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Investigation of The Anti-Hyperglycemic Activities of Aqueous Leaves Extract Of Vernonia Amygdalina (VA) In Rats

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Abstract

Many plants have been discovered to be effective in the prevention and treatment of various diseases This study investigated the anti-hyperglycemic activities of aqueous leaves extract of Vernonia Amygdalina (VA) in rats. 35 wistar rats of weights 175-285g where divided into seven (7) groups of five(5) rats each. Group A was a normoglycemic group and received water and feed ad libitium. Groups B, C, D , E, F and G were non-diabetic and received 80mg/kg of VA extract, Diabetic untreated group, Diabetic and received 40mg/kg of VA extract, Diabetic and received 80mg/kg of VA extract, Diabetic and received 120mg/kg of VA extract and Diabetic and received 5mg/kg of glibenclamide (a standard antidiabetic drug) P.O . The effect of the VA extract on blood glucose level, triglyceride, cholesterol, (HDL), LDL and body weight was estimated and the data were analyzed using SPSS version 20.The extract caused significant ($P<0.05$) and progressive time dependent reduction on blood glucose, serum triglyceride, cholesterol and body weight in both normoglycemic and alloxan-induced diabetic rats, the extract also caused a significant increase on HDL level in both diabetic and non-diabetic rats at all dose. In conclusion; the findings from this study showed that the aqueous leaves extract of VA possess anti-hyperglycemic, lipid lowering and hypoglycemic activities and suggests that 80mg/kg was the most potent dose at which VA demonstrated these activities. The significant reduction in blood glucose, triglyceride and cholesterol levels, body weight and an increase in HDL observed in this study justifies the ethnomedicinal uses of VA in the management of diabetes though effort should be made to optimize the dose for better effectiveness.

Keywords: Antihyperglycaemic, Diabetes mellitus, Vernonia Amygdalina, wistar rats.

1. Introduction

The use of plants as an alternative form of therapy in primary healthcare, communities and developing countries has increased in recent times. [1].Blood glucose level is regulated by insulin secreted by the beta- cells of

pancreas. Diabetes mellitus is a syndrome of impaired carbohydrate, fats, and protein metabolism caused by either lack of insulin secretion or decreased sensitivity of the tissue to insulin. Symptoms of diabetes mellitus include: Polydisia, polyuria, polyphagia, hyperglycemia and glucosuria associated with weight loss [2].Vernonia Amygdalina **(VA)** commonly called bitter leaf is a perennial shrub that belong to the family Asteraceae [3]. It is traditionally used for treatment of diabetes and fever [4]. **VA** has been reported to decrease blood glucose level and also helps to repair the pancreas [5]. Aqueous extracts **VA** leaves induce gastric secretion and dose-dependent contraction of the ileum in guinea pigs [6]. The use of **VA** in Nigeria and South East in particular has been largely restricted to food preparation. In Nigeria, the number of people with diabetes is estimated to about 6 million in 2015 [7]. Anti-diabetic drugs such as metiformin, rosigitazone, glibenclamide have several side effects on the body such as glucose intolerance, insulin resistance, hypoglycemia and psychosis [9]. The utilization of herbal extract from plants found in the fields and forests to treat diabetes related illness has increased over years [10]. Phytochemical analysis revealed the presence of flavonoids, tannins, saponin, polyphenols and alkaloid. Other chemical constituents include: protein, fiber, ether, Na+, Mg+, and K+[11]. Some vitamins (A, C , E, B1, B2), Fe and Niacin have also been identified [12]. The present study is aimed at investigating the anti-hyperglycemic activities of aqueous leaf extract of VA in rats. **VA** is commonly consumed among so many Nigerians especially the Igbos of the South- Eastern region and has also been used traditionally in the treatment of diabetes but there is lack of scientific evidence to substantiate this claim to the knowledge of the researcher. This study was carried out to investigate the anti-hyperglycemic effect of the plant.

2. Materials And Methods

2.1 Plant Collection, Identification, And Authentication

Fresh leaves of Vernonia amygdalina were purchased from Kenyatta market, Uwani Enugu i, Enugu south local government in Enugu state Nigeria. They were identified and authenticated by Mr. Onyeukwu Chijioke of the department of botany, University of Nigeria, Nsukka. A voucher specimen was deposited in the herbarium for further reference with no: (UNH7a).

2.0 Experimental Animals

Thirty - five male (35) wistar rats weighing 175-285g were used. The rats where purchased from the animal house of the Department of Pharmacology & Therapeutics, College of Medicine of the University of Nigeria, Enugu Campus. They were housed in a clean ventilated wire mesh cages at a 12 hrs dark/light cycle in a normal room temperature of 270C and fed with grower mash (Vital feed Ltd, Kaduna) and clean tap water ad libitum They were given one week for acclimatization before the commencement of the experiment. The animals were handled according to the intervention of the Laboratory animal protocols approved by the Research and Ethic Committee of the University of Nigeria, Ituku-Ozalla Enugu State.

2.2 Preparation of the Aqueous Vernonia Amydalina Leaf Extract

Preparation of the extract was done using the method of Akah [13].The leaves were washed and dried under shade for 7 days. It was grounded into powder using electric blender. 5.5 liters of water was added to 1200g of the powder and boiled for 30 minutes under reflux at 80 °C and then allowed to cool for 20 minutes. The mixture was filtered using Whatman No 1 filter paper. The filtrate was concentrated using water bath at a temperature of 50 °C; then evaporated to dryness to give a dark green solid paste with a yield of 135g.

2.3 Phytochemical Screening

The phytochemical screening was done according to the method of Trease and Evans[14].The result of the phytochemical analysis revealed the presence of Saponin and Tapernoids in high concentration, medium concentration of Tannin, Phenol, Glycosides, Resin, Alkaloids and Phenol.

2.4 Determination Of The Acute Toxicity Test

The acute toxicity test was done according to the method of Enegide Chinedu et al[15].

2.5 Induction of Diabtes in the Rats

Diabetes was induced using the method of [16].The rats were fasted overnight, at least 8 hours before the experiment. They were given access to drinking water throughout the experimental period ad libitium. After eight hours, the rats were administered alloxan solution at 10% diluted in normal saline intraperitionally. They were given access to their feed and water after 30 minutes of alloxan administration. The rats were confirmed diabetic after 24 hours using glucometer.

2.6 Experimental Design

Group A = Non-diabetic untreated group (normal control)

Group B = Non-diabetic and received 80mg/kg of VA extract

Group C = Diabetic untreated group

Group D = Diabetic and received 40mg/kg of VA extract.

Group E = Diabetic and received 80mg/kg of VA extract.

Group F = Diabetic and received 120mg/kg of VA extract.

Group G = Diabetic and received 5mg/kg of Glibenclamide (a standard antidiabetic drug) P.O.

2.7 Determination of the Body Weights

The weight of the rats were measured and recorded to the nearest (g) using the electronic weighing scale model no: LP505A made in China.

2.8 Collection of Blood Samples and Analysis of The Parameters

Blood samples were collected from the media canthus of the eye by retro orbital puncture using Heparinized capillary tube and EDTA bottle. Samples were taken at baseline, on day 7th, 14th ,and 21st and the following parameters determined:, lipid profile (Total cholesterol, Triglycerides, High Density Lipoprotein, Low Density Lipoprotein). The lipid profiles was analyzed using the method of [17] in which the collected blood samples were analyzed using the handheld cardiocheck test meter (lipidocare) by Polymer Technology Inc, USA)

2.9 Estimation of the Blood Glucose Levels

At the end of the experiment, the rats were fasted overnight for 10 hours [18]. The fasting blood glucose level was estimated with the tail prick method using glucose oxidase-peroxidase reactive strips (Accu- check, Roche Diagnostic, USA) [18]. 2.0ml of blood was collected from an orbital route of each rat into EDTA bottles for analysis. Then blood glucose levels were determined using glucometer.

3. Statistical Analysis

Data were analyzed using SPSS version 21. Results were expressed as MEAN ± SEM. One way analysis of variance (ANOVA) with Dunnette`s post-hoc test was used to compare the difference between groups. The p values ≤ 0.05 was considered statistically significant.

4. Results and Discussion

Table 1. Result of the Phytochemical Screening

S/NO	SAMPLES	CONCENTRATION
1	Carbohydrates	+++
2	Reducing Sugar	---
3	Terpenoids	+++
4	Saponin	+++
5	Glycosides	++
6	Alkaloids	++
7	Flavonoid	++
8	Phenols	++
9	Tannin	++
10	Resin	++
11	Steroid	---

Keys:+++ = High Concentration; ++= Medium Concentration; --- = Absent.

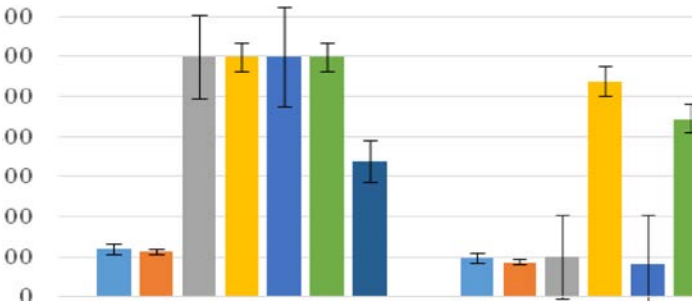

Figure 1: Chart showing the changes in the blood glucose level of VA extract on diabetic and non diabetic rats versus the days.

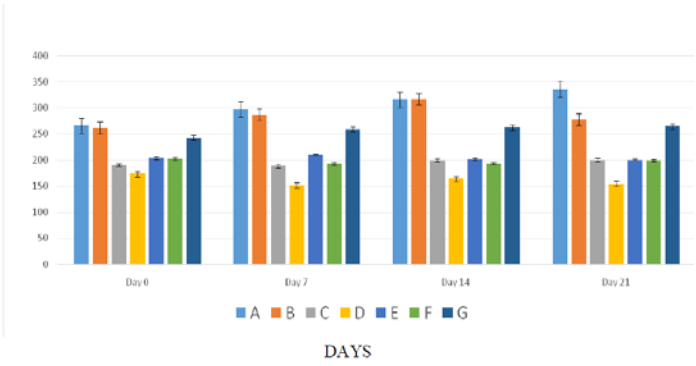

Figure 2: Chart showing the changes of body weight of VA aqueous leaf extract on diabetic and non diabetic Rats versus the days.

Table 1 above depicts the result of the phytochemical screening, It revealed the presence of Saponin and Tapernoids and in high concentration, medium concentration of Tannin, Phenol, Glycosides, Resin, Alkaloids and Phenol, Flavonoids, The major findings of this study were; The phytochemical that is responsible for the anti-hyperglycemic, hypoglycemic and lipid lowering activities of Vernonia amygdalina. Figure 2 shows the effect of VA aqueous leave extract on blood glucose level of diabetic and non-diabetic rats. The potent dose of Vernonia amygdalina that demonstrated these activities. Significant decrease ($p<0.05$) in blood glucose level was observed in group B (non- diabetic treated with 80mg/kg of VA) compared to group A normal control (non-diabetic without treatment) . This may be due to the present of phytochemical; tannin, phenol and saponin in Vernonia amygdalina. Significant decrease ($p<0.05$) in blood glucose level was also observed in group E and G but, it was more pronounced in group E compared to group G. Tannin, was reported to inhibit alpha-amylase, sucrose, as well as the action of SGLUT-1 of the intestinal brush border [19].Our finding is in line with Akah [20].

Figure 2, shows the effect of A VA aqueous leave extract on the body weights of diabetic and non-diabetic rats . There was a significant increase ($p>0.05$) in body weight as observed in group A and B (non-diabetic group) compared to diabetic treated and diabetic untreated group. The decrease in body weights of diabetic groups is in line with symptoms found in hyperglycemia, Polydisia, polyuria, polyphagia and glucosuria which is associated with loss in body weight [2]. There was no significant difference ($p>0.05$) in body weight between diabetic treated Vernonia amygdalina with groups (D, E and F) compared to diabetic untreated group (C). This may be due to environmental and nutritional factor. In diabetes, the obligatory renal water loss combined with the hyperosmolarity tends to deplete intracellular water, triggering the osmoreceptor of the thirst centre on the brain and Polydisia which leads to increase in water intake [21], The catabolic effects then prevail, resulting in weight loss. There was an increase ($p>0.05$) in serum triglyceride

in both non-diabetic control group (A) and non-diabetic treated with VA group (B). This may be due to environmental and nutritional factors. Figure 3 is the chart that shows significant reduction ($p<0.05$) in triglyceride level was observed in group D (diabetic treated with 40mg/kg of Vernonia amygdalina) and E (diabetic treated with 80mg/kg of VA). This may due to the presence of the phytochemical; tannin, phenol and saponin in Vernonia amygdalina. This result agrees with Aka et al. [20] which showed that aqueous leaf extract of VA has anti-hypertriglyceridemic effect on diabetic rats. There was no significant reduction in total cholesterol level of non-diabetic treated (group B) compared to non-diabetic untreated control (group A). Diabetes mellitus is associated with high levels of circulatory cholesterol and other lipids [22]), and this may be due to the nutritional and environmental factors. There was a significant reduction ($p <0.05$) in total cholesterol level of diabetic treated with (40mg/kg) of VA (group D) compared to the diabetic treated with the reference drug (5mg/kg/ of glibenclamide) which was marked on the day 21. This may be due to the presence of tannin, phenol and saponin in VA. The result of the present study agrees with previous reports that the level of total cholesterol decreased in alloxan-induced diabetic rats treated with VA aqueous leaves extract [23, 8].

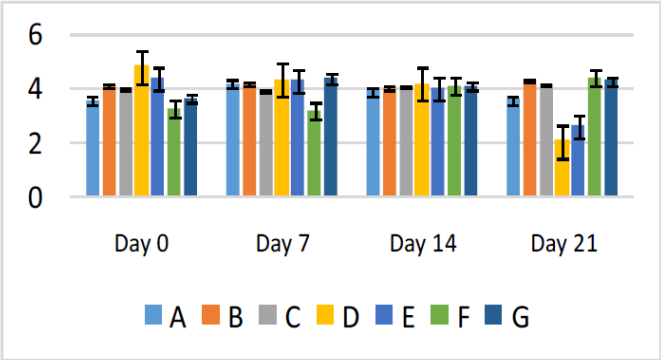

Figure 3: Effect VA aqueous leaves extract in serum triglyceride in diabetic and non diabetic rats.

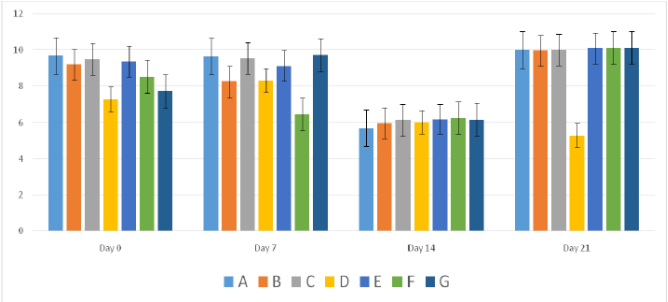

Figure 4: Effect of **VA** aqueous leaves extract in the total cholesterol level in diabetic and non diabetic rats versus the days.

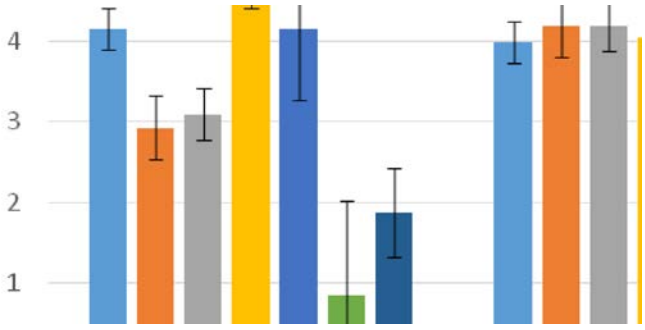

Fig.5: Chart showing the effect of **VA** aqueous leaves extract on High Density Lipoprotein in diabetic and non-diabetic rats**.**

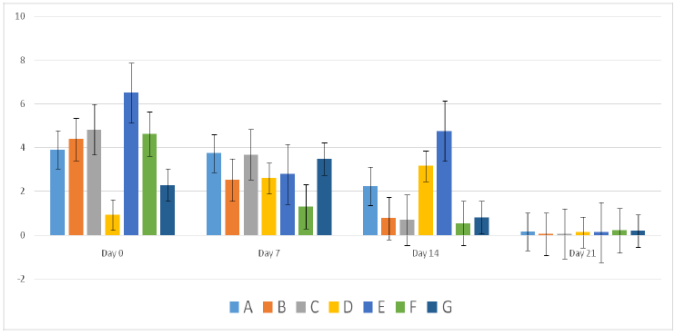

Fig.6: Chart showing the effect of **VA** aqueous leaves extract on Low density Density Lipoprotein in rats' diabetic and non-diabetic rats**.**

Figure 4 depicts the changes in the total cholesterol levels of the diabetic and the non- diabetic rats treated with aqueous leave extract of Vernonia amygdalina. There was a significant reduction ($p<0.05$) in total cholesterol level was noticed only in diabetic treated with (40mg/kg) of VA

(group D) compared to diabetic untreated (group C). This may be due to the presence of the phytochemical; tannin, phenol and saponin in VA. Our result is in agreement with Odoh et al [24]. A significant increase ($p<0.05$) in high density lipoprotein observed on day 7th and 21st in diabetic treated with (80mg/kg) of VA (group E) compared to diabetic treated with the reference drug (5mg/kg glibenclamide) and this may be due to the presence of phytochemical tannin and saponin in VA. There was a marked significant increase on (HDL) level of diabetic treated with (80mg/kg) and (40mg/kg) of VA (group E and D) compared to diabetic untreated (group C) on day 7th and 21st of the experiment though, the increment was more on group E (80mg/kg) of VA. Our finding is in line with Amakiri and Karibi [25]. There was no significant decrease in Low Density Lipoprotein cholesterol level of diabetic treated with Vernonia amygdalina compared to diabetic treated with the reference drug, the statistical analysis shows that there was no significant difference ($p>0.05$). It is documented that LDL-cholesterol level may be used in monitoring the treatment of patients with elevated blood cholesterol levels [26].This may be due to the nutritional and environmental factors. Our result was not in line with the finding of Kate et al [27] where he observed that VA extract reduced total blood cholesterol levels revealing a hypocholesterolemic tendency. It is now widely believed that an important signal for insulin secretion may be the link between glucose and lipid metabolism; and long-term exposure of islet cells to high levels of fatty acids may result in β-cell dysfunction (lipotoxicity) [28].

5. Conclusion

The findings from this study showed that aqueous extract of Vernonia amygdalina possess hypoglycemic, anti-hyperglycemic and lipid lowering activities. Result of the present study suggests that 80 mg/kg was the most potent dose at which Vernonia amygdalina extract demonstrated these activities **.**Since dyslipidemia occurs in most diabetic patients [28], the utilization of lipid-lowering agents is now advocated for diabetic treatment and the findings from this study suggests that Vernonia amygdalina could be useful in this regard and this study justifies the ethnomedicinal use of Vernonia amygdalina in the treatment of diabetes, though effort should be made to optimize the dose for better effectiveness.

6. Acknowledgement

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7. Conflict of Interest

The authors did not have any conflict of interest as at the time of the submission and publication of this article.

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