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Effects of *Vernonia amygdalina* on Biochemical and Hematological Parameters in Diabetic Rats

[1]P.A. Akah, [1]J.A Alemji, [2]O.A Salawu, [1]T.C. Okoye and [3]N.V. Offiah
[1]Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, Nigeria
[2]Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria
[3]School of Veterinary Medicine, Faculty of Medical Sciences, The University of West Indies, St. Augustines, Trinidad.

Abstract: Subacute toxicity studies were carried out on the fraction of the methanol leaf extract of *Vernonia amygdalina* in diabetic rats. Nine fractions (F_1-F_9) were obtained from the MEOH leaf extract of *V. amygdalina* and screened for antihyperglycemic activity in alloxan-induced diabetic rats. The most potent hypoglycemic fraction (F_6) was administered daily to normal and diabetic rats for 28 days at doses of 80, 160 and 320 mg/kg. Blood glucose level, body weight, food and liquid intake, and some biochemical and hematological indices were monitored over the treatment period. At these doses, fraction F_6 significantly ($p<0.05$) increased food intake and body weight and decreased water intake in the diabetic rats. The fraction significantly ($p<0.01$) decreased the levels of triglycerides, low density lipoprotein cholesterol, very low density lipoprotein cholesterol, and increased high density lipoprotein cholesterol level in the diabetic rats. Treatment with fraction F_6 (160 and 320 mg/kg) significantly ($p<0.05$) increased the lymphocyte count and electrolytes levels but lowered the levels of urea and creatinine. The elevated levels of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in the diabetic rats were significantly ($p<0.01$) lowered. The histopathological studies revealed no significant abnormalities in the vital organs in both F_6 and chlorpropamide treated rats. These results suggest that fraction F_6 of *V. amygdalina* is a potent hypoglycemic and hypolipidemic agent which is safe and capable of normalizing biochemical and hematological abnormalities associated with the pathophysiology of diabetes mellitus.

Keywords: *Vernonia amygdalina*, hypoglycemic, hypolipidemic, hepatic enzymes, hematological indices, rats

INTRODUCTION

Diabetes mellitus (DM) is a common disorder associated with increased morbidity and mortality and can be defined as a group of metabolic diseases characterized by chronic hyperglycemia due to defective insulin secretion, insulin action, or both, resulting in impaired carbohydrate, lipid, and protein metabolism (Lebovitz, 1994; Andreoli *et al*., 1990). Pharmacological treatment of DM is based on oral hypoglycemic agents and insulin which have so many side effects (Andreoli *et al*., 1990). In diabetes, the causes and sites of intervention in biochemical process are diverse (Larner, 1985) and high serum total triglyceride level, high level of transaminase, creatinine kinase and urea have been implicated (Anaja, 1985; Leningher, 1998). Differences in the lipid profile of diabetic and non-diabetic individuals are now apparent (Garg and Grundy, 1990, Siegel *et al*., 1996) and lipid abnormalities are common in patients with diabetes mellitus. Dyslipidaemia has been identified as one of the major risk factors for macrovascular complications in diabetes mellitus (ADACS, 1993). The evaluation of medicinal plants used traditionally in treating diabetes is of growing interest (Holman and Turner, 1991; Williams and Pickup, 1991; Kameswara Rao *et al.,* 1997). The World Health Organization also recommended and encouraged this practice especially in countries where access to conventional treatment of diabetes is inadequate (WHO, 1980). The WHO has however emphasized the fact that safety should be the over-riding criteria in the selection of herbal medicine for use in healthcare. The use of *V. amygdalina* in the treatment of DM is very common and some reports led credence to this (Akah and Okafor, 1992; Akah *et al*., 2002, 2004; Gyang *et al*., 2004).

V. amygdalina, Del (Compositae) commonly called "bitter leaf" is a medium sized shrub with petiolate green leaf of about 6 mm diameter and elliptic shape. The leaves have found relevance in traditional folk medicine as anthelmintics, antimalarial, antimicrobial anticancer and as a laxative herb. This study was designed to evaluate the effects of *V. amygdalina* on biochemical and haematological indices in diabetic rats.

MATERIALS AND METHODS

Plant materials: Fresh leaves of *V. amygdalina* were collected in Suleja Local Government Area of Niger State, Nigeria and authenticated by Ibrahim Muazzam of

Corresponding Author: P.A. Akah, Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, Nigeria

the Medicinal Plant Research and Traditional Medicine Dept., National Institute of Pharmaceutical Research and Development (NIPRD), Abuja. A voucher specimen (No. NIPRD/H/15487) was deposited at NIPRD Herbarium.

Animals: Adult Wistar albino rats of both sexes (120-350 g) obtained from the Animal House of the University of Jos and Animal Facility Centre of NIPRD were used. They were fed with standard rat feed, with water *ad libitum*, but starved for 12 hr prior to commencement of experiment. All animal experiments were conducted in compliance with NIH guidelines for Care and Use of Laboratory Animals (Pub. No. 85-23, Revised 1985). This study was conducted partly at the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria and partly at the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka (UNN), Nigeria.

Preparation of fraction F_6: The leaves of *V. amygdalina* were washed, air-dried and crushed into coarse powder using mortar and pestle. About 175 g of powder was loaded into a thimble and continuously extracted with 95% methanol in a soxhlet extractor for 24 h. The solvent was distilled off in the rotary evaporator to obtain a solid residue of 24 g (13.33% w/w). The methanol extract was fractionated by chromatographic methods using hexane/ethylacetate and ethylacetate/methanol (Bobbit, 1994; Touchstone, 1992) to obtain fractions F_1-F_9. Preliminary studies indicated F_6 as the most potent antidiabetic fraction.

Photochemical screening: Standard protocols (Odebiyi and Sofowora, 1978; Trease and Evans, 1989) were used in detecting the presence of different phytochemical constituents in fraction F_6.

Induction of diabetes: Diabetes was induced in rats by a single intravenous injection of freshly prepared solution of alloxan-monohydrate (70 mg/kg) (Pari and Mahesulari, 1999). The rats' fasting blood glucose (FBG) levels were estimated at 0, 2, 4, 6 and 8 h post treatment using One Touch® glucometer (Lifescan, Johnson & Johnson, California). Eight days later rats with blood glucose concentration above 190 mg/dL were considered diabetic and used for the study.

Biochemical and hematological studies: Diabetic rats were divided randomly into 5 groups of 7 rats per group. Group 1 served as the diabetic control (untreated). Groups 2-4 were given fraction F_6 orally at 80, 160 and 320 mg/kg respectively while group 5 received chlorpropamide (250 mg/kg; p.o.). Group 6 served as the normal control. The treatments were given once daily for 28 days. Rats were monitored weekly for body weight and daily for food and water intake. On day 29 the rats were sacrificed and blood was collected by cardiac puncture into EDTA bottles and estimated for haematological parameters viz, packed cell volume (PCV), haemoglobin (Hb), white blood cell counts (WBC), mean corpuscular haemoglobin concentration (MCHC), monocytes, neutrophils, lymphocytes, eosinophils and basophils using an automated dialyser machine (Cell-Dyn, Abbott, US). Another portion of blood was dispensed into plain bottles, allowed to clot and centrifuged at 3500 rpm for 10 min and the clear sera aspirated off for biochemical evaluation viz; urea, creatinine, total bilirubin, conjugated bilirubin, total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density cholesterol (VLDL-C) and triglycerides (TG) using commercial kits obtained from Randox Laboratories, UK. Electrolytes; Na^+, Cl^-, K^+ and HCO^-_3 levels were also estimated by standard methods (Bishop and Fody, 2005).

Histopathological studies: The hearts, kidneys, lungs, testes/uterus, spleens, intestines, stomachs and livers were removed, weighed and observed macroscopically. These organs were fixed in 10% formal saline for at least 48 h, processed routinely and embedded in paraffin wax. Histological sections were cut at 5-6 μm and stained with routine haematoxylin and eosin (HE) and examined.

Statistical analysis: Results were expressed as mean ± SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA). Students't-test at 95% level of significance was used to assess significant difference between the control and treated group.

RESULTS

The phytochemical screening showed that fraction F_6 contained a very significant amount of flavonoids and saponins. It also contained carbohydrates, combined and free reducing sugars, tannins, sterols and balsams. Sub-acute studies showed that fraction F_6 increased feed intake and body weight and significantly ($p<0.05$) decreased water intake in the diabetic rats (Table 1).

Effect on biochemical indices: All doses of fraction F_6 significantly ($p<0.05$) decreased the elevated levels of TG, LDL-C, VLDL-C and increased HDL-C level in the diabetic rats (Table 2).

The diabetic control rats showed elevated serum levels of urea (13.3±1.60) mmol/L and creatinine (94.3±1.91) mmol/L. Treatment with fraction F_6 (80 and 160 mg/kg) significantly ($p<0.05$) lowered the levels of these indices to (10.3±0.93 and 8.8±0.73) mmol/L and (89.98±1.95 and 92.2±3.40) mmol/L. Serum levels of potassium, chloride and bicarbonate were significantly ($p<0.01$) increased (Table 3). The elevated levels of liver function maker enzymes AST, ALT and ALP were significantly ($p<0.01$) lowered. Fraction F_6 at doses of 80 and 320 mg/kg F_6 caused a significant ($p<0.05$) increase in the level of total protein (Table 4).

Table 1: Effect of F_6 on body weight, food and water intake after 4 weeks of treatment

Treatment	Dose (mg/kg)	Body weight (g)		Final Food intake (g)		Wk4 Water intake (ml)	
		Initial	Final	Wk1	Wk4	Wk1	Wk4
Control normal	--	259.6± 16.1	320.1± 1.1	12.9± 0.6	14.6± 8.3	20.4± 0.6	22.5 ±1.0
Diabetic control	--	256.5 ±15.9	201.0± 2.0	8.4± 1.2	9.1± 0.6	19.7 ±1.1	25.±3 1.9
Diabetic +F6	80	254.8± 9.1	267.1± 11.9*	11.1± 0.1*	13.5± 0.7*	19.1 ±2.3	21.4 ±1.0*
Diabetic + F6	160	258.7± 7.3	289.1± 11.8*	11.7 ±2.1*	14.4± 1.5*	20.5±1.8	16.6 ±0.6*
Diabetic + F6	320	254.7± 17.5	277.2± 16.4*	10.4± 0.8	12.6± 0.6*	21.2 ±1,4	17.7 ±1.1*
Diabetic + chlorp	250	264.8± 16.2	294.6 ±23.4*	9.7 ±1.0 1	1.4± 0.7*	20.0± 0.8	18.3 ±0.3*

Chlorp. = Chlorpropamide, Wk1 = Week 1, Wk4 = Week 4. * $p<0.05$ vs diabetic control; No/gp = 7

Table 2: Effect of F_6 on cholesterol level of diabetic rats

Group	Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
Normal control	89.3±8.6	87.0±7.9	56.5±3.0	51.8±4.0	11.85±2.1
Diabetic control	145.1±6.7	169.3±7.6	19.35±2.4	172.8±12.1	23.43±1.8
Diabetic + F_6 (80 mg/kg)	126.1±8.2*	90.1±36.9*	34.51±3.14*	57.7±3.1*	12.31±0.8*
Diabetic + F_6 (160 mg/kg)	98.6±7.4*	94.3±7.1*	44.60±2.31*	54.3±2.6*	12.46±0.9*
Diabetic + F_6 (320 mg/kg)	103.1 6.8*	111.3±7.2*	32.16±1.15*	61.1±3.1*	13.1±1.0*
Diabetic + Chlorp. (250 mg/kg	101.46±6.3*	115.4±8.1*	29.5±2.1*	68.3±3.7*	14.69±1.1*

Chlorp. = Chlorpropamide (n = 7) *$p<0.05$ vs diabetic control

Table 3: Effect of F_6 on serum urea, creatinine and electrolyte concentrations of diabetic rats

Parameter (mmol/L)	Normal control	Diabeti ccontrol	Diabetic + F_6 (80 mg/kg)	Diabetic + F_6 (160 mg/kg)	Diabetic + F_6 (320 mg/kg)	Diabetic + Chlorpropamide (250 mg/kg)
Urea	5.85±0.25	13.3±1.60	10.3±0.93*	8.8±0.73*	12.4±1.09	9.1±0.57*
Creatinine	87.25±4.99	94.3±1.91	89.98±1.95*	92.2±3.40*	94.7±6.60	95.4±4.99
Na^+	151.25±1.82	153.3±0.35	152.1±1.87	153.6±1.50	151.4±1.04	153.9±1.31
K^+	5.16±0.15	4.01±0.12	6.6±0.29*	5.3±0.23*	5.4±0.52*	5.2±0.26*
Cl^-	98.0±0.65	95.2±0.52	96.6±0.90	98.0±0.53*	98.6±0.38*	97.9±0.91*
HCO_3^-	27.0±0.67	25.0±0.51	28.86±1.74*	29.71±0.23*	25.71±0.68	27.71±1.49*

(n = 7) *$p<0.05$ vs diabetic control

Table 4: Effect of F_6 on liver function marker enzymes

Parameter (mmol/L)	Normal control	Diabeti ccontrol	Diabetic + F_6 (80 mg/kg)	Diabetic + F_6 (160 mg/kg)	Diabetic + F_6 (320 mg/kg)	Diabetic + Chlorpropamide (250 mg/kg)
ALP (IU/L)	176.43±9.01	278.5±9.12	260.5±8.15*	189.3±10.1*	245.3±9.10*	240.6±8.71*
ALT (IU/L)	39.42±8.78	211.13±20.15	101.3±19.15*	87.3±18.51*	115.1±19.2*	114±16.25*
AST (IU/L)	57.0±13.38	127.4±7.34	120.3±6.1*	80.4±5.71*	98.3±7.15*	101±6.14*
Total Bilirubin (Umol/L)	14.43±1.23	15.0±1.57	15.1±1.75	16.22±0.61	16.5±2.14	15.2±3.20
Conjugated Bilirubin (Umol/L)	3.94±0.01	2.9±0.16	3.0±0.33	2.7±0.9	3.1±0.65	2.9±0.13
Total Protein (g/L)	72.7±2.50	60.61±1.86	64.7±1.80*	60.8±2.03	72.6±2.07*	66.7±0.46*
Albumin (g/L)	32.1±0.41	30.1±0.68	30.8±0.64	31.7±0.46	30.6±0.51	31.7±0.30

(n = 7) *$p<0.05$, vs diabetic control

Table 5: Effect of F_6 on haematological parameters

Parameter (mmol/L)	Normal control	Diabeti ccontrol	Diabetic + F_6 (80 mg/kg)	Diabetic + F_6 (160 mg/kg)	Diabetic + F_6 (320 mg/kg)	Diabetic + Chlor. (250 mg/kg)
PCV (%)	40.86±3.37	44.8±2.18	40.0±0.71*	43.0±1.57	40.6±1.04**	39.2±3.77*
Hb (g/dL)	12.6±0.27	14.8±0.72	13.4±0.15	13.8±0.23	13.58±0.28	12.8±0.78
MCHC (g/dL)	30.63±0.43	32.9±0.26	33.4±0.26	32.04±0.60	33.5±0.27	32.6±2.49
WBC (X 10^9)	7.14±0.89	8.3±0.98	8.5±2.53	8.5±1.32	8.9±2.87	8.8±2.13
Neutrophil (%)	45.75±1.18	47.8±4.55	47.8±4.55	47.7±2.01	46.6±3.21	47.4±7.28
Lymphocytes (%)	53.38±1.22	50.4±4.69	53.4±4.69	60.1±2.05*	60.6±3.42*	49.8±3.38
Eosinophils (%)	1.06±0.26	1.0±0.00	1.1±0.47	1.0±0.00	1.25±0.15	1.0±0.00
Monocyte (%)	1.17±0.29	1.5±0.35	1.8±0.41	1.43±0.19	1.12±0.17	1.5±0.22
Basophil (%)	0.0±0.00	1.0±0.00	0.00±0.0	00.0±0.0	00.0±0.0	00.0±0.00

Chlorp. = Chlorpropamide, (n=7) *$p<0.05$ vs diabetic control

Effect on haematological indices: At the doses 160 and 320 mg/kg fraction F_6 significantly ($p<0.05$) raised the level of lymphocytes from 50.4±4.69 in diabetic control rats to 60.1±2.05 and 60.6±3.42 respectively in diabetic treated rats. Other haematological indices were not significantly altered (Table 5).

Effects on organ weights: Treatment with fraction F_6 significantly ($p<0.05$) reduced the elevated wet weight heart in diabetic rats (Table 6).

Histopathological investigation: The histopathological studies revealed no significant abnormalities in all the

Table 6: Effect of F_6 on wet organ/body wight ratio

	Treatment and Group					
	Normal control	Diabeti ccontrol	Diabetic + F_6 (80 mg/kg)	Diabetic + F_6 (160 mg/kg)	Diabetic + F_6 (320 mg/kg)	Diabetic + Chlorp. (250 mg/kg)
Heart	0.31±0.022	0.89±0.03	0.34 ±0.01	0.33±0.01*	0.37±0.05	0.34±0.02*
Lungs	0.98±0.17	0.91±0.15	1.09±0.21	0.87±0.09	1.26±0.57	1.26±0.33
Kidney	0.66±0.06	0.81±0.05	0.75±0.06	0.69±0.04	0.75±0.06	0.64±0.09
Intestine	0.40±0.11	0.60±0.21	0.83±0.29	0.87±0.18	0.85±0.10	0.57±0.07
Liver	3.13±0.21	3.98±0.23	3.95±0.24	3.42±0.18	3.71±0.23	3.13±0.21
Testis/ Uterus	0.94±0.15	0.99±0.16	1.16±0.14	0.87±0.16	0.84±0.12	0.99±0.17
Brain	0.61±0.04	0.64±0.03	0.54±0.05	0.68±0.02	0.70±0.03	0.66±0.06
Spleen	0.36±0.03	0.38±0.02	0.42±0.05	0.39±0.05	0.47±0.09	0.41±0.04
Stomach	2.22±0.39	2.51±0.45	2.37±0.41	2.10±0.32	3.16±0.47	2.03±0.32

Chlorp = Chlorpropamide, (n = 7) *p<0.05 vs diabetic control

vital organs in both F_6 and chlorpropamide treated hyperglycemic rats. Diabetic control rats showed myxoid changes in the heart (data not shown).

DISCUSSION

The 28 days sub-acute studies showed that fraction F_6 of *V. amygdalina* increased feed intake and decreased water intake in diabetic treated rats.

In diabetes, the obligatory renal water loss combined with the hyperosmolarity tends to deplete intracellular water, triggering the osmoreceptor of the thirst centre of the brain and polydipsia which leads to increase in water intake (UKPDS, 1998). The catabolic effects then prevail, resulting in weight loss.

High levels of triglycerides, LDL-C, VLDL-C have been associated with heart disease, insulin resistance and diabetic mellitus (Nikkila, 1984). The abnormally high concentration of serum lipids in diabetics is mainly due to increase in the mobilization of free fatty acids from the peripheral fat depots (Bopanna *et al.,* 1997). In this study, the rise in blood sugar was accompanied by marked increase in cholesterol, triglycerides, LDL-C, VLDL-C and reduction in HDL-C. Fraction F_6 and chlorpropamide significantly reduced cholesterol, TG, LDL-C, VLDL-C and significantly increased the HDL-C level in the diabetic treated rats. Aqueous leaf extract of *V. amygdalina* have been shown to have hypolipidemic effect in diabetic rats (Akah *et al.*, 2004). Also Markku (1995) reported that glycemic control is the major determinant of total and VLDL triglyceride concentration. Improved glycaemia control following sulphonylurea therapy was also shown to decrease VDL and total triglyceride levels (Huupponen *et al*., 1984).

Another characteristic feature of severe diabetic is an elevated excretion of urea whose concentration may be five times higher than the normal value (Lehninger, 1998). In this study the elevate serum levels of urea and creatinine in the diabetic rats were reduced to normal values by fraction F_6. In diabetics there is decrease in the level of electrolytes (Na^+, Cl^-) as a result of osmotic diuresis with subsequent loss of water and electrolytes induced by glycosuria (Adrogue *et al.*, 1986). Interestingly the levels of these electrolytes were brought to near normal levels by fraction F_6.

Diabetes mellitus is associated with high levels of circulatory cholesterol and other lipids (Huuponen *et al.*, 1984) and this accounts for the atherosclerosis, arteriosclerosis and severe coronary heart disease which leads to increase levels of transaminases, marker enzymes important in heart and liver damage (Vaishwanar and Kowale, 1976). The levels of AST, ALT and ALP have been reported to be increased in alloxan-induced diabetic rats (Gonzalez *et al.,* 1992, Nwanjo, 2007). In this investigation fraction F_6 significantly ($p<0.05$) reduced elevated levels of ALT, AST and ALP thus improving renal and hepatic functions. This observation is consistent with earlier report on hepatoprotective potentials of leaf extracts of *V. amygdalina* in mice (Iwalokun *et al.,* 2006).

Literature has shown that ingestion of medicinal compounds or drugs can alter the normal range of hematological parameters (Ajagbonna *et al.*, 1999). Significant increase in the lymphocytes induced by fraction F_6 reflects possible imunomodulatory effects of *V. amygdalina* in alloxan-induced diabetic rats.

There was no significant difference in the wet weight of all the vital organs examined in diabetic treated and normal rats. The lack of direct organ toxicity of fraction F_6 may be due to the presence of flavonoids. Potent anti-oxidant and free radical scavenging activities of flavonoids (Hillwel, 1994) could counteract the free radical generation responsible for alloxan-induced diabetes, and may contribute to the very high potency of fraction F_6. Also heterogeneous phytoconstituents of crude extracts have been reported to have synergistic effect (Mazunder *et al*., 2005).

In conclusion, these results confirm the use of leaf extracts of *V. amygdalina* in traditional medicine for the treatment of diabetes mellitus. The results suggest that F_6 of *V. amygdalina* is a safe and potent hypoglycemic and hypolipidemic agent which is capable of normalizing other biochemical and hematological abnormalities associated with diabetes mellitus thus could be prescribed as adjunct to dietary therapy and main therapy for diabetes mellitus.

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