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Amerliorative Effect of Vernonia Amygdalina in Dexamethasone Induced Hyperglycaemia

Christian Onahinon
Co-Researcher, Department of Human Physiology, Benue State University, Makurdi, Nigeria
Dr. Julie Ibu
Chief Inspector, National Youth Service Corps, Nigeria
Dr. Nndunno Akwaras
Senior Registrar, Federal Medical Centre, Makurdi, Nigeria
Dr. Emmanuel Eru
Lecturer, Department of Physiology, Benue State University, Makurdi, Nigeria
Dr. Adugba Augustine
Lecturer, Department of Physiology, Benue State University, Makurdi, Nigeria
Samuel Odeh
Lecturer, Department of Physiology, Benue State University, Makurdi, Nigeria
John Ibu
Lecturer, Department of Physiology, Benue State University, Makurdi, Nigeria

Abstract:
Hyperglycaemia has been found to be one of the main side effects associated with dexamethasone administration. Vernonia amygdalina(VA)-bitter leave is a common shrub consumed in Nigeria. This study was carried out to investigate the ameliorative effect of vernonia amygdalina on dexamethasone induced hyperglycaemia. Twenty (20) adult Wistar albino rats of both sexes weighing 200-250g were randomly allocated into four (4) groups with five (5)animals per group. Group 1 was given normal saline without inducing hyperglycaemia in the group to serve as normoglycaemic control. Animals in group 2, group 3 and group 4 were given prestandardised dose of 1mg/kg body weight dexamethasone for seven days according to the method of Ghaisas et al., 2009. After induction of hyperglycemia, group 2 were given a daily dose of 1mg/kg body weight of dexamethasone with normal saline, group 3., daily dose of 1mg/kg body weight of dexamethasone with 100mg/kg body weight of VA and group 4 daily dose of 1mg/kg body weight of dexamethasone with 200mg/kg body weight of VA. The result showed that the fasting blood glucose of group 1 was 81±2.8 mg/dl. The fast blood glucose of group 2 was 137±15.4 vs 156±20.3mg/dl, group 3 was 129±9.7vs 90±2.7mg/dl and group 4 was 126±7.3 vs 86±4.2mg/dl. There was a significant difference in the fasting blood glucose of group 1compared to group 2, group 3 and group 4 ($p<0.01$) after induction of hyperglycaemia ($p<0.01$). There was no significant difference between group 1 and group3, group1 and group 4($p>0.05$) after VA was co administered with dexamethasone. This shows that Vernonia amygdalina ameliorate dexamethasone induced hyperglycaemia.

Keywords: *Vernonia amygdalina, dexamethasone induced hyglycaemia, fasting blood glucose*

1. Introduction

Diabetes mellitus one of the most common non-communicable diseases globally (IDF, 2013) is a complex and chronic metabolic disorder characterized by a sustained hyperglycaemia with a fasting blood glucose ≥ 126mg/dl, with disturbances of carbohydrate, fat, protein metabolism, high oxidative stress induced by the generation of highly reactive free radicals (Sharma et al.,2010) which result from defects in insulin secretion, insulin action, or both (Kjems et al., 2001). Non insulin dependent diabetes mellitus accounts for more than 90% of diabetic population. The aetiology of type 2 diabetes mellitus in which the b-cells of pancreas are usually functional, very often involves hormonal imbalance (Roith et al., 2000). Hormones such as catecholamine, glucagon, cortisol and thyroxin, either through directly or through their influence on other hormones, affect carbohydrate metabolism to elevate blood glucose level leading to insulin resistance. Insulin resistance has been shown to be present in conditions like Type-II Diabetes, obesity and dyslipidemia. In fact, the hormones of the adrenal cortex are well

known for their diabetogenic effects and are responsible for most steroid diabetes (Gholap and Kar.,2003). Dexamethasone is a synthetic glucocorticoid with anti-inflammatory and immunosuppressant properties. However, long-term use of dexamethasone results in deleterious side effects such as hyperglycemia, hepatosteatosis, and insulin resistance (Vegiopoulos and Herzig., 2007). With these drawback, strategy to achieve therapeutic effect of dexamethasone without its hyperglyceamic adverse effects could be to combine the administration of a dexamethasone with another agent (not targeting glucocorticoid receptor) that can mitigate the gluconeogenic side effects of dexamethasone without altering its therapeutic activity. Also, due to the etiopathogenesis of diabetes mellitus, harmful side effects of synthetic drugs, the inability of existing modern therapies to control all the pathological aspects of the diabetic disorder, enormous cost of modern drugs as well as the poor availability of the advanced therapies for many rural populations in developing countries (Tanaka et al., 1992), alternative strategies to current pharmacotherapy of diabetes mellitus are urgently needed. 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 and could attenuate dexamethasone induced hyperglycaemia.

2. Materials and 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 was 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.

2.2.1. Chemicals

Dexamethasone injection (Alpha pharmaceutical LTD)

2.2.2. Animals

Adult albino rats of Wister strain weighing 200-250g of either sex was 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.

2.2.2.1. Animal Categorization

The animals were allowed 14-day acclimatization period, after which they were randomly allocated into four groups of 4 rats per group: (n=5)

Induction of hyperglycemia.

Hyperglycaemia was also be induced in group 2-4 by daily administration of a prestandardized dose of dexamethasone (1 mg/kg) for 7 consecutive days (Ghaisas et al., 2009). Then group 2, 3 and 4 received continuous dose of dexamethasone with extract and/or placebos.

2.2.2.2. Animal Grouping and Experimental Design

The animals were randomly allocated into 4 groups (n=5)

Group 1 normoglycaemic control
Group 2 continuous daily dose of 1mg/kg+normal saline
Group 3 continuous daily dose of 1mg/kg +100mg/kg of VA
Group 4 continuous daily dose of 1mg/kg +200mg

3. Results

Group	Before Dexamethasone FBS±SEM (Mg/Dl)	7days After Dexamethasone FBS± SEM (Mg/Dl)	After N.Saline/VA FBS±SEM (Mg/Dl)	% Change FBS
1	81±2.8	-	-	-
2	73±2.6	137 ±15.4	156 ±20.3	13.9%
3	77±4.1	129 ±9.7	90 ±2.7	-25.6%
4	77±2.4	126 ±7.3	86 ±4.2	-31.8%

Table 1: Fasting Blood Glucose of Wistar Albino Rats before and 7 Days after Givin Dexamethas One and 14days after Giving Dexamethasone with Normal Saline/Va Extract

Table 1 shows the fasting blood glucose of wistar albino rats before administration of dexamethasone. The fasting blood glucose of the rats used falls within physiological range and there was no significant difference within and between groups as shown in table 2 (Levene test of homogeneity) $p>0.05$ Table 1 also showed the fasting blood glucose of the rats after they were given1mg/kg body weight of dexamethsone. There was a significant increase in the fasting blood glucose of the rats ($p<0.05$). After induction of hyperglycaemia, there was no significant difference in homogeneity of variance ($p>0.05$).

Table 1 also showed the fasting blood glucose of the hyperglycaemic rats after receiving dexamethasone with normal saline/VA extract.

Test of Homogeneity of Variances				
	Levene Statistic	df1	df2	Sig.
FBS before Dex	1.021	3	16	.410
FBS after dex	1.700	3	16	.207

Table 2: Test of Homogeneity of Variances

Table 2 Levene test of homogeneity of Variance in the fasting blood glucose before administration of 1mg/kg body weight of dexamethas one and 7days after daily administration of dexamethas one. There was not significant difference in homogeneity before and after induction of hyperglycaemia.

ANOVA					
FBS after DEX+AGENTS					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	18941.600	3	6313.867	11.439	.000
Within Groups	8831.600	16	551.975		
Total	27773.200	19			

Table 3: Analysis Of Variance after Giving Dexamethasone Induced Hyperglycaemic Wistar Abino Rats Co-Administration of Dexamethasone with Normal Saline/Va Extract

From table 3, there was a significant difference in the fasting blood glucose between group 2, 3 and 4($p<0.01$). Turkey Post Hoc test was used to determine where the significant difference was.

Homogeneous Subsets FBS after DEX+AGENTS				
	Groups of Wistar albino rats	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	Normoglycemic control	5	80.6000	
	20mg/100g VA	5	86.2000	
	10mg/100g VA	5	89.8000	
	Diabetic control	5		156.2000
	Sig.		.924	1.000

Table 4: Turkry Homogeneous Subsets of the Fasting Blood Glucose of Dexamethasone Induced Hyperglycaemic Wistar Abino Rats Co-Administration of Dexamethasone with Normal Saline/Va Extract

Means for Groups in Homogeneous Subsets Are Displayed

a. Uses Harmonic Mean Sample Size = 5.000

From the table there was a significant difference between group 2 and group1, group 3 and group 4 ($p<0.01$). There was no significant difference in fasting blood glucose between group 1, group 3 and group 4 ($p>0.05$)

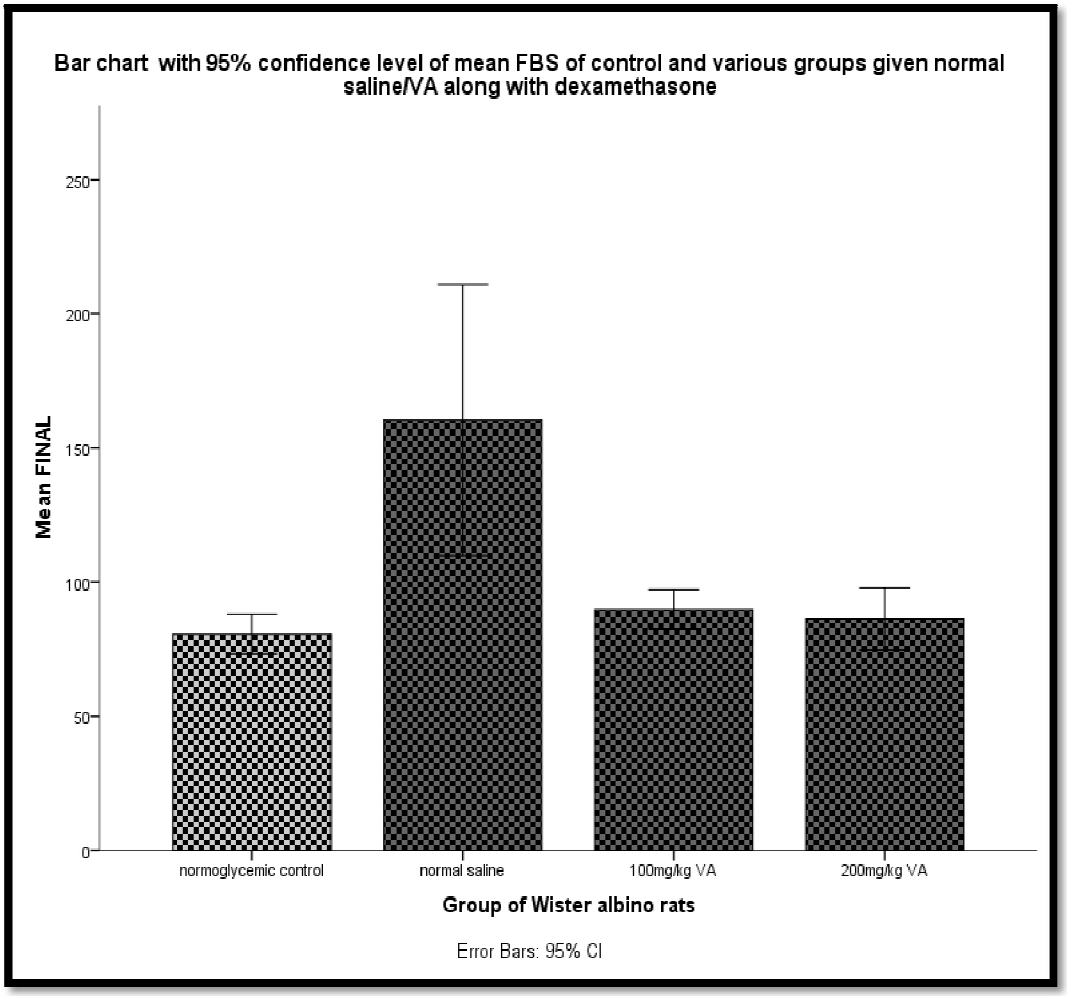

Figure 2

The bars in figure 2 showed the significant difference between the dexamethasone+normal saline group and dexamethasone +10mg/100g VA group and dexamethasone+20mg/100g VA group.

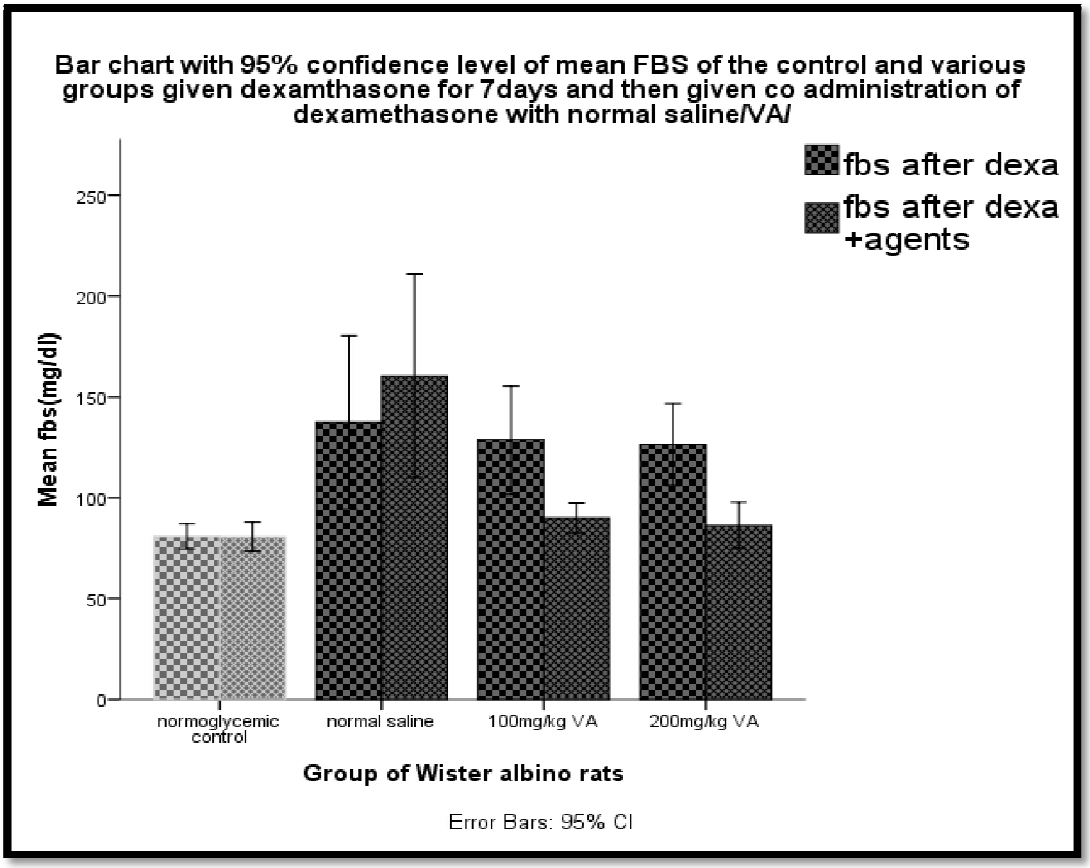

Figure 3

Figure 3 showed that there was further increase fasting blood glucose of the diabetic control group given normal saline. This implies that continuous administration of dexamethasone without appropriate antihyperglycaemic agent increases the fasting blood glucose.

Figure 3 shows that 10mg/100g VA and 20mg/100gVA ameliorate the hyperglycaemic effect of dexamethasone. There was no significant difference between 10mg/100g VA and 20mg/100g VA ($p>0.05$).

4. Discussion

The result from this study showed that dexamethasone significantly increased fasting blood glucose of Wistar rats after seven (7) days ($p<0.01$). This was also reported by Gholap and Kar., 2005; Ghaisas et al., 2009. There was further increase in fasting blood glucose after 14days with continuous administration of dexamethasone which showed that administration of dexamethasone without appropriate agent to ameliorate its diabetogenic effect worsened insulin resistance .This agrees with the work of Robert et al.,1999 who demonstrated that Prolonged glucocorticoid exposure is associated with development of severe insulin resistance and metabolic dysfunction. Glucocorticoids have been shown to increase hepatic gluconeogenesis, decrease peripheral glucose uptake into muscle and adipose tissue, breakdown of muscle and fat to provide additional substrates for glucose production which is as a result of insulin resistance, caused by the alteration in binding of insulin to its receptor (receptor defect) or by the impairment of the intracellular response to insulin (post receptor defect) (Gholap & Kar, 2005).

However, continuous co-administration of Vernonia amygdalina with dexamethasone to hyperglycaemic Wistar rats after 14 days resulted in a significant decrease in fasting blood glucose($p<0.01$) and ameliorated dexamethasone induced hyperglycaemia. There was no significant difference between the euglycaemic group and the group given co- administration of dexamethasone with Vernonia amygdalina. There was no significant difference between 100mg/kg VA and 200mg/kg VA ($p>0.05$).

Vernonia amygdalina have been shown to possess antihyperglycamic effect (Akah et al., 2002). VA have also been shown to reverse oxidative stress imposed on pancreatic beta cell by alloxan (Owolabi et al.,2011). The ameliorating effects of VA in dexamethasone induced hyperglycaemia could be 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 hypoglycaemic 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). Flavonoid have also been shown to be potent antioxidant agent that impair the generation of free radicals (El-Abhar, and Schaalan, 2014).

Therefore, the result of this study indicates that Vernonia amydalina may serve as a prophylactic treatment of insulin resistance (the hallmark of type 2 diabetes) and may prevent the development of diabetes in prediabetic patients and prevents the development of secondary complications.

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