					Diabetic Post-treatment									
												Mid-term study (4 weeks administration)					End-term study (8 weeks administration)				
		L	N	M	E	O	L	N	M	E	O	L	N	M	E	O	L	N	M	E	O
DCNT	-	85	15	8	0	0	66	13	7	0	0	65	13	7	0	0	63	12	6	0	0
DCT	2.5	80	11	7	1	0	76	9	7	1	0	77	8	8	1	0	79	10	10	2	0
AAML	100	79	9	12	1	0	63	8	10	0	0	68	8	10	0	0	74	9	11	0	0
	200	78	15	14	0	0	67	12	11	0	0	71	12	11	O	0	77	14	12	0	0
	400	80	21	13	2	0	63	17	11	1	0	69	18	12	I	0	76	20	13	2	0

Values are mean ± S.E.M, n = 5; *P<0.05 compared to Diabetic Pre-treatment values (significant, Student's *t*-test); AAML - Alkaloid fraction of *Acanthus montanus* Leaf, DCNT – Diabetic control non-treated was a diabetic control and received the vehicle. DCT - Diabetic control treated and received glibenclamide.

Table 5. Effect of AAML on biochemical parameters in alloxan induced diabetic rats during prolonged treatment

Parameter	Treatment	Dose (mg/kg)	Concentration			
			Pre-Diabetic	Diabetic Pre-treatment	Diabetic Post-treatment	
					Mid-term study (4 weeks administration)	End-term study (8 weeks administration)
AST (iu/l)	DCNT	-	36,08 ± 0.10	47.30± 3.6	48.01± 1.75	51.16 ± 0.61
	DCT	2.5	32.07 ± 0.04	43.50 ± 3.2	42.50 ± 0.38	40.02 ± 0.19
	AAML	100	28. 09 ± 1.05	39.15± 1.01	38.23± 0.50	33.02 ± 0.52*
		200	39.00 ± 0.30	51.02 ± 0.09	49.07± 0.79*	42.60± 1.15*
		400	20.44 ± 0.02	34.08 ± 0.04	32.16 ± 1.04*	21.60± 2.16*
ALT (iu/l)	DCNT	-	19.00 ± 2.18	35.70 ± 1.09	39.01 ± 1.75	41.40 ± 0.34
	DCT	2.5	16.02 ± 0.13	31.20 ± 0.52	29.72 ± 0.22	28.02 ± 0.19
	AAML	100	18. 25 ± 1.20	33..06 ± 1.12	31.00 ± 0.60*	29.36 ± 0.52*
		200	22.00 ± 0.08	38.41 ± 0.36	37.07 ± 0.45*	28. 18 ± 1.33*
		400	13.60 ± 0.13	27.50 ± 0.70	25.86 ± 1.04*	15.20 ± 0.01*
ALP (iu/l)	DCNT	-	78.20 ±1.12	115. 20 ± 0.32	116.50 ± 0.50	116.94 ± 0.61
	DCT	2.5	69.50 ± 0.40	101.00 ± 0.52	101.65 ± 0.72	100.08 ± 0.19
	AAML	100	80. 40 ± 0.60	119.05 ± 1.42	118.94 ± 1.32	116.45 ± 0.03*
		200	72.20 ± 0.38	99.50 ± 0.08	96.84 ± 0.36*	85. 20 ± 1.08*
		400	85.10 ± 0.12	121.00 ± 0.05	118.58 ± 0.13*	98.32 ± 1.04*
Triglyceride (mg/dl)	DCNT	-	102.30 ± 2.10	130.30 ± 3.62	131.00± 2.70	132.82 ± 2.63
	DCT	2.5	113.47 ± 0.94	131.50 ± 3.20	128.47± 2.31	124.60 ± 3.90
	AAML	100	107. 35 ± 4.2	132.15 ± 2.10	129.65± 0.79*	123.60 ± 2.31*
		200	95.50 ± 0.34	119.00 ±1.91	117.10±0.05*	102.61 ±0.53*
		400	114.60 ± 2.50	125.35 ± 1.01	121.47± 2.21*	116.60 ± 3.90*
Urea (mg/dl)	DCNT	-	34.08 ± 0.04	47.00± 2.04	49.30 ± 2.71	52.00 ± 1.12
	DCT	2.5	38.20 ± 0.30	59.56± 3.20	55.64± 0.56*	49.10 ± 2.04*
	AAML	100	40.05± 1.10	61.38 ± 2.10	57.31± 1.02*	52.00 ± 0.20*
		200	37.45 ± 1.08	51.30 ± 0.60.	43.24.± 1.50*	39.20 ± 3.90*
		400	36.02 ± 0.15	53.42 ± 1.91	48.00±0.02*	37.11 ±0.53*
Total cholesterol (mg/dl)	DCNT	-	137.41 ± 2.01	187.2± 1.31	188.05±1.49	189.40±2.06
	DCT	2.5	110.07± 4.52	162.15± 1.61	163.5± 0.41	160.50± 4.32
	AAML	100	131.95± 2.01	170.2± 1.42	168.87± 0.97	159.80±0.90*
		200	121.50 ± 1.30	180.7 ± 3.10	175.4 ± 35.10*	154.90 ± 1.90*
		400	120.00±0 1.40	168.7 ± 4.80	159.0 ± 29.52*	131.60 ± 0.50*
Creatinine (mg/dl)	DCNT	-	1.68 ± 0.52	1.80 ± 0.15	1.88 ± 0.60	1.92 ± 0.40
	DCT	2.5	1.02 ± 0.25	1.32 ±1.04	1.22 ± 0.17	1.10 ± 0.12
	AAML	100	2.02 ± 0.17	2.37 ± 0.17	2.25 ± 0.16	2.10 ± 0.15
		200	1.40 ± 0.93	1.71 ± 0.02	1.60 ± 0.13	1.50 ± 0.3s
		400	2.30 ± 0.21	3.20 ± 0.16	2.98± 0.26*	2.48 ± 0.10*

Values are mean ± S.E.M, n = 5; *P<0.05 compared to Diabetic Pre-treatment values (significant, Student's *t*-test); AAML - Alkaloid fraction of *Acanthus montanus* Leaf, DCNT – Diabetic control non-treated was a diabetic control and received the vehicle. DCT - Diabetic control treated and received glibenclamide.

Table 6. Effect of AAML on urine parameters in alloxan induced diabetic rats during prolonged treatment

Treatment	Dose (mg/kg)	Diabetic Post-treatment					
		Mid-term study (4 weeks administration)			End-term study (8 weeks administration)		
		Specific gravity	pH	Protein (g/L)	Specific gravity	pH	Protein (g/L)
DCNT	-	0.97 ± 0.03	7.45 ± 0.38	1.63 ± 0.82	1.21 ± 0.10	6.90 ±0.44	1.98 ± 0.40
DCT	2.5	1.00 ± 0.37	7.60 ± 0.22	1.95 ± 0.06	1.01 ± 0.41	7.40 ± 0.53	1.98 ± 0.11
AAML	100	0.99 ± 0.12	7.50 ± 0.72	2.00 ± 0.02	1.00 ± 0.01	7.75 ± 1.05	2.10 ± 0.31
	200	1.01 ± 0.56	7.00 ± 0.12	2.40 ± 0.34	1.02 ± 0.30	7.50 ± 0.60	2.47 ± 0.56
	400	1.02 ± 0.21	7.80 ± 1.06	1.85 ± 0.12	1.02 ± 0.05	8.30±0.42*	1.86 ± 0.45

Values are mean ± S.E.M, n = 5; *P<0.05 compared to Diabetic Pre-treatment values (significant, Student's *t*-test); AAML - Alkaloid fraction of *Acanthus montanus* Leaf, DCNT – Diabetic control non-treated was a diabetic control and received the vehicle. DCT - Diabetic control treated and received glibenclamide.

DISCUSSION

From results obtained in the acute toxicity test, AAML can be said to be safe when administered intraperitoneally. Administered up to 5000 mg/kg, the extract did not produce any mortality and visible signs of delayed toxicity. It can therefore be classified as relatively non-toxic. AAML at all doses investigated did not produce significant changes in body weight over the 8 weeks period of treatment in the subchronic toxicity test. In respect of vital organs, there were significant reductions in the weight of the heart (100, 200, 400 mg/kg) in the end-term study (8 weeks). However, no significant changes in weight were observed in respect of the kidneys and spleen. Generally, reductions in body weight gain and internal organ weights are simple and sensitive indices of toxicity after exposure to toxic substances (Raza *et al.*, 2002; Teo *et al.*, 2002, Tan *et al.*, 2008). In this study, there were no reductions in body weight gain and gross examination of the organs observed did not show detectable abnormalities. In the case of the liver, there was no elevation in levels of associated enzymes to suggest toxicity. The effect on body and organ weights is thus a pointer to the safety of prolonged intrapritoneally administration of AAML. AAML did significantly affect all the haematological and biochemical parameters at the dose of 200 and 400 mg/kg but no effect was observed for the urinary parameters in the 4 week study when compared to control. However, in the 8 week study, all the haematological and biochemical parameters were significantly ($p < 0.05$) affected at all doses studied. There was no significant effect on the urinary component except for the pH

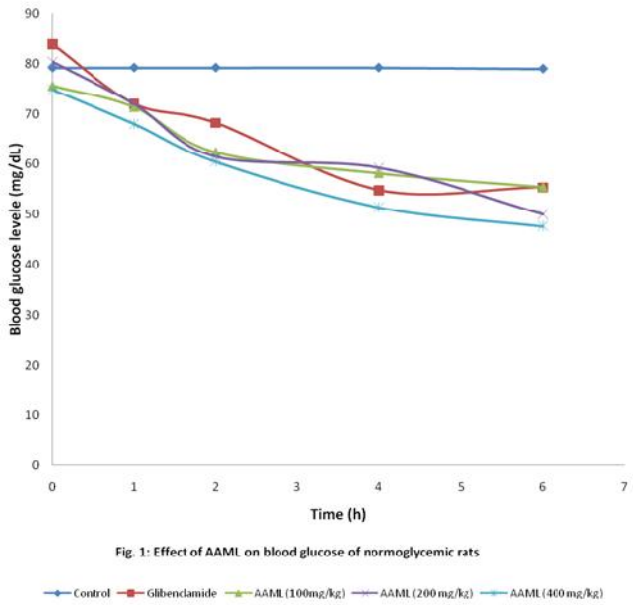

Fig. 1: Effect of AAML on blood glucose of normoglycemic rats

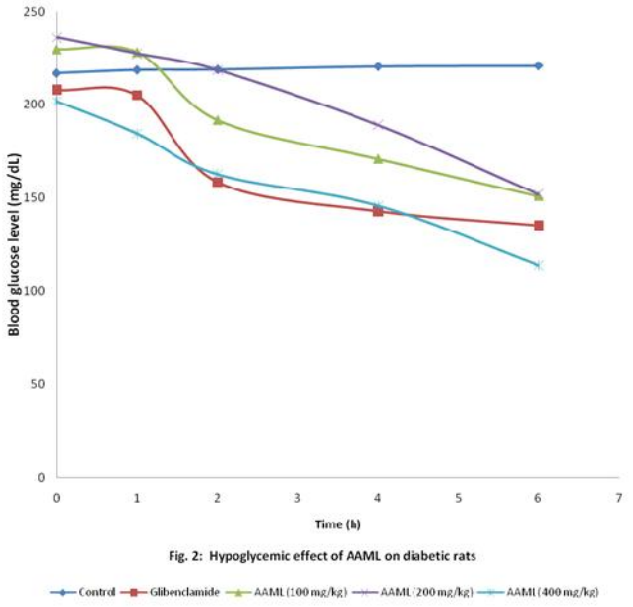

Fig. 2: Hypoglycemic effect of AAML on diabetic rats

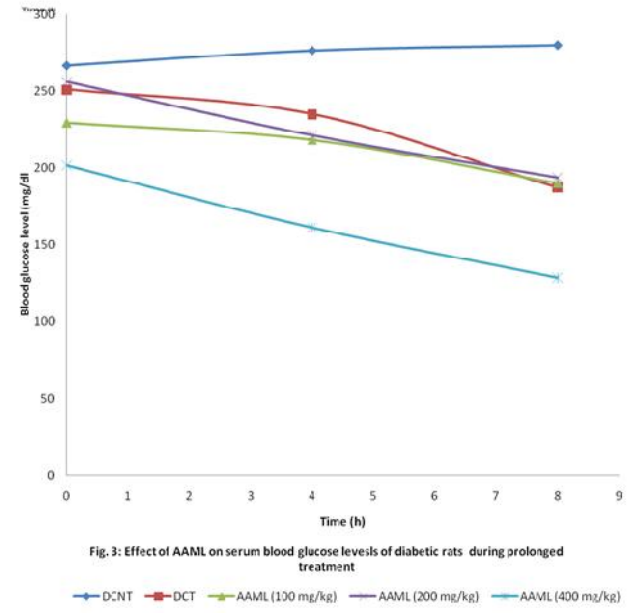

Fig. 3: Effect of AAML on serum blood glucose levels of diabetic rats during prolonged treatment

which was significant at the dose of 400 mg/kg. However, all animals in this group appeared normal and healthy and no mortality was recorded all through the duration of the experiment. The proportion of neutrophils was increased with an accompanying increase in the proportion of lymphocytes. Neutrophils are the first defense responders to microbial infections (bacterial or fungal) and are associated with other inflammatory processes. Lymphocytes on the other hand generate antibodies that bind to pathogens to enable their destruction and are more involved in defense against intracellular bacteria, virus infected cells and tumour cells. The AAML can be said to enhance immediate response to microbial attack and inflammatory processes. Increases in the levels of these enzymes above normal are reliable indices of liver toxicity (Odoh, *et al*., 2008; Hayes, 1989) or altered integrity of cellular membrane and cell death or lysis (Olagunju *et al.*, 2000). AST is not a highly specific indicator for liver injury because it is found in other tissues like the heart, muscles, kidney, brain, and RBC, unlike ALT which is fairly specific being found largely in the liver.

The reductions in the levels of AST and triglycerides therefore suggest that the AAML is not toxic to the liver or damaging to the integrity of the bile duct, but possibly suggesting the greatest potential for hepatoprotection and protection against atherosclerosis and its accompanying risk of cardiovascular diseases. The lowering of the AST level may also suggest a non-toxic but protective effect on the other organs in which the enzyme is well expressed. The significant reduction of serum total cholesterol level is suggestive of a potential hypocholesterolaemic effect which helps in reducing the predisposition to atherosclerosis and cardiovascular diseases like myocardial infarction, stroke, and peripheral vascular disease. Creatinine, urea, and uric acid determinations are critical as markers of kidney function (Newman and Price, 1999). The AAML did not affect serum urea level which if elevated may suggest azotemia as a consequence of renal impairment. This condition usually leads to hyperkalaemia, hypocalcaemia, and anaemia among other conditions.

Conclusion

It can be concluded from results obtained in this study that the AAML is relatively safe when administered intraperitoneally. Prolonged administration revealed effects which suggest that it may possess immunostimulant, hepatoprotective, and hypocholesterolemic properties.

Acknowledgement

The authors are grateful to Mr Austin Ngene of Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Nigeria, Nsukka for the heamatological, biochemical and urinary analysis conducted.

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