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Original Research Article

Effect of *Vernonia amygdalina* and *Balanites aegyptiaca* Aqueous Leaves Extracts on Blood Glucose Level and Lipid Profile of Streptozotocin Induced Diabetic Rats

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Abstract: This study evaluated the effect of combined treatment of aqueous extracts of *Vernonia amygdalina* and *Balanites aegyptiaca* leaves on blood glucose and lipid profile in streptozotocin induced diabetic rats. Twenty four (24) Wistar male rats weighing 100-150g were randomly distributed into four groups. The first group served as the normal control while the remaining three groups were induced with diabetes using streptozotocin at 60 mg/kg body weight. Group two served as diabetic control and the remaining groups were treated with the combined extracts and *V. amygdalina* alone at 500 mg/kg body weight respectively. The rats were treated orally once daily for 28 days. At the end of the experimental period, blood glucose level and lipid profile were measured. The result showed that there was a significant reduction ($p<0.05$) in the blood glucose level of all the treated groups compared to the diabetic control. The results also showed that HDL-C was significantly increased ($p<0.05$) in all treated groups compared to the untreated diabetic group. It is concluded that aqueous extracts of *V. amygdalina* and *B. aegyptiaca* leaves possess strong hypoglycemic activity with very low antihyperlipidemic activity.

Keywords: *Balanites aegyptiaca*, Blood Glucose Level and Lipid Profile *Vernonia amygdalina*.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycaemia and glycosuria due to absolute or relative lack of insulin. It is also defined by the World health organization (WHO), defined diabetes as a fasting venous plasma glucose concentration greater than 7.8 mmol/l (140mg/dl) or greater than 11.1 mmol/l (200mg/dl) two hours after a carbohydrate meal or two hours after an oral ingestion of the equivalent of 75g glucose, even if the fasting concentration is normal [1]. The prevalence of diabetes is increasing rapidly worldwide; about 422 million people have diabetes [1]. The World Health Organization has predicted that this number will be doubled in the next twenty years. There are two types of diabetes. Type 1 diabetes is an auto-immune disorder in which the immune system wrongly destructs beta cells of the pancreas resulting in insulin deficiency and subsequently, high blood glucose. Type 2 diabetes results from inadequate insulin secretion and/or inefficient action of insulin. Dyslipidemia is one of the most common complications of Diabetes mellitus. It plays a significant role in the development of premature atherosclerosis, coronary insufficiency and myocardial infarction [2]. Lipid profile abnormalities in diabetes are mediated through derangements in a variety of regulatory processes, especially insulin deficiency, thereby rendering diabetic patients more prone to hypercholesterolemia and hypertriglyceridemia [3].

Treatment of hyperlipidemia in diabetes involves improving glycemic control, getting exercise

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and using lipid-lowering diet and drugs [4]. At present, dyslipidemia is most commonly treated with lipid-lowering drugs, some of which are associated with serious adverse side effects. Thus, the effects of dietary components on plasma lipid metabolism have recently received considerable attention, highlighting the importance of natural products as lipid metabolism regulators [5].

Balanite aegyptiaca is a tree known as 'desert date' and its distributed in drier parts of Africa and South Asia. It contains a wide variety of compounds such as essential amino acids, saponins, flavonoids, alkaloids and carbohydrates [6]. *B. aegyptiaca* has been used in the Egyptian folk medicine as anti-diabetic agents. Aqueous extract of fruits showed spermicidal activity without local vaginal irritation in human being, up to 4% sperms becoming sluggish on contact with the plant extract and then immobile within 30 s; the effect was concentration-related. Protracted administration of the fruit pulp extract produced hyperglycemia-induced testicular dysfunction in dogs. The seed is used as expectorant, antibacterial, and antifungal. Fruit is used in whooping cough, also in leucoderma and other skin diseases. Bark is used as spasmolytic [7].

V. amygdalina (bitter leaf) belongs to the family Astreraceae which has a characteristic feature of bitterness in taste. It is a small shrub that can grow up to three meters and native to tropical Africa. The plant is also well distributed in Asia and more commonly found near drainage line and in natural forests [8]. The leaves of the plant are used for making soup and stew in tropical Africa and majorly used ethno-botanically for the treatment of different ailments [9]. *V. amygdalina* is a medicinal plant used in folk medicine for treatment of several diseases. The bitter taste of *V. amygdalina* can be reduced by boiling or soaking in water [10]. Several bioactive alkaloid saponins and tannins are present in the plant leaves. These bioactive components made out of *V. amygdalina* leaf acts as an antimicrobial agent in brewing industries [11]. Several ailments such as fever, kidney problems, hiccups, and stomach discomfort can be treated ethno-medically using extracts of *V. amygdalina* leaves and roots [10]. The plant has also an amazing antiparasitic efficacy in zoo pharmacognosy as it is easily recognized and used for self-medication by parasitized chimpanzees [12].

MATERIALS AND METHODS

Collection and Identification of Plant Sample

The leaves of *Vernonia amygdalina* and *Balanites Aegyptiaca* were obtained within Dutsin-ma Local Government, Katsina State, Nigeria. They were authenticated at the Department of Biology, Federal University Dutsin-ma, Katsina State.

Experimental Animal

Twenty four male (24) Albino Wistar rats weighing 100g to 150g were purchased from Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna. The animals were housed in hygienic wooden cages and maintained under standard environmental conditions in the animal facility of Federal University Dutsin-ma, Katsina State. They were allowed to acclimatize for two weeks and fed with standard pellet and water *ad libitum*.

Preparation of the Aqueous Leaves Extract

The leaves of *Vernonia amygdalina* and *Balanites Aegyptiaca* were washed with distilled water and left to air dry and then ground to powder using a mortar and pestle. 200g of the powdered samples each were soaked in distilled water and then filtered. Dose corresponding to 500mg/Kg body weight prepared.

Diabetes Induction

Diabetes was induced using streptozotocin at 60mg/kg body weight. The streptozotocin was reconstituted in normal saline in 1g: 10ml. The induction was carried out using insulin syringe and hyperglycemic condition was confirmed by fasting blood glucose level which doubles the basal value.

Experimental Design

The 24 Wistar rats were randomly distributed into four (4) groups. Each group was treated as follows:

- **Group I:** Normal control rats, fed with normal feed only
- **Group II:** Diabetic control rats, fed with normal feed only
- **Group III:** Diabetic treated rats treated with *Vernonia amygdalina* and *Balanites Aegyptiaca* (DTB_iBa500mg body weight) combined aqueous leaf extracts.
- **Group IV:** Diabetic treated rats treated with bitter leaf (DTBi500mg body weight) only.

Administration of Extracts

The plant extracts (DTB_iBa 500mg/kg body weight) and (DTB_i 500mg/kg body weight) were administered to the various group test respectively. Feed and water were withdrawn prior to administration of the extract and restored after. The rats were observed for death or change in behavior throughout the experiment. Administration of the extracts were done once daily for 28 days using the gavage.

Collection of Blood Sample for Analysis

The animals were sacrificed at the end of the administration period using chloroform as partial anesthetic. Blood sample was then collected through the abdominal aorta using 5 ml syringe into plain containers. The blood sample was centrifuge at 3000 rpm for 15 minutes after which the serum was kept in clean plain container for analysis of the lipid profile (i.e., total cholesterol, triglyceride, LDL, and HDL levels).

Determination of the Biochemical Parameters

Determination of the Blood Glucose Level

The blood samples used to check for glucose level were collected from the tip of the tail of the rats a using glucometer.

Determination of Lipid Profile

The assay of lipid profile was determined using automated machine at Federal Medical Centre Katsina.

Statistical Analysis

The results are expressed as means ± SEM of six independent determinations. The means were compared by one-way ANOVA followed by Duncan post hoc test. Value of P less than 5% ($P<0.05$) was considered statistically significant.

RESULTS

Blood Glucose Level

The result of the fasting blood glucose is presented in Table 4.1 below. The result shows a significant increase ($p<0.05$) in fasting blood glucose in diabetic group as compared to the basal throughout 4 weeks after induction. Administration of the combined extract (DTBiBa500mg) reduced fasting blood glucose of the treated rats from week 1 to week 4 as compared to the diabetic control.

Table 1: Effect of combined *Vernonia amygdalina* and *Balanite aegyptiaca* aqueous leaves extract on blood glucose level of streptozotocin-induced diabetic rats

Blood Glucose Level					
Groups	**Basal**	**Week 1**	**Week 2**	**Week 3**	**Week 4**
NC	77.33 ± 4.07^{ab}	79.17 ± 4.50^{ab}	66.67 ± 5.16^{a}	66.67 ± 5.16^{a}	84.17 ± 7.20^{b}
DC	75.83 ± 3.59^{a}	268 ± 39.98^{bc}	254.67 ± 24.72^{bc}	312.83 ± 20.87^{c}	213.67 ± 34.73^{b}
Grp iii	70.33 ± 2.33^{a}	232 ± 40.16^{b}	181.33 ± 23.90^{b}	193.67 ± 46.36^{b}	$156.50 \pm$
Grp vi	64.67 ± 4.06^{a}	140.67 ± 11.13^{b}	118.83 ± 31.71^{ab}	96.67 ± 5.78^{ab}	80.33 ± 0.33^{a}

Values are expressed as are means ± SEM (n=6). Values in the same column with different superscript represent significant differences ($p<0.05$) with the basal. SEM- Standard error of mean, NC- Normal control, DC- Diabetic control, Grp A- Diabetic group treated with combined aqueous extract of Bitter leaf and *Balanite aegyptiaca*, Grp B- Diabetic group treated with aqueous extract of Bitter leaf.

Serum Lipid Profile

Table 4.2 shows the results of the serum lipid profile of the combined aqueous extracts of *Vernonia amygyladina* and *Balanite aegyptiaca* on steptozotocin induced diabetic rats. The result show that there was a significant increase ($p< 0.05$) in total cholesterol (T-Chol),) and high density lipoprotein cholesterol (HDL-C) in the diabetic group compared to the normal control. However, there was no significant difference in triglyceride (TG) and low density lipoprotein cholesterol (LDL-C) between the diabetic group and the normal control. The result also revealed that there were no significant difference in T-chol, TG and LDL-C in rats treated with combined aqueous extracts (DTBiBa500mg) and *Vernonia amygdalina* (DTBi500mg) alone as compared to the diabetic control. However, there was a significant increase in HDL-C in the treated groups compared to the diabetic control, with DTBi500mg been significantly lower than DTBiBa500mg.

Table 4.2: Effect of combined *Vernonia amygdalina* and *Balanite aegyptiaca* aqueous leaves extract on lipid profile level of streptozotocin-induced diabetic rats

Serum lipid profile (mg/dl)				
Groups	**T-CHOL**	**TG**	**HDL-C**	**LC**
NC	144.17 ± 1.14^{a}	64 ± 1.69^{a}	33.17 ± 1.14^{a}	94.5 ± 3.55^{a}
DC	164.5 ± 2.35^{b}	78.5 ± 8.38^{ab}	41.5 ± 0.92^{b}	88.67 ± 8.53^{a}
Grp iii	165.67 ± 8.29^{b}	94.33 ± 7.17^{b}	61.33 ± 2.40^{d}	82.00 ± 5.00^{a}
Grp iv	161.33 ± 4.10^{b}	89 ± 7.37^{b}	52.33 ± 1.45^{c}	98 ± 0.58^{a}

Values are expressed as means ± SEM (n=6). Values in the same column with different superscript represent significant differences ($p<0.05$) with the respective controls (diabetic and normal control). T.cho l- total cholesterol, TG- triglyceride, HDL-C- High density lipoprotein cholesterol, LDL-C- Low density lipoprotein cholesterol.

DISCUSSION

Traditional antidiabetic plants might provide a useful source of new oral hypoglycemic compounds for development as pharmaceutical entities, or as simple dietary adjuncts to existing therapies [13]. Diabetes induced by streptozotocin can induced specific necrosis of pancreas and reduced synthesis of insulin. Usually, cells of pancreas normally maintain blood glucose concentrations within a narrow range by modulating their insulin secretion rate in response to the glucose concentrations; apoptosis of pancreatic cells is believed to be the primary factor which ultimately results in hyperglycemia [14]. Thus, the results of the glucose level (Table 4.1) showed that there was a significant increase in the glucose level of the diabetic induced animals compared to those of Normal Control. Significant decrease in blood glucose level was observed in the group treated with combined aqueous extract compared to the Diabetic Control. Improvement of the diabetic conditions in rats treated with the extracts is possibly due

to recovered endocrine pancreatic tissue at both structural and functional levels. This can lead to elevated insulin level and improved insulin sensitivity that lowers the concentration of glucose in blood. Insulin inhibits hepatic glucose production, stimulates both of glucose uptake and metabolism by muscle and adipose tissues and increases liver glycogen content [15].

In addition to the antidiabetic properties of these plants, each of the plants have been reported of different activity geared towards alleviation of complication usually associated with diabetes. The findings of this present study also confirmed that *Vernonia amygdalina* extract alone reduce blood glucose level significantly ($p<0.05$) in steptozotocin induced diabetic rats. This can be attributed to the bioactive molecules present in the indigenous plant. This report is in accordance with that of Donatus *et al.,* [16], which reported the antihyperglycaemic effect of *Vernonia amygdalina* on streptozotocin induced diabetic rats. According to Ukpabi *et al.,* [15], Vernonia amygdalina is rich in alkaloid, tannins, saponins, flavonoids and glycosides. Secondary metabolites of plants such as the ones listed above possess some alpha-glucosidase inhibitors and competitively inhibit intestinal brush border enzymes with an eventual reduction in digestion and absorption of carbohydrates from the gut-postprandial hyperglycemia, hence resulting in an effective glucose control [17]. The hypoglycemic effect observed in the present study could be due to depression of key gluconeogenic enzymes or the increase in the levels of glucose transport and stimulation of uptake in peripheral tissues [18]. It could as well be that these plant extracts may have the potential of preserving the cells of islets of langerhans, which in turn result in an increase in insulin activity [19].

Diabetes mellitus can lead to fatty liver, hypercholesterolemia and hypertriglyceridemia. Moreover, elevated cholesterol levels are associated with diabetic nephropathy. From the results of the lipid profile (Table 4.2), it was also observed that there was a significant increase ($p<0.05$) T-CHOL level of the diabetic untreated group. Treatment with plant extracts resulted in significant increase in HDL-C. This is in accordance with newly published studies of Ajiboye *et al.,* [20]. Increase in HDL-C could be due to the effect of cuisine a component of *V. amygdalina*, which reduces low density lipoprotein cholesterol while also boosting good high density lipoprotein cholesterol [21]. However, no significant difference was observed in Tchol, TG and LDL-C levels in treated groups in this study. One of the possible reasons for this may be that the extract does not possess a strong antihyperlipidemic activity. The concentration of active principles at the given doses might not be capable in controlling hyperlipidema. The active principles in the extract might take time to reach sufficient concentration at the target site.

CONCLUSION

This study has demonstrated that the combined aqueous extracts of the *Vernonia amygdalina* and *Balanite aegyptiaca* has a strong antihyperglycemic property by lowering serum blood glucose levels of streptozotocin induced diabetic rats. However, its antihyperlipidemic activity is low. Though, *Vernonia amygdalina* extract alone is more effective than the combined extract, may be there is need to increase the extract doses of the combination.

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