Preliminary Phytochemical Analysis And The Effect Of Ethanolic Leaf Extract Of *Vernonia amygdalina* On Blood Glucose Concentration And Hematological Parameters In Alloxan-Induced Diabetic Albino Rats

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ABSTRACT

The study was conducted to determine the preliminary phytochemical analysis and the effect of ethanolic leaf extract of *Vernonia amygdalina* on hematological parameters in Alloxan-induced diabetic albino rats. The phytochemical constituents of an ethanolic leaf extract of *V. amygdalina* indicates the presence of secondary metabolites like tannins, flavonoids, alkaloids, glycosides,. cyanogenic glycosides, anthraquinones, terpenoids, saponin, and polyphenol.. Adult albino rats weighing between 140-170 g were induced intraperitoneally with alloxan. The albino rats were grouped into five groups (five animals per group). Group I rats were not induced with alloxan , Group II animals were diabetic, serves as the negative control and were giving distilled water *ad libitum*, Group III serves as positive control and was treated with glibenclamide, Group IV and V were treated with 150 and 250 mg/kg body weight of ethanolic leaf extract of *V. amygdalina* respectively. The extracts were given to the animals orally for 14 days. At the end of the experimental period, the animals from each experimental group were starved for 16 hours and sacrificed by cervical dislocation. The weight of diabetic untreated rats (Group II) were significantly ($P<0.005$) reduced when compared to other groups. The group of rats given glibenclamide, 150 and 250mg/Kg B.W of *V. amygdalina* extract showed a significant decrease ($P<0.05$) of blood sugar level on day 7 and day 14 respectively when compared to the untreated rats. This suggests that the leaf extract possesses anti-diabetic effect (hypoglycaemic effect). The extracts of *V. amygdalina* significantly increase RBC, HGB, HCT and the WBC value is significantly reduced in the treated group compared to the untreated group. This shows that the extract is not hematotoxic.

Keywords: Alloxan-induced diabetic rats, hypoglycaemic effect, haematological parameters and *Vernonia amygdalina.*

Introduction

Diabetes mellitus (DM) is a complex and chronic disease associated with a myriad of debilitating complications, causes of which are usually multi-factorial. It causes elevation of blood glucose, resulting from a partial or complex cessation of insulin secretion or synthesis, or peripheral resistance to insulin action. The effective therapeutic approach should be multimodal and in this light, several traditional medicinal herbs have been preferred given the plethora of active ingredients present in a single herb (Tiwari and Rao, 2002, Atangwho *et al.*, 2009). Plants have been used as a source of food and medication and are a source of raw materials for industries. Many parts of plants such as fruits, seeds, barks, roots, fruits and flowers have been used as medicaments to cure various diseases that afflict humans and other animals (Phyllistin *et al.*, 2000). Plants have been known to contain abundant phytochemicals, antimicrobials and pharmacologically active principles, which include: anthraquinones, flavonoids, saponins, polyphenols, tannins and alkaloids (Sofowara, 1993; Evans, 1985; Erasto *et al.*, 2006). Several herbal preparations from different parts of plants (leaves, roots, barks and twigs) have become popular for the treatment of a variety of diseases such as diabetes mellitus, breast cancer, hypertension, etc. (Pinto and Rivlin, 1999; Li and Schellhorn, 2007). One of such plants suspected to possess medicinal value is *Vernonia amygdalina*. The plant grows throughout tropical Africa and is common in the South-Eastern part of Nigeria, where it has also been domesticated in some parts. The taxonomic classification of *Vernonia amygdalina* is as follows: Kingdom: plantae, Division: Angiosperms, Order: Asterales, Family: Asteraceae, Genius: Vernonia, Species: *V. amygdalina*, Botanical Name: *Vernonia amygdalina*. It has a variety of names in various languages. "Bitter leaf" in English language, "Shuwaka" in Hausa language, "Onugbu" in Igbo language, it is called "Etidot", in Efik, Ijaw and Ibibio, "Ewuro" in Yoruba language, "Oriwo" in Edo and "Chusa-doki" in Hausa (Egedigwe 2010). This study was conducted to determine the preliminary phytochemical analysis and to study the effect of an ethanolic leaf extract of *Vernonia amygdalina* on hematological parameters in Alloxan-induced diabetic albino rats.

Methodology

Collection and identification of ethanolic leaf extract of *Vernonia amygdalina*

The leaf of *Vernonia amygdalina* were gotten from Ikorodu market in Lagos State, Nigeria and authenticated by Miss Shokefun, a botanist from the Department of Science Laboratory Technology (Environmental Biology Unit), Lagos State Polytechnic Ikorodu, Lagos-Nigeria.

Preparation of ethanolic leaf extract of *Vernonia amygdalina*

The leaf were air dried under shade in the Biochemistry laboratory. The dried leaf were pounded to coarse powder in a mortar and then to fine powder with a blender. Extraction

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was carried out by dispersing 300g of the grounded plant material in 1L of 80% ethanol and shaking was done with GFL shaker for 72 hours. This was followed with vacuum filtration and evaporation at a temperature not exceeding 40°C. The concentrate was heated over a water bath to obtain a solvent-free extract, which was stored in a refrigerator at 4°C.

Phytochemical analysis of ethanolic leaf extract of *Vernonia amygdalina*

Phytochemical tests for bioactive constituents were carried out on portions of the residual material using standard phytochemical procedures (Trease and Evans (1985), Harborne (1973), and Sofowora (1993).

Administration of alloxan

Male albino rats of about eleven weeks old with weight range of 140-170g were made diabetic by injecting them with alloxan intraperitoneally. Development of diabetes was confirmed after 72 hours of alloxanisation by using "Accuchek Active Glucometer" (Roche Diagnostics) and blood glucose test strips. The animals were grouped into five groups. Each group contain five animals.

Grouping of animals

The animals were grouped as follows:

Group I - control (non-diabetic rats)
Group II- Negative control (diabetic without treatment)

Group III- Positive control (diabetic + glibenclamide)

Group IV- Diabetic + 150mg/Kg B.WT of *Vernonia amygdalina*

Group V- Diabetic + 250mg/Kg B.WT of *Vernonia amygdalina*

Determination of hematological parameters

The total red blood cell (RBC), hemoglobin concentration (HGB) ,white blood cell count (WBC), platelet count and other hematological parameters were determined in the blood using ADVIA 60 Closed Tube (CT) Automated Hematology System in Yaba, Psychiatric Hospital, Lagos, Nigeria.

Collection of blood samples for plasma preparation

The rats were sacrificed by cervical dislocation. Blood samples were collected by ocular punctures into heparinized tubes. The blood was later centrifuged for 10 min at 3000rpm using a centrifuge. The clear supernatant was used for the estimation of lipid profiles and liver function tests.

Data Analysis

Data analysis was done using the GraphPad prism computer software version 5. Students't'-test and one-way analysis of variance (ANOVA) were used for comparison. A P-value < 0.05 was considered significant.

Result

Table I: Phytochemical screening of ethanolic leaf extract of *Vernonia amygdalina*

Phytoconstituent	Qualitative abundance
Tannins	++
Flavonoids	++
Alkaloids	+
Glycosides	++
Cyanogenic glycosides	+
Anthraquinones	+
Terpenoids	++
Saponin	+
Polyphenols	++

(**+**) present at low levels, (**++**) present at moderate levels.

Phytochemical screening of ethanolic leaf extract of *Vernonia amygdalina* shows the presence of secondary metabolites like tannins, saponins, flavonoids, polyphenol, alkaloids, glycosides, cyanogenic glycosides, anthraquinones and terpenoids (Table 1). The presence of these secondary metabolites in Vernonia amygdalina may be responsible for the hypoglycaemic effect of the extract.

Figure 1 shows that there is a drastic decrease in the body weight of diabetic untreated rats (group II) compared to other groups.
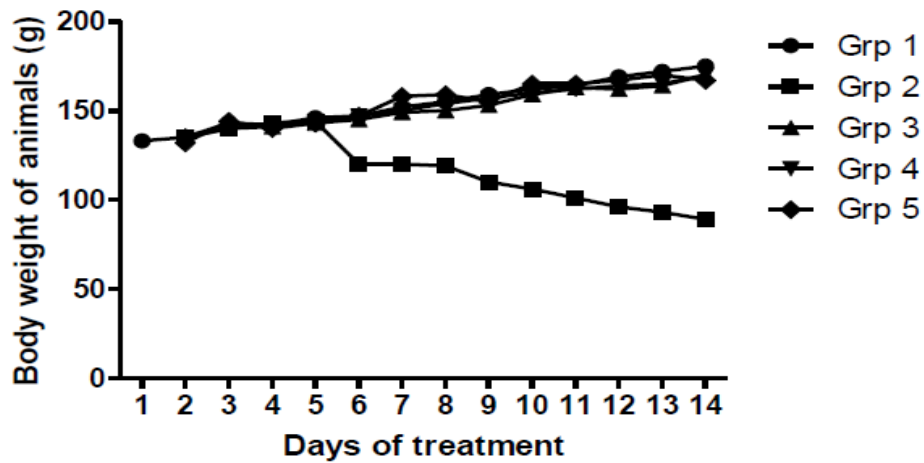
Figure 1 Body weight of animals after treatment with glibenclamide, 150 and 250 mg/kg B.W of *Vernonia amygdalina*

Figure 2 below shows the initial blood sugar level before induction. This indicates that all the animals used for the experiments are healthy.
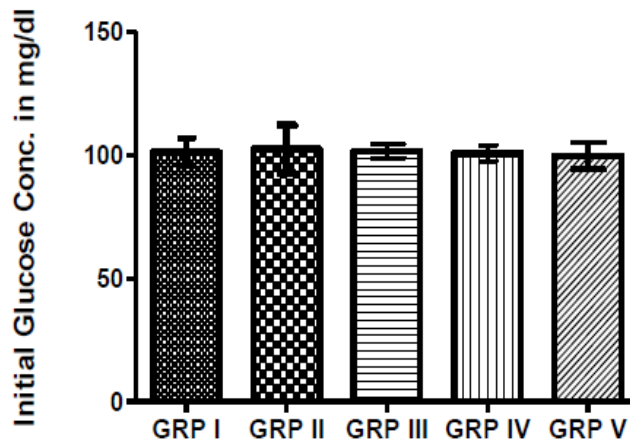

Figure 2. Initial blood glucose concentration values in mg/dl for normal, Diabetic untreated, Diabetic treated with glibenclamide, 150 and 250mg/kg body weight of seed extract of *Vernonia amygdalina* .

Figure 3 shows the blood sugar concentration after induction. Only group 1 animals were not induced with alloxan. This indicates that all the animals in group II -V were diabetic after induction with alloxan.
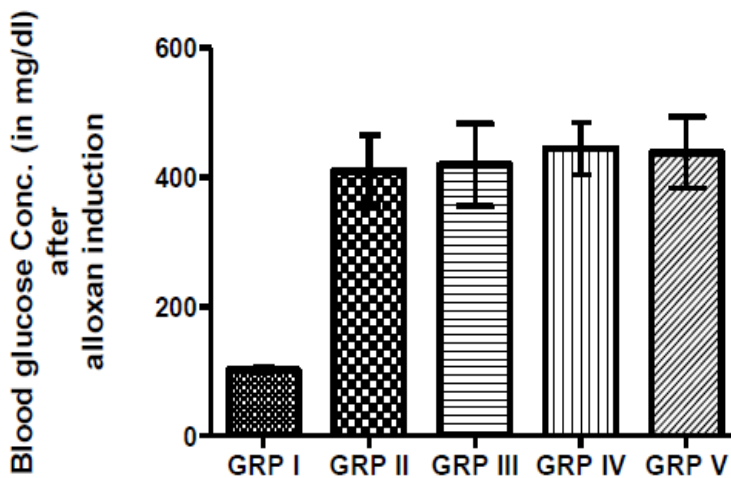

Figure 3. Blood glucose concentration values in mg/dl after alloxan induction for all the experimental groups.

Figure 4 below shows the blood glucose concentration values in mg/dl after 7 days of treatment. Group III -V were treated with glibenclamide, 150 and 250 mg/kg B.W of *Vernonia amygdalina* respectively and they all have a hypoglycaemic effect on the animals induced with alloxan.
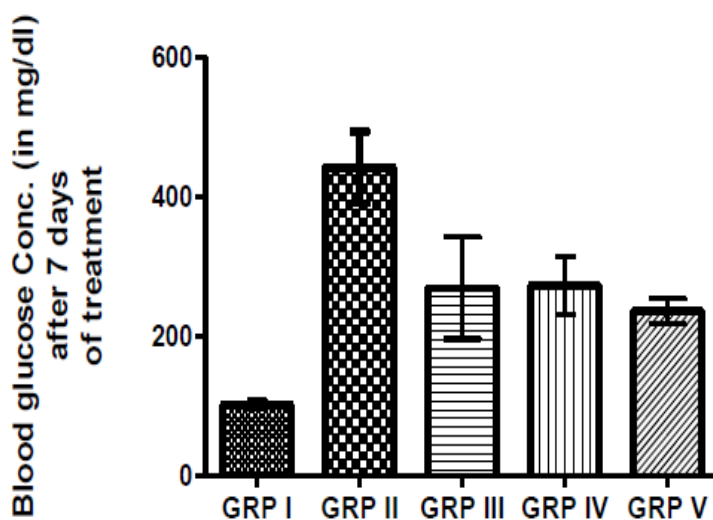
Figure 4. Blood glucose concentration after 7 days of treatment for all the experimental groups.

Figure 5 below shows the blood glucose concentration values in mg/dl after 14 days of treatment. Group III -V were treated with glibenclamide, 150 and 250 mg/kg B.W of *Vernonia amygdalina* respectively and they all have hypoglycaemic effect.
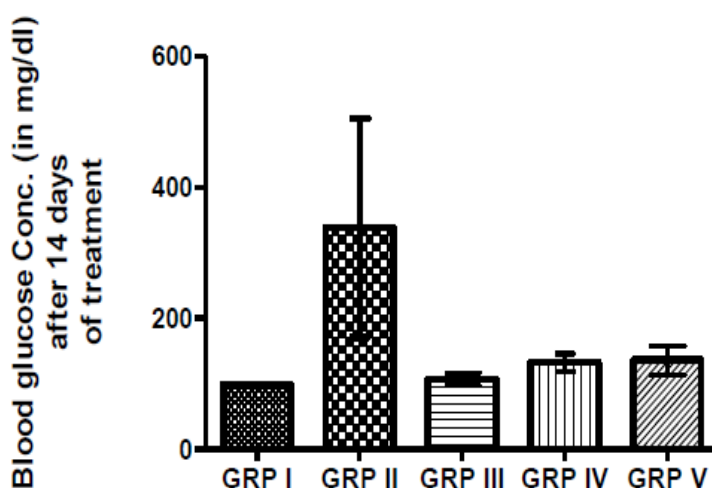

Figure 5. Blood glucose concentration after 14 days of treatment for all the experimental groups.

Table II: Effect of ethanolic leaf extract of *Vernonia amygdalina* on hematological parameters

Hematological parameters	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E
WBC ($\times 10^9$/L)	9.1 ± 2.2*	12.5 ± 2.1	10.6 ± 1.7*	10.2 ± 2.4*	9.8 ± 3.2*
HGB g/dl	11.9 ± 2.2*	8.6 ± 2.1	10.8 ± 1.8*	11.4 ± 3.2*	12.1 ± 2.1*
RBC ($\times 10^{12}$/L)	12.3 ± 2.4*	8.5 ± 2.5	10.1 ± 2.2*	11.3± 2.2*	11.5 ± 2.6*
HCT %	41.4 ± 2.1*	34.1 ± 2.2	40.4 ± 2.2*	42.5 ± 2.6*	44.2 ± 2.2*
MCV fl	55.1 ± 2.4	50.2 ± 2.7	53.1 ± 2.4	58.5 ± 2.2	58.2 ± 1.8
MCH pg	18.5 ± 1.1	17.3 ± 1.3	17.1 ± 1.8	17.2 ± 2.2	18.6 ± 1.5
MCHC g/dl	29.9 ± 1.2	30.1 ± 1.2	30.6 ± 1.2	30.9 ± 1.7	29.6 ± 1.4
RDW-CV %	16.2 ± 0.5	16.2 ± 0.9	16.29 ± 1.1	16.9 ± 1.8	16.8 ± 1.6
RDW-SD fl	33.1 ± 1.6	32.9 ± 0.7	33.9 ± 0.6	33.7 ± 0.9	33.7 ± 1.2
MPV fl	7.5 ± 0.7	7.6 ± 0.8	7.4 ± 0.4	7.4 ± 0.8	7.5 ± 1.3
PDW	16.7 ± 0.9	16.1 ± 0.7	15.8 ± 0.3	15.6 ± 0.4	15.4 ± 0.5

The values are the Means ± SD for five rats in each group. Hemoglobin (HGB), Red blood count (RBC), Hematocrit (HCT),Mean cell volume(MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration(MCHC), Red Blood Cell Distribution Width Coefficient of Variation (RDW-CV), Red Blood Cell Distribution Width Standard Deviation (RDW-SD), Mean platelet volume (MPV),platelet Distribution Width (PDW).

Discussion

Medicinal plants constitute an effective source of both traditional and modern medicines, herbal medicine have been shown to have genuine utility and about 80% of rural population depends on it as primary health care. Phytochemical screening of ethanolic leaf extract of *Vernonia amygdalina* shows the presence of secondary metabolites like tannins, saponin, flavonoids, polyphenol, alkaloids, glycosides, cyanogenic glycosides, anthraquinones and terpenoids (Table 1). Igile *et al.* (1994) reported that the leaves of bitter leaf contain sesquiterpene lactones, tannins sterols, saponin, glycosides, and flavonoids. It has also been reported that the plant extract is generally non-toxic, but excess consumption could have a purgative effect. Phenols are reported to inhibit alpha (α) amylase, sucrase, as well as the action of sodium glucose transporter (S-GLUT-1) of the intestinal brush border cells and this is responsible for their anti-diabetic action (Tiwari and Rao, 2002). *Vernonia amygdalina* extract contains active ingredients such as vernoniosides, glucosides, saponin, flavonoids and phenol (Jisaka *et al.*, 1993). These phytochemicals maybe responsible for their pancreatic repair caused by streptozocin damage in experimental animals.

The untreated diabetic animals (group II) showed significantly weight loss ($P<0.05$) when compared to the healthy and treated animals (Figure 1). This may be due to loss in muscle adipose tissue protein and fatty acids (Granner, 1996). Ahmed *et al*, 2005 reported significant weight reduction in untreated diabetic animals.

Figure 2 shows the initial blood sugar level before induction of the control group and other groups. From the result obtained, all the animals used for the experiments are healthy. Figure 3 shows the blood glucose concentration after diabetes induction. The result obtained indicates that all the animals in group II -V were diabetic after induction with alloxan. Animals in group II and III serve has the negative and positive control while group IV and V animals were treated with 150 and 250mg/Kg B.WT of ethanolic extract of *Vernonia amygdalina* respectively. Experimental findings show that after 7 days of treatment, group III -V animals blood glucose level significantly reduce ($P<0.05$) when compared to untreated diabetic rats (Figure 4) . Figure 5 shows the blood glucose concentration values in mg/dl after 14 days of treatment. Group III -V animals show significant ($P<0.05$) hypoglycemic effect after treatment with glibenclamide and an ethanolic leaf extract of *Vernonia amygdalina*.

Hematological and biochemical indices have been reported to be a reliable parameter for assessment of the health status of animals (Tiwari and Rao 2002). The extract (*Vernonia amygdalina*) prevented a drastic reduction in HGB, RBC and HCT values, features typical of anaemia. This observation was supported by a report stating that anemia was characterized by decreased values of HGB, RBC and hematocrit (Aleksandro *et al* 2009). This is an indication of the hematopoietic effect of *Vernonia amygdalina*. There were significant increases ($P < 0.05$) in the WBC count in the untreated group compared to group I and all the groups treated with glibenclamide and *Vernonia amygdalina* (Table II). There was no significant change ($P<0.05$) in the MCV, MCH and MCHC values in the entire experimental groups. No significant changes occurred in MPV, PDW, RDW-CV and RDW-SD.

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