INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH & DEVELOPMENT

ISSN 2278 – 0211 (Online)

Effect of Vernonia Amygdalina Found on Alloxan Induced Diabetes Mellitus

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Abstract:
Vernonia amygdalina (VA) is a common shrub widely consumed in Nigeria. This study was done to investigate the effect of VA on alloxan induced hyperglycemia in Wister albino rats. Adult albino rats of Wister strain of both sexes weighing 200-250g were randomly allocated into 4 groups with five (5) rats per group. Group 1 was given normal saline without induction with alloxan to serve as the control while group 2, 3 and 4 were induced with alloxan 6.5mg/100gbody weight of alloxan monohydrate after the method of Osikwe et al., 2015. After induction of hyperglycemia, group 2 were given normal saline, group 3, 10mg/100g body weight of VA and group 4, 20mg/100g body weight of VA for a duration of 2 weeks. The result showed that the fasting blood glucose of group 1 was 81±2.8 mg/dl. The fasting blood glucose of group 2 was 242±8.3 vs295±9.5 mg/dl, group 3 was 304±3.8 vs 203±2.4 mg/dl and group 4 253±12.5 vs 247±22.9 mg/dl. There was a significant difference in basal blood sugar level after induction between group 2 and group 3, and between group 2 and 4 p<0.01. 10mg/100g of VA significantly reduced fasting blood glucose compared to diabetic control. There was also significant difference between group 2 and group 4 (p<0.05) however, there percentage change in fasting blood glucose after 10mg/100g VA and 20mg/100g of VA were 34.7% and 11.6% respectively. The fact that 20mg/100g VA exhibited 11.6% which was significantly less than 34.7% (p<0.01) showed that V.amygdalina exhibits saturation phenomenon.

Keywords: *Vernonia amygdalina, hyperglycaemia, fasting blood glucose, alloxan induced diabetes*

1. Introduction

Diabetes mellitus (DM) is now one of the most common non-communicable diseases globally (IDF, 2009). The term diabetes mellitus describes a metabolic disorder of multiple etiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (Kjems et al., 2001). The effects of diabetes mellitus include long–term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as polydipsia, polyuria, polyphagia, blurring of vision, and weight loss (Feldman 1998). In its most severe forms, ketoacidosis or a non–ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death. Often, symptoms are not severe, or may be absent, and consequently hyperglycemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made. The long–term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, Charcot joints, and features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease (Kjems et al., 2001).

Currently, available therapies for diabetes include “insulins, insulin secretagogues (sulfonylureas, meglitinides), insulin sensitizers (biguanides, thiazolidinedione), agents that enhance incretin secretion and action (incretin analogues, incretin mimetics, dipeptidyl peptidase IV (DPP-IV) inhibitors), agents that decrease gastrointestinal glucose absorption (alpha glucosidase inhibitors, alpha amylase inhibitors, sodium-glucose co-transporter (SGLT-1) selective inhibitors), agents that promote renal glucose excretion (sodium-glucose co-transporter (SGLT-2) inhibitors) and others (amylin analogue, bile acid sequesterants, bromocriptine) (Verspohl, 2012).

Presently, there is growing interest in herbal remedies due to the multimodal approach required in the management of diabetes mellitus couple with the cost, non availability, side effects associated with the oral hypoglycemic agents (Tiwari and Rao, 2002). Ethnobotanical and ethnopharmacological surveys report that more than 1200 plants are being used in many ethnic societies around the world in traditional medicine for their alleged hypoglycemic activity (Akah et al.,2002) and Vernonia amygdalina is one of them.

2. Materials Method

Fresh leaves of V. amygdalina were collected from the natural habitat in Makurdi, Benue State, Nigeria. The leaves were confirmed by a taxonomist from the Department of Botany in the Faculty of Science, Benue State University and was allocated a voucher number and deposited in the herbarium of the department.

Figure 1: Leaves of Vernonia Amygdalina Used in the Study

2.1. Preparation of Extract

The leaves were sorted out to obtain only the fresh leaves and washed with distilled water without squeezing to remove debris and dust particles. They were shade dried for ten days and then, the dried leaves were pulverized with LG electric grinding machine. A portion, (600 g) of the powdered leaves were soaked in 2400ml of 70% ethanol for 72hours with the solution thoroughly stirred twice daily. The extracts were then be filtered with WHATMAN no1 filter paper. The filtrate was air dried and then reconstituted with distill water later.

2.2. Chemicals

Alloxan monohydrate (Sigma, St Louis MO, USA) was used to induce diabetes in the rats.

2.3. Animals

Adult Wister albino rats weighing 200-250g of either sex were purchased from the disease-free stock of the animal house of the College of Health Sciences, Benue State University, Makurdi and used for the study. They were maintained in normal and standard laboratory conditions of temperature 28^{o}C and relative humidity (with 12-hour light dark cycle)and adequate ventilation. The animals were fed with commercial diet (Vital Feed Nig.Ltd.) and water *ad libitum*. Food was withheld 8 hours before the experiments, but they had free access to water. Permission for the use of animals and animal protocols were obtained from the Animal Ethics Committee of Benue State University Makurdi, prior to the experimentation.

2.4. Animal Categorization

The animals were allowed 14-day acclimatization period, after which they were randomly allocated into four groups of 5 rats per group: (n=5)

2.5. Induction of Diabetes

Diabetes was induced on group 2, 3 and 4 by intraperitoneal injection of alloxan monohydrate (6.5mg/100g) in normal saline (0.9%NaCl), Osikwe et al.,2015. 50% glucose solution was given to prevent initial hypoglycemia caused by alloxan. Diabetes was confirmed three days later in alloxan- induced animals showing fasting blood glucose (FBG) level ≥ 126 mg/dl by using glucometer to monitor the blood sample from the tail vein.

3. Animal Grouping and Experimental Design

The animals will be randomly allocated into 4 groups of 5 animals per group (n=5)

Group1: non diabetic control given normal saline

3.1. Alloxan Induced

Group 2: diabetic control given normal saline
Group 3: given 10mg/100g VA
Group 4: given 20mg/100g VA.

Group	Before Alloxan Fbs±Sem (Mg/Dl)	After Alloxan Fbs± Sem (Mg/Dl)	After N.Saline/Va Fbs±Sem (Mg/Dl)	% Change Fbs
1	81±2.8	-	-	-
2	81±2.1	242 ±8.3	295 ±9.5	23.4%
3	84±2.4	304 ±3.8	198 ±2.4	-34.7%
4	84±3.1	292 ±12.3	258 ±22.9	-11.6%

Table 1: Fasting Blood Glucose of Wister Albino Rats Before and After Induction with Alloxan and After Treatment with Vernonia Amygdalina
MEAN ±SEM n=5

Table1 shows the fasting blood glucose of Wister albino rats before induction. It can be seen that the fasting blood glucose of the rats were within the physiologic range and there was no significant difference between the groups are shown in table 2 (levene test of homogeneity). $p > 0.05$

Also, there was no significant difference in homogeneity of variance after induction with alloxan. Table 2, also showed that there was no significant difference between the groups before treatment, $p>0.05$

Test of Homogeneity of Variances of FBG of groups				
	Levene Statistic	df1	df2	Sig.
After N.saline/VA	2.495	2	12	.124
After induction with alloxan	1.090	2	12	.367
Before induction with alloxan	.851	2	12	.451

Table 2: Levene Test of Homogeneity of Variances in Fast Blood Glucose Levels among Groups

After Normal Saline/Va					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	21448.933	2	10724.467	10.345	.002
Within Groups	12440.400	12	1036.700		
Total	33889.333	14			

Table 3: Analysis Of Variance after Treatment of Alloxan Induced Diabetic Rat with Vernonia Amygdalina

Table 3 showed the Analysis of Variance (ANOVA) after treatment of diabetic rats in group 2, 3 and 4. The result shows that there was a significant difference between groups. $P <0.05$.
Turkey's post Hoc analysis was used to determine where the significant difference was.

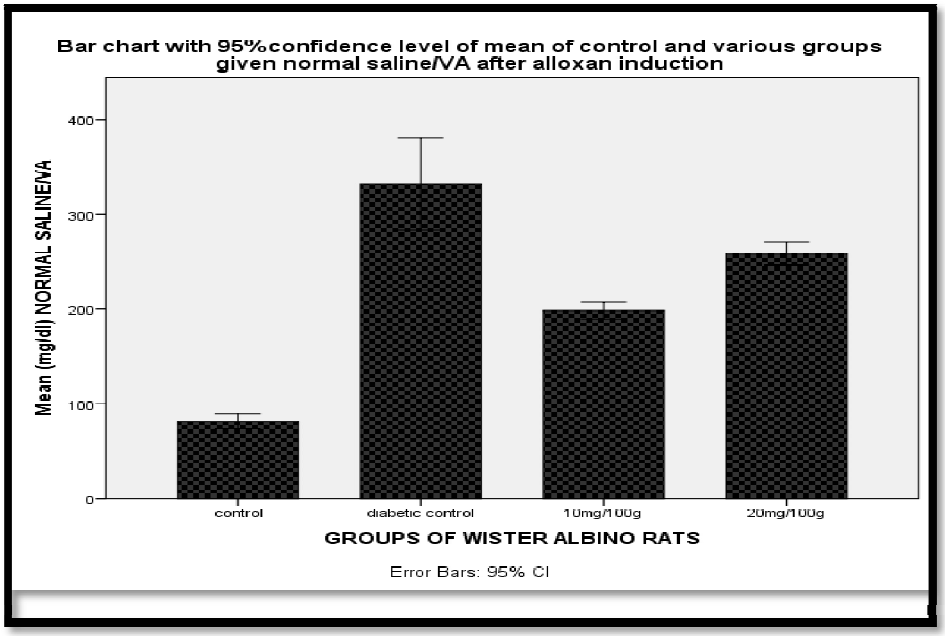

Figure 2

From fig 2, it can be seen from the bar chart that there was a significant difference between group 3 (treated with 10mg/100g of VA) compared to the control treated with normal saline.

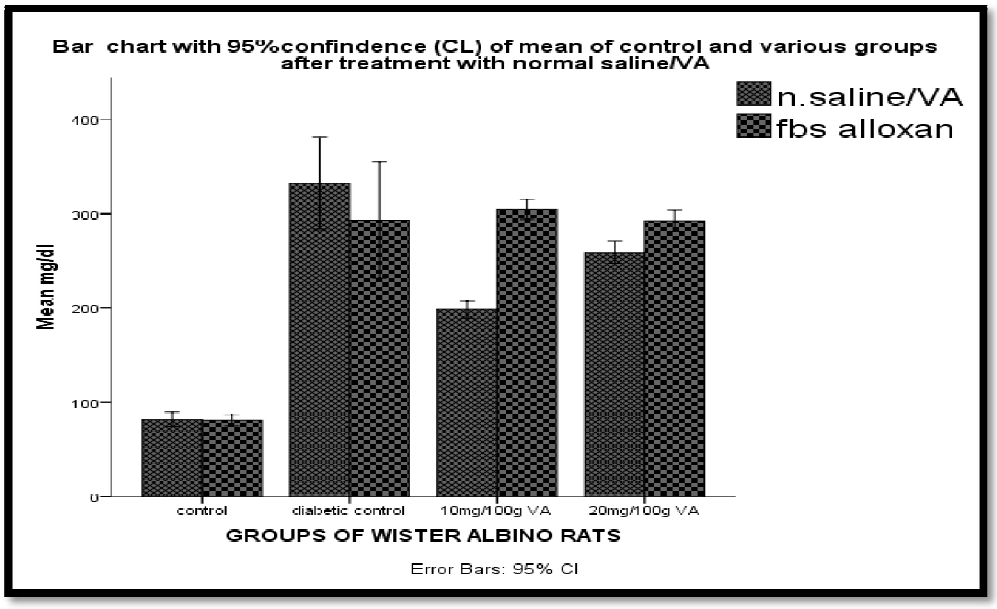

Figure 3

Fig 3 showed that there was further increase of fasting blood glucose of the diabetic control group given normal saline as diabetes deteriorate if not managed with hypoglycemic agent.

From fig 3, it can be seen that although group 3is significantly different from the group 2, there was however a significant difference between the fasting blood glucose between group 3 and group 1 after treatment as $p<0.01$.

Fig3 also showed that higher dose of VA offered no statistically significant advantage over 10mg/100g of VA. $P>0.05$ as it exhibit saturation phenomenon.

4. Discussion

The quest for good glycemic control to prevent microvascular and macrovascular complication (Min and Park2010) has led to the search of readily available option. From the present study Vernonia amygdalina seems to be one of such option.

Alloxan induced diabetes is known to be mediated through the generation of free radicals which destroys the pancreatic beta cell (Akah et al 2011). The generation of free radicals is also responsible for the oxidative stress that plays pivotal role in

the development of diabetes complication both microvascular and cardiovascular complication (Ferdinando and Michael, 2010). The increased super oxide production is central and major mediator of diabetes tissue damage (Ferdinando and Michael, 2010).

The result obtained from this study showed a significant ($P<0.01$) reversal of hyperglycaemia induced by alloxan by 10mg/100g of administered ethanol extract of Vernonia amygdalina. This agrees with the study of Udem et al.,2017 which stated that the maximum effective dose of semi purified Vernonia amygdalina of 100mg/kg possesses significant antihyperglycemic effect.

The reduction of fasting blood glucose by VA in alloxan induced diabetes is as a result of several active constituents that have been reported to be present in VA extract (Mukwaya et al.,2016). These phytochemicals include Saponins, Phenols, Tannins, Flavonoids, Terpenes and Glycosides (Mukwaya et al., 2016). It has been shown that flavonoids possess remarkable hypoglycemic effect (Cazarolli et al., 2008). This effect has been linked to its ability to impair glucose absorption and improve glucose tolerance (Cazarolli et al., 2008). Flavonoids have also been shown to be potent antioxidant agent that impair the generation of free radicals (El-Abhar, and Schaalan, 2014).

This study also showed that although there was a significant difference ($p<0.01$) between the alloxan control group and group treated 10mg/100g of VA, there was however no good glycemic control with VA. ($p>0.05$) compared to the fasting blood glucose before induction. This agrees with several studies (Udem et al,.2017; Mukwaya et al.,2016; Jide et al.,2013) that good glycemic control is achieved when combine with other hypoglycemic agents

The result from this study also showed that higher dose of VA offers no significant advantage over 10mg/100g of VA this agrees with Mukwaya et al., 2017 that stated that 100mg/kg of semi purified VA is the maximum effective dose of VA in diabetic use.

The fact that 20mg/100g VA did not offer improvement on the glycemic condition after alloxan induction compared to 10mg/100g indicates that VA at higher dose exhibits saturation phenomenon.

5. Conclusion

Vernonia amygdalina significantly reduces fasting blood glucose of alloxan induced diabetes mellitus in Wister albino rat but did not achieve good glycemic control alone. To achieve good glycemic control, Vernonia amygdalina should be use with other appropriate antihyperglycemic agents.

Recommendation: Need for public Health Education

- There is need to create awareness among the Nigerian public about the practical application and usefulness of the findings in the present study.
- Health Education with regard to the finding that Vernonia amygdalina (bitter leave) will be useful to patients with Diabetes mellitus should be pursued vigorously.
- The general public should be made to realize that too much bitter leave will be less useful since this study shows Saturation phenomenon.

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