



EFFECT OF AQUEOUS EXTRACT OF PHASEOLUS VULGARIS L. (RED KIDNEY BEANS) ON ALLOXAN-INDUCED DIABETIC WISTAR RATS

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ABSTRACT

Aqueous extract of Phaseolus Vulgaris L. (Red Kidney Beans) was investigated for its effects in alloxan induced-diabetic rats. Twenty four albino rats were randomly allocated into four groups (A-D) of six rats each such that group A (diabetes control) received 0.5 mL distilled water, group B (diabetes) received 400 mg/kg bwt of extract, group C (normal control) received 0.5 mL of distilled water while group D (normal) received 400 mg/kg bwt of extract, all extract were orally administered once daily for 14 days. Diabetes was induced in groups A&B by single interperitoneal injection of 150 mg/kg alloxan monohydrate. Phytochemical screening indicated the presence of alkaloids, balsam, flavonoids, saponins, tannins, cyanogenic glycosides, terpenes and steroids. The hypoglycaemic potential of Phasoelus vulgaris L. was expressed in diabetes treated rats. Blood glucose, total protein, albumin and cholesterol levels of the diabetes treated rats and normal treated rats were not significantly ($p>0.05$) altered when compared with the control rats. However, these values were significantly ($p<0.05$) increased in diabetes untreated rats. Aqueous extracts of Phaseolus vulgaris L. at 400 mg/kg body weight also significantly reduce ($p<0.05$) the values of ALT, AST and ALP when compared to high values of the enzymes observed in diabetes untreated rats. Extract had no significant ($p>0.05$) effects on PCV and Hb in all groups when compared to the normal control. The study showed that the aqueous extract of Phasoelus vulgaris L. leaves possess hypoglycaemic, antidiabetic properties and ameliorating the high levels of marker enzymes observed in diabetes untreated rats.

Keywords: Phaseolus vulgaris, Diabetes, Antidiabetic potentials, Marker enzymes, Phytochemicals.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases in which a person has high blood sugar, either because the pancreas does not produce enough insulin, or because cells do not respond to the insulin that is produced. Diabetes mellitus is classified into four broad categories; type 1, type 2, gestational diabetes and "other specific types".The "other specific types" are a collection of a few dozen individual causes [1].Diabetes mellitus type 1 is a form of diabetes mellitus that results from autoimmune destruction of insulin-producing beta cells of the pancreas[2]. The classical symptoms are polyuria (frequent urination), polydipsia (increased thirst), xerostomia (dry mouth), polyphagia (increased hunger), fatigue, and weight loss[3]. Diabetes mellitus type 2 is a metabolic disorder that is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency[4]. The classic symptoms are excess thirst, frequent urination, and constant hunger. The development of type 2 diabetes is caused by a combination of lifestyle and genetic factors [5]. There are a number of medications and other health problems that can predispose one to diabetes [6]. Some of the medications include: glucocorticoids, thiazides, beta blockers, atypical antipsychotics[7]. Onset of type 2 diabetes can be delayed or prevented through proper nutrition and regular exercise [8].

Phaseolus vulgaris L. (Red Kidney Beans), have a notable place in the folklore throughout the world and in the traditions of many cultures such as its antidiabetic activity [9][10][11]. Raw kidney beans contain the toxinphytohaemagglutinin, which is destroyed by boiling for at least ten minutes [12]. The kidney bean seed is a diuretic and hypotensive,preclinical investigations have unanimously reported how the acute, repeated administration of extracts of *Phaseolus vulgaris* L, as well as some of their isolated ingredient reduced food intake, body weight and lipid accumulation in lean and obese laboratory animals. Several authors are keen in the interesting pharmacological activity of the leaves of *Phaseolus vulgaris* [13][14]. Currently, the data on antidiabetic properties and bioactive constituent of *Phaseolus vulgaris* are limited in open literature. Hence, the present study was undertaken to determine the phytochemical constituents of aqueous extract of *Phaseolus vulgaris* L. leaves (Red Kidney Beans) and also to study its effects on some biochemical and haematological parameters in alloxan induced-diabetic wistar rats.

MATERIALS AND METHODS

Chemical and Reagents:

Alloxan monohydrate used was products of Sigma Chemical Company, St. Louis, USA. Cholesterol, triglycerides, glucose, total protein and albumin assay kits used were produced by Randox Laboratories Ltd, UK. All other chemicals were of analytical grades and prepared in glass apparatus using distilled water.

Plant Material:

The leaves of *Phaseolus vulgaris* L. (Red Kidney Beans) were collected at Massallacin-Jumma'a Street, Jos, Plateau State, Nigeria in July 2011and was authenticated by Dr. M.C. Okonkwo of Department of Forestry

Technology, FCF Jos, Plateau State, Nigeria.

Experimental Animals:

Twenty four (24) wistar rats were obtained from the animal holding unit, University of Jos, Nigeria. They were randomly distributed into four groups of six rats each and were fed with standard commercial feeds (vital feeds, Nigeria).

Preparation of Extract:

The dried leaves of Phaseolus Vulgaris were pounded using pestle and mortar. 20 grams of the powdered leaves were poured into 200 mL of distilled water and placed on a hot plate. Mixture was boil for 20 minutes, allowed to cool and sieved with Whatman No.1 filter paper. Filtrate was then concentrated at 50°C and stored in an air tight container which was later reconstituted to give the required dose of 400 mg/kg body weight.

Administration of Alloxan:

Alloxan monohydrate solution was administered at a single dose of 150 mg/kg body weight intraperitoneally. Diabetes was confirmed by determining the blood glucose concentration after 48 hours of alloxan injection. The rats with blood glucose level >7.0 mmol/L were selected for the study.

Experimental Design:

Group A	Diabetes Control (0.5 mL distilled water/day)
Group B	Diabetes + Extract (400 mg/kg bwt/day)
Group C	Normal Control (0.5 mL distilled water/day)
Group D	Normal + Extract (400 mg/kg bwt/day)

Each group consist of six animals (n = 6).

Sample Collection:

On the 15th day, the rats were anaesthetised with diethyl ether, the neck area was quickly cleared of fur and skin to expose the jugular veins. Venous blood was thus collected into a plain sample container for three animals, allowed to clot and the serum was clearly remove and used for the assays. Blood sample from the last three were separately collected into an anti-coagulant and were used for haematological assay.

Biochemical Assays:

Packed Cell Volume (PCV), Haemoglobin (Hb) concentration, White Blood Cell (WBC) and Platelet count were determined using Mindray Haematology Analyser (Mindray BC-2300, Guangzhou Shihai Medical Equipment Co., Ltd, China). Serum glucose, total protein, albumin, total cholesterol and triglycerides (TG) were determined by using commercial kits. Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were measured spectrophotometrically using a modified method described by ^[15].

Statistical Analysis:

Results were expressed in mean \pm Standard deviation. The data were statistically analyzed using one-way ANOVA with multiple comparisons versus control group. Values of $p < 0.05$ were considered as significant. The alloxan-induced diabetes groups and normoglycemic groups were analyzed separately for statistical significance.

RESULTS

Freshly prepared *Phaseolus vulgaris* L. extracts were subjected to preliminary phytochemical screening for various constituents. The active principles detected included alkaloids, balsams, cyanogenic glycosides, flavonoids, saponins, tannins, terpenes and steroids (table 1).

The blood glucose, total protein, albumin and cholesterol levels of the diabetes treated rats and normal treated rats were not significantly altered when compared with the control rats. However, these values were significantly increased in diabetes untreated rats (table 2). Extract significantly reduced cholesterol levels of treated rats when compared to the diabetes untreated rats while a significant increase was observed in triglyceride levels of diabetes control and diabetes treated rats when compared with normal control (table 2).

Aqueous extracts of *Phaseolus vulgaris* L. at 400 mg/kg body weight significantly reduce the values of ALT and AST when compared to high values of the enzymes observed in diabetes untreated rats. Levels of ALP were statistically altered in all groups when compared to the control rats (table 3).

Extract had no significant effects on PCV and Hb in all groups when compared to the normal control. Extract was able to reverse the significant increase observed in platelet count of diabetes treated rats when compared to diabetes untreated; extract also significantly reduce platelets levels in normal treated rats when compared to the control (table 4).

Phytochemicals	Aqueous extract
Alkaloids	+
Balsam	+
Cyanogenic Glycosides	+
Flavonoids	+
Saponins	+
Tannins	+
Terpenes and Steroids	+

Table 1: Phytochemical Screening

Note: + = Present, - = Absent

Parameters	Experimental Groups			
	Group A (Diabetes)	Group B (Diabetes + Extract)	Group C (Normal)	Group D (Normal + Extract)
Glucose (mmol/L)	16.8±0.30*	6.0±0.36	6.4±0.10	5.3±0.20
Total Protein (g/L)	78.1±3.96*	68.2±2.35	70.0±1.38	67.5±2.36
Albumin (g/L)	38.0±0.36*	35.7±1.62	35.9±1.78	35.2±0.95
Cholesterol (mmol/L)	9.6±0.76*	4.8±0.40	4.4±0.46	3.8±0.20
Triglycerides (mmol/L)	2.8±0.20*	1.5±0.27*	1.1±0.10	0.7±0.10*

Table 2: Effect of Phaseolus vulgaris L. extract on some cell biomolecules

Values are mean ± SD of 3 replicates; *Values are statistically significant when compared with normal control at p<0.05

Parameters	Experimental Groups			
	Group A (Diabetes)	Group B (Diabetes + Extract)	Group C (Normal)	Group D (Normal + Extract)
ALT (U/L)	95.0±3.61*	43.0±2.00	46.0±2.65	40.8±2.65
AST (U/L)	96.0±2.65*	67.0±3.61	68.0±1.00	59.0±3.61*
ALP (U/L)	221.4.0±5.57*	196.6±5.96*	177.3±4.29	130.5±7.99*

Table 3: Effect of Phaseolus vulgaris L. extract on some marker enzymes

Values are mean ± SD of 3 replicates; *Values are statistically significant when compared with normal control at p<0.05

Parameters	Experimental Groups			
	Group A (Diabetes)	Group B (Diabetes + Extract)	Group C (Normal)	Group D (Normal + Extract)
PCV (%)	45.0±1.00	46.0±2.00	44.0±1.00	45.0±1.00
Haemoglobin (g/dL)	16.1±0.36	16.8±0.66	16.5±0.50	16.5±0.27
WBC (10 ² cells/mm ³)	112.5±3.76*	72.0±1.32*	104.5±1.80	54.5±1.32*
Platelets (10 ³ cells/mm ³)	665±21.93*	460±25.83	470±20.00	410±5.00*

Table 4: Effect of Phaseolus vulgaris L. extract on some haematological parameters

Values are mean ± SD of 3 replicates; *Values are statistically significant when compared with normal control at p<0.05

DISCUSSION

Aqueous extracts of Phaseolus vulgaris L. was able to maintain a non-significant level of glucose in diabetes treated and normal treated rats when compared to normal control, this anti-diabetic effect may be due to increased release of insulin from the β -cells of pancreas. Alloxan monohydrate is one of the chemical agents used to induce diabetes mellitus. It induces diabetes by partial 'chemical' destruction of the β -cells of Islets of Langerhan's leading to hyperglycemia. This results in decreased insulin levels leading to type 1 diabetes mellitus. The antihyperglycemic effects of Phaseolus vulgaris might be either stimulating pancreatic β -cells to secrete more insulin (insulin secretor) or increased insulin sensitivity in peripheral tissues, to include adipose tissue, muscle and liver to clear blood glucose at faster rate. The exact mechanism of action of Phaseolus vulgaris L is not well-understood. Literature suggests the involvement of two possible mechanisms of action in the reducing effect of Phaseolus vulgaris extracts on glycemia, these mechanisms focus on the role of phytohemagglutinin and α -amylase inhibitors. Pancreatic α -amylase is an enzyme that catalyzes hydrolysis of α -(1,4)-glycosidic bonds of starch polymers [16]. Thus, inhibition of α -amylase results in the suppression of starch metabolism and in turn, a decrease in glycemia [17]. It has also been reported that α -amylase inhibitors delay gastric emptying, producing feelings of satiety [18], thus resulting in reduced food intake. Phytohemagglutinin is known to bind to the stomach epithelial cells and to the brush border membrane of small intestine, cecum, and colon [19]. This binding results in the stimulation of the release of cholecystokinin and glucagonlike peptides [20][21], two hormones playing a relevant role in digestive processes.

The above findings suggest that extracts of *Phaseolus vulgaris* L. may constitute potentially interesting, novel remedies for the treatment of metabolic syndrome such as diabetes.

Phaseolus vulgaris L. is increasingly gaining attention as a functional or nutraceutical food, due to its rich variety of phytochemicals which have a potential benefits on health [22]. Important biological activities have been described from common beans like the enhancement of bifidogenic effect [23]; anti-oxidant [24]; anticarcinogenic^[13] effects. The dosage administered during the study was found to be active in reducing blood glucose level in both diabetes and normoglycemic rats, hence expressing its hypoglycemic potential. The reason for this active hypoglycemic activity of *Phaseolus vulgaris* L. might be due the availability of some phytoconstituents, which include alkaloids, flavonoids, tannins, terpenoids and saponins^{[25][26]}. It has also been reported that terpenoids [27], saponins^[28], are precise bioactive components responsible for the antidiabetic activity of some plants.

Diabetes mellitus is also associated with hyperlipidaemia with profound alteration in the concentration and composition of serum lipid [29] such as total cholesterol and triglyceride as reported in this work. Changes in the concentrations of the lipid with diabetes mellitus contribute to the development of vascular disease [30][31]. Fatty acids, an important component of cell membranes, are eicosanoid precursors and are therefore required for both the structure and function of every cell in the body [32]. Extract was able to improve total cholesterol level of diabetes rats at the administered dosage, extract was also able to reduce the levels of TG which was however statistically significant when compared to normal control.

Hyperalbuminemia, hyperuremia and hypercreatininemia are conditions which have been reported to occur in alloxanized diabetic rats [33]. Aqueous extracts was able to ameliorate this diabetes condition on albumin content with a similar effect on total protein content of diabetes and normoglycemic treated rats.

Aqueous extracts from *Phaseolus vulgaris* L. had no significant effects on percentage PCV and haemoglobin in all treated groups. However, significant effects were recorded in White Blood Cell (WBC) and platelets count. Extracts considerably improved the platelet counts at the end of the administration period when compared to the diabetes untreated.

The study corroborates the increases in levels of AST, ALT and ALP as previously reported in alloxan-induced diabetes rats [34]. These are marker enzymes for hepatocellular damage; extract was able to reverse their increases to a better extent in ALT and AST. This suggests that aqueous extracts may have ameliorated the drug-induced 'chemical' damage to the liver cells as observed in diabetes untreated rats.

CONCLUSION

From this study, *Phaseolus vulgaris* L. affirms its hypoglycemic and antidiabetic potential; it may be used in complementary medicine to treat diabetes population. Further study is required in isolation, purification and characterization of its bioactive component(s) and possible toxicity studies.

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