

Glucose Lowering Effects and Histomorphological Changes of *Vernonia amygdalina* on Pancreatic Compromised Wistar Rats using Alloxan Monohydrate.

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Abstract

Vernonia amygdalina commonly known as bitter leaf is a medicinal plant used in trado-medicine for the treatment of various health conditions. This study was aimed at evaluating the effects of graded doses of *Vernonia amygdalina* leaves extract on blood glucose levels in alloxan induced diabetic Wistar rats. Thirty five (35) adult male Wistar rats (170g - 200g) were randomly divided into 5 groups. Group 1 served as the control group while group 2 served as the diabetic group induced with alloxan only. Group 3 – group 5 animals were induced with 100mg/kg of alloxan and were further treated with 200mg/kg, 400mg/kg of extracts and 150mg/kg of metformin respectively for 14 days. Blood samples were collected by retro-orbital puncture at the end of 7 and 14 days into plain heparinized sample bottles and glucose analysis was carried out using standard hematological techniques. Experimental animals were euthanized by cervical dislocation; pancreas was harvested, fixed, processed and stained according to standard histological procedures. Stained tissue images were captured using digital micrometer eyepiece. Results were expressed as mean \pm standard deviation. Results of data obtained were computed using SPSS version 20 and analyzed using one way ANOVA. A $p < 0.001$ was considered statistically significant. The study showed a significant decrease in blood glucose levels of test animals and significant increase in body weight of experimental animals in a time and dose dependent manner. The extracts also showed the presence of flavonoids, saponins, tannins, phenols and cardiac glycosides as major components of the plant. In addition, histopathological findings demonstrated a restorative effect of *Vernonia amygdalina* on the diabetic pancreas of experimental animals treated with this agent. Hence, acute administration of *Vernonia amygdalina* on alloxan induced diabetic rats showed a time and dose dependant glucose lowering levels and pancreatic cell regeneration.

Keywords: glucose lowering, histomorphology, *vernonia*, *amygdalina*, pancreas, wistar rats

Introduction

Diabetes mellitus (DM) is a well-known public health issue with high mortality and morbidity rates. This obviously has led to increased investigation into the potential causes as well as possible remedies of DM. Giugliano *et al*^[1] reported increased susceptibility to lipid peroxidation as well as disturbances in lipid profile due to diabetes mellitus. Diabetic patients have shown high increased oxidative stress as observed by high free radicals.^[2] Retinopathy, atherosclerosis, nephropathy have been shown as long term complications of type 2 diabetes.^[3,4,5]

Plants have been explored in traditional medicine for the speeding up of natural healing processes.^[6] Varieties of these plants and their formulations have been reported to be imperative in ethno-medicine for the treatment of many diseases in developing countries such as Nigeria and have also been used as dietary supplements.^[6,7]

Vernonia amygdalina has been described as a medicinal plant that belongs to the genus “*Vernonia*” and specie “*amygdalina*”.^[8] It is a major vegetable that is widely distributed abundantly in the west coast of Africa where it grows wild as a domestic browse plant.^[8] In Nigeria it is commonly called “Bitter Leaf” in English, “Onugbo” in the eastern part of Nigeria, “Shuwaka” in northern Nigeria and “Ewuro” in western Nigeria.^[9] This agent is also called “Etidot” by the Ibibios, Efik and Ijaws while in Edo state, it is called “Eriwo”.^[9]

Studies have reported that this agent is imperative in the treatment and management of many ailments such as asthma, schistosomiasis, malaria, measles, diarrhea, tuberculosis, abdominal pain and fever and cough.^[10, 11] It has also been demonstrated to exhibit anti-hyperlipidemic activities on diabetic and non-diabetic rats.^[12] Its renal-protective and hepato-protective effects on the kidneys and livers of alloxan induced diabetic rats have also been reported in 2007.^[12]

Various studies have demonstrated the phytochemical constituents of this plant; it was reported to be composed of saponins, sesquiterpenes, lactones and flavonoids.^[13, 14] Another study revealed the presence of steroid glucosides such as vernoniosides A₁, A₂, A₃, A₄, B₁, B₂, B₃, D and E.^[13]

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Furthermore, due to its active ingredients, Ebong and his colleagues in 2008, described it as having strong anti-diabetic potentials.^[15]

Most plants which have glucose lowering properties do so by acting on blood glucose.^[13] This is either by acting on the pancreatic beta cells so as to inhibit the blood glucose level^[14, 15] or by interfering with glucose absorption in the GIT.^[16]

Considering the morbidity and mortality associated with Diabetes mellitus and its high cost of management, clinicians are continuously searching for new drugs with glucose lowering properties that can provide benefit for patients and reduce cost of treatment. Thus, this study aimed at evaluating the effects of graded doses of *Vernonia amygdalina* leaves extract on blood glucose levels in alloxan induced diabetic Wistar rats.

Materials And Methods

Animal breeding

Thirty-five (35) adult male Wistar rats with range of body weight of 170g – 200g and age of about 3 months were purchased from the Faculty of Basic Medical Sciences animal house of the University Nigeria Nsukka, and raised in the Animal House unit of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria. Handling of the experimental animals followed standard procedures and regulations.

Plant Collection and Extraction

Mature fresh leaves of *Vernonia amygdalina* were collected from local garden in Abraka village and authenticated at the herbarium section of Botany Department Delta State University Abraka.

The fresh leaves were air dried and grounded into powder before extraction. The powdered plant leaves were extracted with 95% ethanol and then allowed to stand for about 72 hours. Filtration was done using Whatman filter paper 1 and the filtrate was concentrated using rotary evaporator at temperature 45°C to obtain a paste like extract.

Induction of Diabetes Mellitus

Experimental animals were fasted overnight and diabetes was induced via intra-peritoneal injection of 120mg/kg of alloxan monohydrate (Sigma, St. Louis Mo, USA). Glucose oxidase method using spectrophotometer was employed in confirming diabetes after 3 days of alloxan induction and only rats with blood 200 mg/dl were included in this study.

Estimation of Blood Glucose

At 7 days post-induction of diabetes, blood samples were collected by retro-orbital puncture method into white plain containers. Serum analysis was carried out ten minutes after blood collection. Analysis

of blood glucose was assayed using the glucose oxidase method and a spectrophotometer at 546nm.

Research Design

This was an experimental and observational study that entailed five groups (1 - 5). A total of thirty-five (35) adult male rats were weighed, sorted and assigned to a group containing seven rats each. Animals were fed with commercially formulated rat chow and water *ad libitum* under standard condition (12 hours light and 12 hours darkness, temp: 28-31°C; humidity: 50-55%) and were allowed to acclimatize for two days before administration according to animal handling guidelines stated by Animal research ethics (2009).^[20] The experiment lasted for 14 days with body weight of the rats measured using a weighing balance before the induction of diabetes, at the 7th and 14th day of administration.

Dosage and Drug administration

Extract of *Vernonia amygdalina* and metformin were administered orally using an orogastric tube at dosage of 200 and 400mg/kg to test animals as shown below.

Group 1 = Normal rats fed on rat chow and distilled water

Group 2 = Diabetic rats fed on rat chow and distilled water

Group 3 = Diabetic rats treated with 200mg/kg *Vernonia amygdalina*

Group 4 = Diabetic rats treated with 400mg/kg *Vernonia amygdalina*

Group 5 = Diabetic rats treated with 150mg/kg Metformin

Treatment of Diabetes Mellitus with Oral Glucose lowering Drug

Known glucose lowering drug Metformin (Merck, Germany), was purchased and used in this study. The drug was administered orally once daily in the time for 14 days. One tablet of metformin 500 mg was dissolved in 5mls to obtain a concentration of 100mg/ml. Metformin drug was administered per oral through an oro-gastric tube at a dosage of 150mg/kg.^[21]

Collection of Blood Samples and Histological procedures.

Blood samples were collected through retro-orbital puncture after an overnight fast at the 7th day and cardiac puncture on the 14th day post administration of plant extract and glucose lowering drug. Blood samples were collected into plain sample bottles, allowed to clot for about 2 hours, centrifuged (3000rpm for 10min) to remove cells and recover serum which was then used for glucose analysis. The pancreas was harvested, fixed in 10% formalin and was processed according to standard histological procedures. Haematoxylin and

Eosin Stained tissue images of the pancreas were captured using digital microscopic eyepiece "SCOPETEK" DCM 500, 5.0 mega pixel connected to computer

Phytochemical Screening

Phytochemical screening of vernonia amygdalina was done according to the methods as described by Trease and Evans.^[22]

Statistical Analysis

Results were expressed as mean \pm standard deviation. Results of data obtained were computed using SPSS version 20 and analyzed using one way ANOVA. A p-value of less than 0.001 was considered statistically significant.

Results

The phytochemical analysis reveals the following as the major constituents of the vernonia amygdalina: Alkaloids, Anthraquinones, Flavonoids, Saponins, Tannins, Terpenoids, Phenols and Cardiac Glucosides.

The results showed a significant decrease in body weight of diabetic rats when compared with the normal after the 14th day duration of the experiment as shown in Table 1. Subsequent administration of *Vernonia amygdalina* extract caused significant increase in all treated groups when compared with the control ($p < 0.001$) at day 7 and day 14. The patent drug metformin caused a fluctuating non-significant increase and decrease in the body weight when compared with the normal control Table 1.

As shown in Table 2, *Vernonia amygdalina* at both doses of 200mg/kg and 400mg/kg showed consistent significant decrease in blood glucose concentration on day 7 and day 14 ($p < 0.01$) and also significant decrease when day 7 and day 14 were compared with day 3 ($p < 0.01$).

Figure 1 showed abundant serous acinar cells distributed in an exocrine pancreatic parenchyma cells. The serous cells are made up of deeply basophilic bland round to oval nuclei with an extensive eosinophilic cytoplasm. Also seen were congested blood vessels and eosinophilic protein cast in the lumen of the interlobular ducts. Sections from group 1 animals

Table 1: Effect of *Vernonia amygdalina* on body weight of experimental animals.

Values were expressed in Mean \pm SD. * $p < 0.001$ indicate significant changes compared with

GROUPS	TREATMENTS	DAY 1 (g) (BASAL)	DAY 3 (g)	DAY 7 (g)	DAY 14 (g)	% CHANGE
1	Normal control	174.29 \pm 5.35	181.43 \pm 8.10	184.29 \pm 7.87	188.57 \pm 8.10?	3.90%
2	Diabetic control	180.00 \pm 0.00	182.86 \pm 11.17	181.43 \pm 8.10	175.71 \pm 5.34	-3.91%
3	200mg/kg	182.86 \pm 9.51	180.00 \pm 8.16	198.57 \pm 8.10?	220.00 \pm 11.54?	22.22%
4	400mg/kg	187.14 \pm 13.80	177.14 \pm 4.88	195.71 \pm 17.18?	214.29 \pm 7.87?	20.97%
5	Metformin	180.00 \pm 11.21	190.00 \pm 0.00	192.85 \pm 7.56	182.86 \pm 4.88	-3.76%

diabetic control group.

Table 2: Effects of *Vernonia amygdalina* leaf extracts on blood glucose concentration of alloxan induced diabetic rats.

GROUPS	TREATMENT	DAY 1 (mg) (BASAL)	DAY 3 (mg)	DAY 7 (mg)	DAY 14 (mg)	% CHANGE
1	Normal control	82.43 \pm 7.76	86.79 \pm 7.76*	82.76 \pm 5.33*	94.20 \pm 11.75*	8.54%
2	Diabetic control	75.57 \pm 6.16 ^a	396.87 \pm 84.94	362.36 \pm 38.93	326.61 \pm 36.47	-17.70%
3	200mg/kg	83.86 \pm 4.60 ^a	446.59 \pm 42.59	209.50 \pm 28.35* ^a	172.43 \pm 36.35* ^a	-61.39%
4	400mg/kg	83.29 \pm 11.48 ^a	401.11 \pm 71.31	151.93 \pm 16.49* ^a	195.21 \pm 37.89* ^a	-51.33%
5	Metformin	83.00 \pm 7.87 ^a	403.02 \pm 147.93	186.24 \pm 62.81* ^a	139.75 \pm 40.32* ^a	-65.32%

Values were expressed in Mean \pm SD. * $p < 0.001$ indicate significant changes compared with diabetic control group and ^a $p < 0.001$ indicate significance when compared with day 3

revealed the normal pancreas cytoarchitecture while group 2 animals displayed severe acinar cells degeneration. Observed features in group 3 and 4 were keeping with exocrine pancreatic cells restoration.

Discussions

The present study showed statistically no significant decrease in body weight in the groups administered alloxan while the normal control group showed a significant increase in body weight at the end of the study. Maximum changes in body weight were observed in the groups treated with varying doses of *Vernonia amygdalina*. This significant weight gain could be ascribed to the nutritive value of this agent as previously reported that due to the nutritive components of its leaves they are used as condiments in cooking Nigerian dishes.^[7,19,21]

The present study indicated that leaf extracts of *Vernonia amygdalina* showed dose and time dependent effects in decreasing blood glucose level in test animals. These notable significant decreases were evident in the group that received the lowest dose of the extract and the group that was treated with metformin and thus confirmed the anti-diabetic/glucose lowering effects of ethanolic extracts of *Vernonia amygdalina*. The ethanolic extract of *Vernonia amygdalina* at 200mg/kg showed 61.39% reduction while at 400mg/kg there was 51.33% reduction in blood glucose at the end of 14 days period. Also similar reduction (65.32%) was observed in the group treated with metformin drug. This resultant observation is in agreement with the findings of other authors who reported similar results. Some studies reported significant reduction in blood glucose level of alloxan induced diabetic rats treated with *Vernonia amygdalina* after 14 days duration.

These anti-diabetic properties of *Vernonia amygdalina* may be due to the presence of active components of this plant also reported in this study. Phytochemical analysis screening revealed the presence of flavonoids, alkaloids, saponins, tannins and cardiac glycosides in higher concentrations, terpenoids, terpenoids and **anthraquinones** as trace elements. Flavonoids have been reported to act on the β -cells of islets of Langerhans and thus stimulating the production of glucose.^[25] Subsequently, studies have also revealed tannin, a major phyto-component of bitter leaf to possess a glucose lowering effect.^[26,27] One of the mechanisms of action of *Vernonia amygdalina* glucose lowering ability is the simultaneous suppression of gluconeogenesis and potentiation of glucose oxidation via the pentose phosphate pathway almost exclusively in the liver.^[28]

Histopathological observations, revealed a dose/time dependent serous acinar cells regeneration compared to the diabetic group that exhibited focal areas of degeneration especially in the ductal

epithelium. The pancreatic tissue architecture of animals administered alloxan revealed the presence of colloids or eosinophilic debris in the pancreatic ducts and as such suggested degeneration of the pancreas. In addition, histopathological findings revealed the presence of lymphocytic infiltrates within the pancreatic tissue stroma suggesting a marked destruction of the insulin producing beta cells of the islets with marked vascular congestion in the experimental animals treated with alloxan.

Furthermore, histoarchitectural and cellular features of test animals pretreated with *Vernonia amygdalina* revealed pancreatic acinar restoration which indicated an increase in pancreatic parenchyma cellularity with a rapid reduction in blood vessel congestion and colloids. This notable observation agrees with a study that noted a protective effect of this agent with minimal necrotic changes seen in experimental groups and supports the findings that *Vernonia amygdalina* has glucose lowering potentials.^[29]

Conclusion

Acute administration of *Vernonia amygdalina* on alloxan induced diabetic rats showed a duration and dose dependent glucose lowering effect on blood glucose levels and pancreatic cell regeneration. This could be due to the presence of flavonoids and tannins which was demonstrated to be an active component of this agent upon phytochemical screening and have been established as having glucose lowering properties.

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