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Evaluation of antidiabetic effect of ethanol extract of *Phaseolus vulgaris* SEEDS ON alloxan-induced Wistar diabetic rats

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Abstract

This work was designed to evaluate the anti-diabetic efficacy of ethanol extract of *Phaseolus vulgaris* seeds in normal and in alloxan -induced diabetic rats. The blood glucose level of the rats was checked before the administration of alloxan using One Touch Glucometer and test strips. The rats were then fasted for 16 hours, but with free access to water after which they received an intraperitoneal injection of alloxan 200 mg/kg body weight. The rats were orally given 20 ml each of 75% glucose solution after 3 hours to prevent hypoglycemia. The animals were allowed free access to food and water after alloxan administration. After 48 hours of the alloxan administration, blood was collected orbito rectally and their glucose level was checked using One Touch glucometer and test strips. The rats were divided into six groups containing five rats each. The groups were administered 150 mg, 250 mg and 350 mg doses of ethanolic extract of *Phaseolus vulgaris seed* per kg body weight respectively. Administration in all instances was by gavage using intubation cannular. These treatments were repeated for five consecutive days. To a positive control group of five rats was administered 5 mg of glibenclamide (a standard anti-diabetic drug) per kg body weight for five consecutive days. Another group of five rats used as negative control did not receive any treatment. The non-diabetic group received neither alloxan nor the extract. The 24-hour acute toxicity test of the orally administered ethanolic extract of *Phaseolus vulgaris* seeds showed that the extract is non-toxic because no death was recorded. Antidiabetogenic potential of the extract was investigated by pretreatment of two groups of rats with 350 mg/kg of the extract for one and two weeks respectively. The result showed that all the doses were significantly ($P<0.05$) effective in reducing blood sugar level of alloxan induced hyperglycaemic rats when compared to the control diabetic rats that were treated with glibenclamide (a standard antidiabetic drug). However, the best reduction in blood glucose level was observed with ethanolic extract at a dose of 150 mg/kg$_{bw}$. The antidiabetogenic groups showed a significant reduction($P<0.05$) in the glucose levels post induction of Alloxan. These results suggest that the seed extract of *Phaseolus vulgaris* may possess hypoglycemic effect and could be of benefit in the treatment and management of diabetes mellitus, controlling the blood sugar as well as in preventing or delaying the onset of diabetes mellitus.

Keywords: Alloxan; Hyperglycemia; *Phaseolus vulgaris;* Glibenclamide

1. Introduction

Diabetes mellitus is a chronic condition in which a person has a high blood sugar level, either because the body does not produce enough insulin, or because body cells do not properly respond to the insulin that is produced. Diabetes mellitus

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is a serious metabolic disease which has several complications including diabetic nephropathy, diabetic neuropathy, coronary heart disease and hypertension (Boll,1963). . The manifestation of diabetic nephropathy is associated with a poor prognosis of affected patients (Chattopadhyay,1999).

Type 1 diabetes results from the body's failure to produce insulin and requires the person to inject insulin. It is partly inherited and triggered by certain infections. Type 1 diabetes mellitus is also called Insulin Dependent Diabetes Mellitus (IDDM). The majority of Type 1 diabetes is the immune-mediated nature, where beta cells are lost due to T-cells mediated autoimmune attack (Chattopadhyay,1998).

Type 2 diabetes mellitus also called Non-insulin Dependent Diabetes Mellitus (NIDDM) or adult-onset diabetes is a metabolic disorder that is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency. Unlike type I diabetes mellitus, there is very little tendency toward ketoacidosis though it is not unheard of (Fimognari *et al*., 2006). Gestational diabetes mellitus occurs when a pregnant woman who have never had diabetes before, have a high blood glucose level during pregnancy. Gestational diabetes usually results after delivery (Daisy *et al*., 2009). About 20-50% of affected women develop type II diabetes later in life (Eshrat, 2003) .

Consumption of calorie rich diet, obesity and sedentary life style have lead to tremendous increase in the number of diabetics worldwide especially in Asia (Klein, *et al*., 2007). Patients with diabetes mellitus are more likely to develop and die from microvascular complications than the non diabetic population (Neil, 2003). In extreme cases, circulation of limbs is affected, potentially requiring amputation. Loss of hearing, eyesight, and cognitive ability has also been linked to this condition (Chopra,1956).

Though pharmaceutical drugs like sulfonylureas (chlorpropamide, glibenclamide, glipizide, glyburide, micronase, tolazamide, tolbutamide), biguanides (metformin), meglitinides, thiazolidinediones, alpha glucosidase inhibitors, dipeptidyl peptidase inhibitors and ergot alkaloids are used for the treatment of diabetes, these are either too expensive or have undesirable side effects or contra indications (Rang, *et al*., 1991). In recent years, there has been renewed interest in plant medicine (Ladeji, *et al*., 2003) and plant-based therapies Hypoglycaemic effect was observed with *Phaseolus vulgaris* when given as a seed extract, in normal as well as diabetic rabbits.(Chattopadhyay,1999)

The use of medicinal plants to prevent and treat diabetes mellitus successfully over the years has attracted the attention of scientists globally. In the rural communities, many people depend solely on medicinal plants for the treatment of diabetes due to its easy accessibility, affordability and availability even when the efficacy of the herbal remedy has not been established (Andrews, 2001). Many traditional plant treatments for diabetes mellitus are used throughout the world and some of these plants have been assayed while a good number of them are yet to receive scientific scrutiny (Agunu, *et al*., 2005). *Phaseolus vulgaris* is one of the most versatile medicinal plants having a wide spectrum of biological activity. In the rural area, the traditional healers make use of the seeds of *Phaseolus vulgaris* to treat diabetes. Scientific reports also support the hypoglycemic activity of *Phaseolus vulgaris* seeds (Klein, *et al*., 2007).

2. Materials and Methods

2.1. Determination of median lethal dose (LD50) of *Phaseolus vulgaris*

The Median Lethal Dose (LD_{50}) was determined using Wistar Albino Mice. Test animals were randomly divided into three (3) groups of three mice each and administered graded doses of 10 mg,100 mg,1000 mg of ethanolic extract of *Phaseolus vulgaris* per kg body weight respectively. When there was no mortality recorded, another three groups of one mice each were administered with an increased dose of 1600 mg, 2900 mg and 5000 mg. The *Phaseolus vulgaris* extract was administered by gavage using an intubation canular and were monitored for 24 hours for changes in behaviour and mortality, however no mortality was recorded.

2.2. Investigation of anti-diabetic properties of ethanolic extract of *Phaseolus vulgaris* seeds on alloxan induced wistar rats

The blood glucose level of the rats was checked before the administration of alloxan using One Touch Glucometer and test strips. The rats were then fasted for 16 hours, but with free access to water after which they received an intraperitoneal injection of alloxan 200 mg/kg body weight (Etuk, 2010). The rats were orally given 20 ml each of 75% glucose solution after 3 hours to prevent hypoglycemia. The animals were allowed free access to food and water after alloxan administration. After 48 hours of the alloxan administration, blood was collected orbito rectally and their glucose level was checked using One Touch glucometer and test strips. Diabetes was confirmed to have been induced when the glucose level was observed to be far much higher than normal (above 140mg/dl).

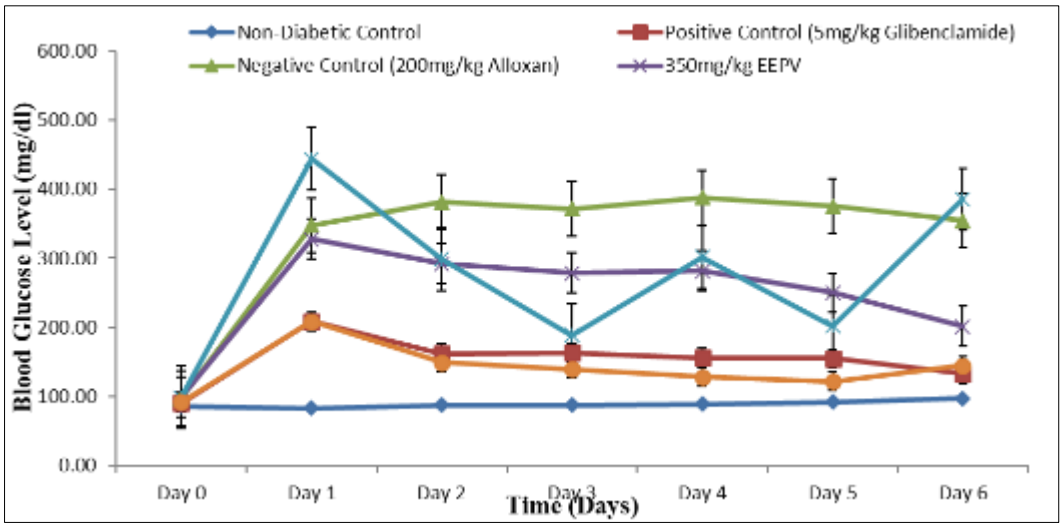
The diabetic rats were divided into six groups each containing five rats. To one group was administered 150 mg/kgbw, 250 mg/kgbw and 350 mg/kgbw doses of ethanolic extract of *Phaseolus vulgaris seed* per kg body weight respectively. Administration in all instances was by gavage using intubation cannular. These treatments were repeated for five consecutive days. To a positive control group of five rats was similarly administered 5 mg of glibenclamide (a standard anti-diabetic drug) per kg body weight for five consecutive days. Another group of five rats used as negative control did not receive any treatment.

2.3. Determination of the anti-diabetogenic effect of extract

Exactly 350 mg/kg of the ethanol seed extract was administered to two groups of rats by gavage using intubation cannular. These groups were administered the extract for one week and two weeks respectively. Consequently diabetes was induced after pretreatment. Blood glucose levels of rats were determined before the administration of the extract and consistently for the one and two weeks of repeated dose before induction of diabetes. After treatment with alloxan the blood glucose level was again determined to establish to what extent the extracts prevented onset of diabetes.

3. Results

3.1. Blood Glucose Levels



(Data represented as Mean ± SEM)

Figure 1 Blood glucose profile of treatment with 5 mg/kg glibenclamide, 150 mg/kg EEPV, 250 mg/kg EEPV and 350 mg/kg EEPV per kg body weight following the induction of diabetes using 200 mg/kg Alloxan

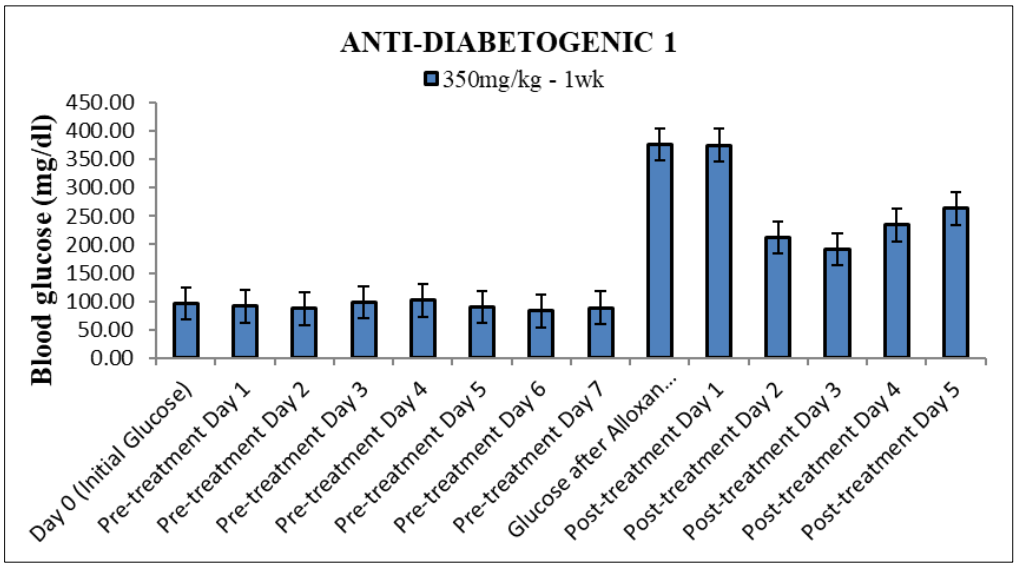


Figure 2 Blood glucose profile of the test groups prior to and after diabetes induction (Data represented as Mean values± S.E.)

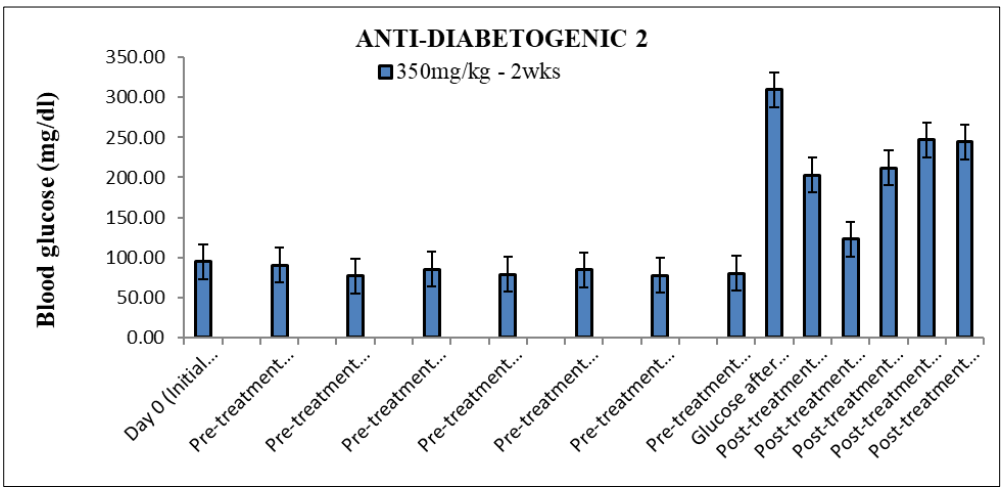


Figure 3 Blood glucose profile of the test groups prior to and after diabetes induction. (Data represented as Mean values± S.E.)

4. Discussion

Diabetes mellitus (DM) is the commonest endocrine disorder that affects more than 100 million people worldwide (6% of the population) and in the next 10years it may affect about five times more people than it does now (Abdullahi *et al*., 2001) WHO Expert Committee on diabetes encourages further investigation into traditional methods of treatment and also emphasizes the need to ensure safety and quality control of ingredients used. The presence of abnormal high blood glucose level is the criterion on which the diagnosis of of diabetes is based. The therapeutic goal of treatment within each type of diabetes is to normalize insulin activity and blood glucose levels in an attempt to reduce the development of the vascular and neuropathic complications. The acute toxicity study of the orally administered ethanolic extract of *Phaseolus vulgaris seed* showed that the extracts are not toxic, the extent of hypoglycemic effect varied considerably among different groups of rats given different doses ethanolic extract of *Phaseolus vulgaris* seed.

Throughout the treatment days, ethanolic extract of *P. vulgaris* at 350 mg/kg showed reductions in glucose levels but was not statistically significant ($p>0.05$) when compared to the negative control group. The reduction in the glucose level by the extract at the dose of 350 mg/kg was consistent which could be as a result of dose dependency. At 250

mg/kg of the ethanolic extract of *P.vulgaris,* there was a reduction from the first day of treatment till the last day but was not statistically significant, the extract had a sharp reduction on day three and day five of the same dose which proved the extract had a better effect on day three and five while there was a consistent and statistically significant reduction ($p>0.05$) of the extract at 150 mg/kg when compared to the negative control group. This result proved that the ethanolic extract of *P.vulgaris* had the best effect at 150 mg/kg and its glucose level reduction was dose dependent.

From figure 3, the result of the anti-diabetogenic studies of *P.vulgaris* showed that the extract had anti-diabetogenic properties as the animals did not respond strongly to diabetic induction after treatment with 350 mg/kg of the extract. The groups showed a significant reduction($P<0.05$) in the glucose levels post induction of alloxan.

5. Conclusion

The results of this study indicate that the ethanolic extract of *Phaseolus vulgaris* seeds may be useful for the treatment of diabetes. Also the study has shown that the ethanolic extract of *Phaseolus vulgaris* seeds were very safe at the biologically active doses.

Although the mechanism of action was not investigated, a number of plants were found to possess hypoglycemic effects and the possible mechanism suggested for such hypoglycemic actions could be through the increased insulin secretion from β-cells of islets of Langerhans or its release from bound insulin. That is to say such hypoglycemic effects of plant extracts could also be due to their insulin like actions.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The Department of Biochemistry Ethical Review Committee, Ebonyi State University, Abakaliki, Nigeria, approved the study design and assigned approval Number.

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