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EVALUATION OF ANTILIPIDEMIC POTENTIALS OF *PHASEOLUS VULGARIS* ON ALLOXAN-INDUCED DIABETIC RATS

A. R. Nwaji [*1], C. U. Igwe [2], E. R. Neife [1], E. O. Ngwu [3] and U. A. Inwang [1]

Department of Physiology [1], College of Medical Sciences, Alex Ekwueme Federal University, Ndufu-Alike, Ebonyi, Nigeria.
Hospital Services Unit [2], Ministry of Health, Kingdom of Saudi Arabia.
Department of Environmental Health [3], Faculty of Public Health, University of Ibadan, Ibadan, Nigeria.

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Correspondence to Author:
Mr. Azubuike Raphael Nwaji

Department of Physiology,
College of Medical Sciences,
Alex Ekwueme Federal University,
Ndufu-Alike, Ebonyi, Nigeria.

E-mail: zubbynwaji@yahoo.com

ABSTRACT: Healthy diet has been known to play a key role in managing and preventing diseases. The health benefits of consuming common beans (*Phaseolus vulgaris*) are widely acknowledged and are ascribed mostly to their high dietary fiber contents and polyphenols. Various animal experimental studies have shown that polyphenol in common beans has antioxidant properties and other biological activities. This study was designed to evaluate the antilipidemic potentials of Phaseolus vulgaris on experimentally-induced diabetic rats. 20 male rats of the Wistar strain were divided into 4 groups (n=5). Group A was the normal control; group B was the diabetic control; while groups C and D were diabetic and treated with 400mg and 800mg/kg body weight of aqueous extract of *Phaseolus vulgaris* seed, respectively. Blood samples were collected on the last day of the experiment for biochemical assay. Results showed a significant increase ($p<0.05$) in total cholesterol (TC), triglyceride (TG), and low-density lipoprotein (LDL-c) levels of diabetic rats when compared to the control group. Treatment of diabetic rats with *Phaseolus vulgaris* showed a significant reduction in the TG and very low-density lipoprotein (VLDL-c) levels. However, there were no significant changes in the TC, high-density lipoprotein (HDL-c), and LDL-c when compared with the control and diabetic control. In conclusion, the results of this study showed that aqueous extract of *Phaseolus vulgaris* seed exhibited hypolipidemic effect on serum TG and VLDL-c but not on HDL-c, LDL-c and TC of the alloxan-induced diabetic rats. Thus, may not be concluded to have antilipidemic potential.

INTRODUCTION: Diabetes mellitus, a group of metabolic diseases characterized by lipid abnormalities and increased blood levels of glucose, has been reported to result from defects in insulin secretion, insulin action, or both [1, 2].

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Prevalence has been increasing steadily all over the world and as a result of this trend; it is gradually becoming an epidemic in some countries [3].

Diabetic dyslipidemia is a term used to describe the pathophysiology surrounding the effects of insulin resistance on abnormal lipid levels. Dyslipidemia in diabetes commonly manifests as raised low-density lipoprotein cholesterol (LDL-c), decreased high-density lipoprotein cholesterol (HDL-c) levels, or elevated triglyceride (TG) levels [4]. Ozder, [5] reported widespread lipid abnormalities in the

course of diabetes-triggered dyslipidemia. Man has always looked for a way to fight and control disease, and a lot of remedies have been used by man. A healthy diet has been known to play a key role in preventing and managing cardiovascular disease and type 2 diabetes. Various scientific studies reveal that nearly every foodstuff, including herbs and spices, potion, and their nutritional components in addition to bioactive substances, impacts the outcome of chronic diseases, the risk associated with them, and the overall health status of an individual [6].

Legumes have been reported to contain bioactive molecules that represent multiple interests exploited in different domains. For instance, the Common beans (*Phaseolus vulgaris*) is a legume and contains polyphenolic compounds and oligosaccharides known for their beneficial effects on health [7-10]. Diaz-Batalla *et al.* [11] also reported that beans contain other phytochemicals such as saponins, phytates and phenols. The benefits of bean in relation to health are widely recognized and are attributed mainly to their dietary fiber content [9]. Various animal experimental studies have shown that common bean polyphenol possesses antioxidant properties and has various other biological activities, including anti-diabetic, cardioprotective, and others.

Studies have also suggested that the beans diet reduces low-density lipoprotein (LDL), increases high-density lipoprotein (HDL) levels, and positively affects risk factors for metabolic syndrome, thus decreasing the risk of cardiovascular diseases, obesity, and diabetes [12]. A lot of research has also shown the occurrence of dyslipidemia in a patient with diabetes. Hence, there is a need to investigate the antilipidemic properties of Polyphenol enriched common beans. Therefore, this study was designed to evaluate the antilipidemic potentials of Phaseolus vulgaris on experimentally-induced diabetic rats.

MATERIALS AND METHODS:

Collection and Identification of Seed: Healthy packs of the common beans (*Phaseolus vulgaris*) were bought from local markets at Ikwo in Ebonyi State, Nigeria. A botanist did seed identification and authentication at the Department of Biology, Alex Ekwueme University Ndufu-Alike.

Extraction of the Seed: The seeds were cleaned and selected from unwanted materials. Bad seeds were promptly removed by hand picking, and the good seeds were shade dried under the sun, and then crushed into powder using a grinding machine. About 50g of the powder was crammed within the thimble and extracted using water (500mL) as solvent (aqueous extract) for sixteen hours, and with the help of a rotary evaporator at reduced targeted pressure and temperature of 60±10ºC. The desired amount of extract was attained. The extract was kept at the freezer and used for the study.

Experimental Animals: Twenty (20) adult male rats of the wistar strain, about eight weeks old, weighing between 140-200g obtained from the Animal Breeding Unit of the Department of Physiology, Faculty of Basic Medical Sciences, Alex Ekwueme Federal University Ndufu-Alike, were used as the experimental animals. The rats were kept in cages for two weeks and allowed to acclimatize in the Animal House of the Department of Physiology, Faculty of Basic Medical Sciences and were allowed free access to food and water *ad libitum.* The protocol was in line with the guidelines of the National Institute of Health (NIH Publication 85-23, 1985) for laboratory animal care and use.

Induction of Diabetes: Following two weeks of acclimatization, a freshly prepared solution of alloxan was injected intraperitoneally to the experimental rats at a 160mg/kg body weight at a fasting state. Blood samples were collected from the tail vein after three days of induction of diabetes. Blood glucose concentration was analyzed using a glucose meter (Accu-answer ®, India) before the commencement of the administration of the extract. The alloxan-treated rats with evidence of hyperglycemia (fasting blood glucose level >200mg/dL) were considered diabetic and included in the experiment.

Experimental Design: The animals were randomly distributed into four groups (A B, C and D) of five animals each, as shown below. The supplemented diet was stored in an air-tight refrigerated container and then dispensed to the rats daily. The study lasted for 14 days. During this period, each animal received normal rat chow daily and had unrestricted access to water.

Grouping of Experimental Animals:

Groups	Treatment
A (Control)	Received normal rat chow
B (Diabetic control)	Diabetes rats (not treated)
C (Treated)	Diabetic rats + 400 mg/kg body weight of *Phaseolus vulgaris*
D (Treated)	Diabetic rats + 800 mg/kg body weight of *Phaseolus vulgaris*

Sample Collection and Analysis: After the treatment period, the rats were fasted overnight, anesthetized, and bled by cardiac puncture. The blood sample was transferred to serum separator tubes, allowed to clot, and centrifuged for 10 minutes at 3000 rpm.

The sera were carefully removed and placed into clean and appropriately labelled sample containers and stored frozen until the time of biochemical analysis.

Biochemical Assay: Total cholesterol and HDL–c were analyzed by automatic analyzer using a Randox commercial diagnostic kit. The TG was by Teco diagnostic kits per the procedures described in the manufacturer's operation manual.

VLDL and LDL were estimated using Friedewald's equation as reported in our previous work [1]:

$$VLDL = TG/5(mg/dl)$$

$$LDL = TC - (HDL+VLDL)$$

All the laboratory analysis was performed at the Clinical Chemistry Laboratory Department of Alex Ekwueme Federal University Teaching Hospital Abakaliki (AE-FUTHA) Ebonyi State, Nigeria.

Statistical Analysis: The graph pad prism version 7.0 software was used for the data analysis. One-way analysis of variance (ANOVA) and student's t-test was used to compare the mean difference across the groups. All the data were presented as mean ± SEM with a p-value <0.05 considered statistically significant.

RESULTS:

Effect of *Phaseolus Vulgaris* on Lipid Parameters of Diabetic Rats: The effect of *phaseolus vulgaris* on Total Cholesterol (TC), Triglyceride (TG), High-Density Lipoprotein cholesterol (HDL-c), Very Low-Density Lipoprotein cholesterol (VLDL-c), Low-Density Lipoprotein cholesterol (LDL-c) level of diabetic rats are shown in **Table 1** and **Fig. 1-5** below.

All the values given are expressed as mean ± SEM. Asterisks or hash signs in the table and bar charts indicate the values significantly different from the control values or treatment group of all the measured variables.

Fig. 1 shows the effect of *Phaseolus vulgaris* on TC level. There was a significant ($p<0.05$) increase in the TC level of the diabetic rats. However, no significant difference was observed between the control and the treated groups.

As indicated in **Fig. 2** and **4**, there was a significant increase in the TG and VLDL-c of the diabetic rats compared with the control. However, there was a significant reduction in the TG and VLDL-c levels of rats following treatment with 400 and 800mg/kg of the extract of *Phaseolus vulgaris* when compared with the diabetic group.

Oral administration of *phaseolus vulgaris* extract had no significant effect ($p<0.05$) on the HDL-c and LDL-c levels of the experimentally diabetic rats, as shown in **Fig. 3** and **5.**

TABLE 1: EFFECT OF *PHASEOLUS VULGARIS* ON LIPID PARAMETERS OF DIABETIC RATS. VALUES ARE EXPRESSED AS MEAN ± SEM (N=5)

	Parameters				
Groups	TC (mg/dL)	TG (mg/dL)	HDL-c (mg/dL)	VLDL-c (mg/dL)	LDL-c (mg/dL)
A (Normal control)	58.3±6.3	41.1±4.6	57.2±5.4	10.7±2.9	8.8±16.3
B (Negative control)	90.1±5.7**	114.7±22.5**	54.7±8.1	22.9±4.5*	12.5±11.1
C (Diab + 400mg/kg)	74.8±6.8	53.3±14.7#	58.6±4.8	8.8±1.0#	7.4±11.7
D (Diab + 800mg/kg)	74.2±10.5	43.9±4.8#	66.8±2.8	8.2±0.9##	-19.2±8.7

Key: n=5; ## P<0.01, # P<0.05 Vs Diabetic ctrl; **p<0.01, *p<0.05 Vs Normal ctrl. TC- total cholesterol, TG- triglyceride, HDL-c-high density lipoprotein cholesterol, VLDL-c- very low-density lipoprotein cholesterol, LDL-c- low-density lipoprotein cholesterol.

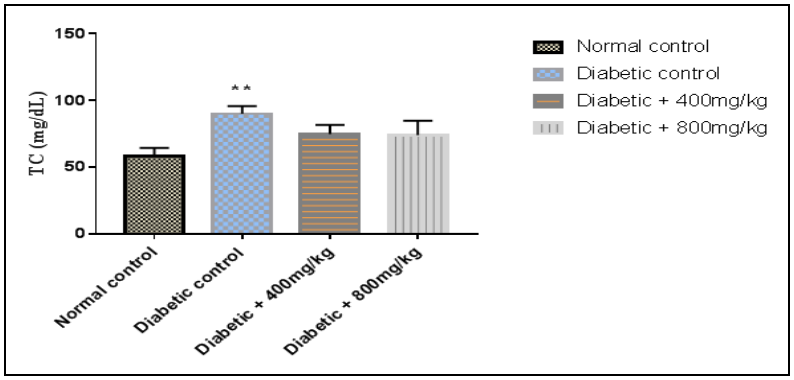

FIG. 1: SHOWING THE TOTAL CHOLESTEROL LEVEL OF NORMAL CONTROL, DIABETIC GROUPS AND THE DIABETIC GROUPS TREATED WITH DIFFERENT DOSES OF *PHASEOLUS VULGARIS* (N=5; **P<0.01).

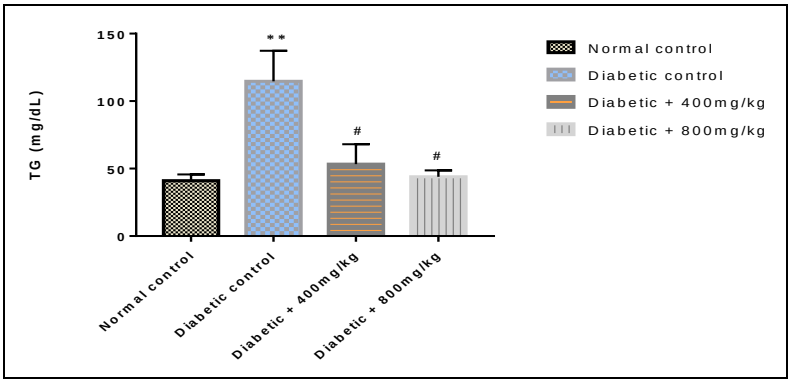

FIG. 2: SHOWING THE TRIGYCERIDE LEVEL OF THE NORMAL CONTROL, DIABETIC GROUPS AND THE DIABETIC GROUPS TREATED WITH DIFFERENT DOSES OF *PHASEOLUS VULGARIS* (N=5; **P<0.01 VS CTRL; #P<0.05 VS DIABETIC CTRL).

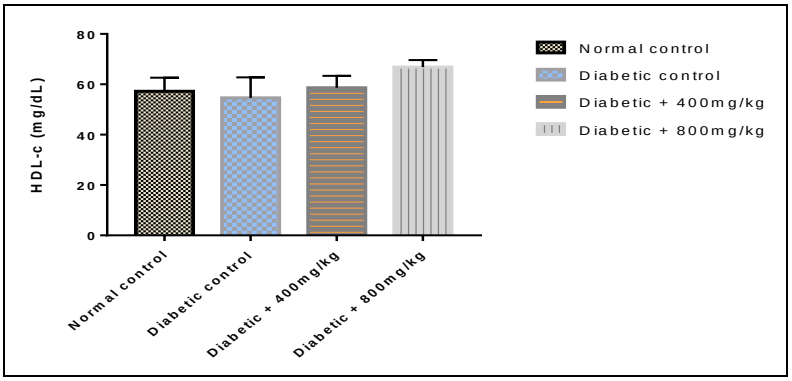

FIG. 3: SHOWING THE HIGH DENSITY LIPOPROTEIN CHOLESTEROL LEVEL OF NORMAL CONTROL, DIABETIC GROUPS AND THE DIABETIC GROUPS TREATED WITH DIFFERENT DOSES OF *PHASEOLUS VULGARIS* (N=5).

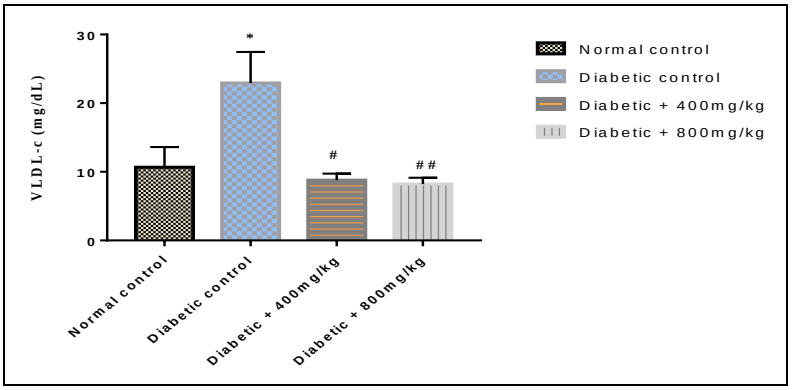

FIG. 4: SHOWING THE VERY LOW DENSITY LIPOPROTEIN CHOLESTEROL LEVEL OF NORMAL CONTROL, DIABETIC GROUPS AND THE DIABETIC GROUPS TREATED WITH DIFFERENT DOSES OF *PHASEOLUS VULGARIS* (N=5; *P<0.05 VS CTRL; #P<0.05, ##P<0.01 VS DIABETIC CTRL).

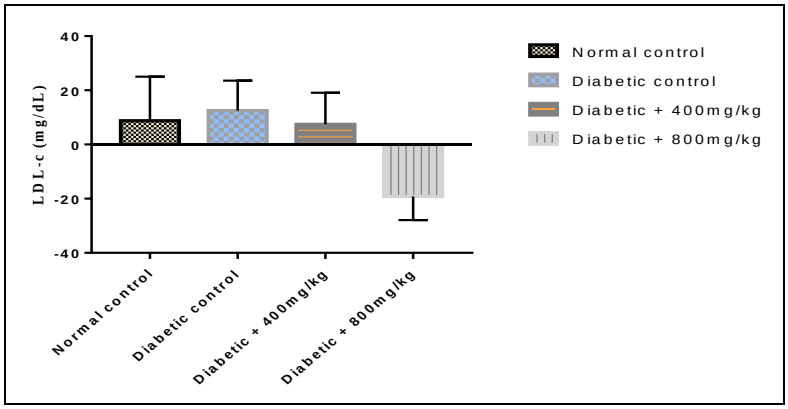

FIG. 5: SHOWING THE LOW-DENSITY LIPOPROTEIN CHOLESTEROL LEVEL OF NORMAL CONTROL, DIABETIC GROUPS AND THE DIABETIC GROUPS TREATED WITH DIFFERENT DOSES OF *PHASEOLUS VULGARIS* (N=5).

DISCUSSION: *Phasolus vulgaris* is increasingly gaining attention as a food with nutraceutical properties, due to its rich variety of Phytochemicals which have a potential benefit on health and also in the prevention of cardiovascular and metabolic diseases [13-16]. It has been used to improve diabetic complications due to its low glycemic index and phytochemical contents [17]. Oral administration of an aqueous or ethanolic extract of *Phasolus vulgaris* pods has been reported to have hypoglycemic and hypolipidemic effects. In this case, the aqueous extract increases insulin levels and decreases the activity of gluconeogenic enzymes of the liver. It has also been reported to increase the levels of antioxidant enzymes [18, 19]. In the present study, we investigated whether the *Phasolus vulgaris* extract has any effect on the lipid profile of alloxan-induced diabetic rats.

The results of this study showed an increase in the TC, TG and LDL-c levels of diabetic rats. However, treatment of diabetic rats with *Phaseolus vulgaris* showed a reduction in the TG and VLDL-c level in a dose-dependent manner. This is consistent with Egbuna *et al.* 20. It also agrees partly with Diego *et al.* [15] who reported that induction of diabetes increases cholesterol, LDL and about a 50% increase in the TG levels of the diabetic group when compared to the healthy animals. In contrast, a decrease of about 22 % TG levels was observed following treatment. However, Diego *et al.* [15] reported a reduction of 29.9% TC and 56.1% LDL after being fed the bean diet. The results of this study also show no changes in the TC, HDL-c and LDL-c of the diabetic rats following treatment with the extract compared with the control and diabetic control. This finding agrees with the reports of Liu *et al.* [21] who observed no differences in the plasma lipid profile when high-fat-fed rats were treated with brown beans. Egbuna *et al.* [20] also reported no difference in treated rats' mean values of TC and HDL. Luka *et al.* [22] reported that the extract of *Phaseolus vulgaris* improved total cholesterol level of diabetes rats at the administered dosage and significantly reduced the level of TG when compared to the normal control group. This agrees partially with our findings. This finding is also partly in pact with the reports of Diego *et al.* [15] who observed no difference in the HDL levels between the healthy, preventive-treatment and diabetic groups. But disagrees in part where he reported that HDL (a "healthy lipoprotein"), increased in the treatment group. A slight increase with no statistical significance has also been reported [20]. This result partly does not agree with Bazzano *et al.* 23's report, which established that kidney bean is a very good source of cholesterol-lowering fiber. Fibre has been known to help reduce fat- cholesterol levels. The cholesterol-lowering effect of dietary fiber has been attributed to its ability to inhibit the intestine from absorbing bile acids, steroids, and the total excretion of steroids [24]. These variations observed may be related to different bean species, geographical location, the dose of the extract used, and the duration of treatment.

CONCLUSION: The results of this study showed that the aqueous extract of *Phaseolus vulgaris* seed exhibited hypolipidemic effect on serum TG and VLDL-c but not on HDL-c, LDL-C, and TC of the alloxan-induced diabetic rats. Thus, it may not be

concluded to have antilipidemic potential. However, this study needs further research to include studies at the cellular level to determine the mechanisms of improvement in the lipid profile more specifically.

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