EFFECT OF AQUEOUS EXTRACT OF BEAN POD ON ALLOXAN INDUCED DIABETES RATS

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Abstract

*Phaseolus vulgaris* is herbaceous annual plant grown worldwide for its edible bean. Serum glucose for Alloxan induced diabetes rats grouped into four was determined. It was determined after treated with bean pod extract. Group 1 (normal control) has serum glucose level 4.40 ± 0.10 this value shows significant decrease at p< 0.05. When compared with Group II (Negative control I) which confirmed diabetes induction. Serum glucose of Group III (treated with 3.0 g/kg) has serum glucose of 5.22 ± 0.41 this value shows significant increase when compared with Group IV (Negative control II). Also serum glucose level of Group III (treated with 6.0 g/kg) was 5.94 ± 0.61 which also shows significant increased at p< 0.05 when compared with Group IV (Negative control III). This shows that bean pod extract has hyperglycemic effect (i.e. cannot manage/cure diabetes). Phytochemical screening of bean pod extract was carried out and found to have contain, tannins and glycosides which are polymers of carbohydrates this may likely contributed to rise in serum glucose when metabolized.

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1. Introduction

The common bean, *Phaseolus vulgaris* is herbaceous annual plant grown worldwide for its edible bean. The leaf occasionally used as leaf vegetables, and the straw was used for fodder. The common bean was classified as a dicotyledonous, Bean are legumes acquiring nitrogen through an association with Nitrogen fixing bacteria. (Raemackers, 2001). The mature dried seed of *Phaseolus vulgaris* contains per 100 g of edible matter, 10 g water, 22.6 g protein, 1.4 g fat, 62 g carbohydrate, 4.3 g fiber, 3.7 g ash,120 mg calcium, 323 mg phosphorous, 8.2 mg iron, 10 µg β-Carotene, 0.37 mg thiamine and 0.16 mg riboflavin. The compositions of green pods are 91 g water, 1.8 g protein, 0.2 g fat, 6.6 g carbohydrates, 1 g fabric and 0.7 g ash, 43 mg calcium, 48 mg phosphorous, 1.4 mg iron, 750 µg β carotene, 0.8 mg thiamine, 0.12 mg riboflavin, 0.5 mg niacin and 2.7 mg ascorbic acid. The fresh leaves contain 3.6 g protein, 110 mg vitamin C and high content of precursor of vitamin A (Raemackers, 2001). Ethno medical use of *Phaseolus vulgaris* is that it was found to cure importance disease like Typhoid fever, malaria and diabetes mellitus.

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Diabetes is a syndrome characterized by disordered metabolism and abnormally high blood sugar (hyperglycemia) resulting from insufficient level or ineffective of the hormone insulin. The characteristics symptoms are excessive blood glucose levels, excessive thirst and increased fluid intake (polydipsia), blurred vision due to high blood glucose, effects on the eyes optics. Basically there are two types of diabetes mellitus, known as Type 1 and Type 2 (Champe and Harvey, 1994). Type 1 (insulin dependent diabetes mellitus) was characterized by loss of insulin producing Beta cells of the Islet of Langerhans, the pancreas, leading to a deficiency of insulin. The main cause of this beta cells lost is T-cell mediated autoimmune attack which usually begins before age of twenty. Type - 2 (Non-insulin dependent Diabetes mellitus). This type was characterized due to insulin resistance or reduced insulin sensitivity combined with reduced insulin secretion. The defective responsiveness of body tissue to insulin was almost certainly involves the insulin receptor in cell membrane. The research work was aimed to evaluate the anti-diabetic property of aqueous extract of bean pod on chemically induced diabetes rats.

2. Materials and method

Collection of Plant sample

A fresh dried bean pod was purchased from Sharada market, Kano city, Nigeria. The sample was grinded into powder using motor and pestle, which was kept in sterile polythene bag and stored at room temperature.

Preparation of aqueous extract

Aqueous extract of beans pod was prepared by weighing and soaking 100 g of powdered beans pod in 1 L (1000 ml) of distilled water and kept for about 24 hours (w/v). The mixture was then filtered using Whatman filter paper. The residue was dried and reweigh again. The concentration of the filtrate was determined as the difference in weight per final volume of the solution using the relation.

Concentration (g/ml) = Initial weight of sample - Final weight of sample/Final volume

The volume of aqueous extract of bean pod administered was based on the weight of the rat. The amount given to each rat was obtained using the following relation.

Volume (ml) = Dose (mg) × Weight of rat (g)/Concentration of extract (g/ml) × 1000

Experimental Design

Twenty six (26) Albino rat were purchased from animal house, Faculty of Pharmaceutical Science, Ahmad Bello University, Zaria. The rats were placed in metal cage in Animal room of Physiology, Department of Bayero University, Kano. They were allowed to acclimatized and fed for one week prior to the commencement of the experiment. The rats were divided into four groups; Group I and II consisted of 3 rats each, while Group III and IV contained 10 rats each. The experiment was done in two phase of three days interval.

Treatment of Albino Rats

All animals were fed on normal diet for 7 days of acclimatization. Diabetes was induced by an interperitoneal (IP) injection of 100 mg Alloxan/kg body weight. In which the Alloxan was dissolved in freshly prepared 10 mmol/L of sodium citrate pH 4.5. The rats were fast for at least 16 hrs prior to blood glucose level determination. In the first day of experiment, animal in Group II, III and IV was induced with diabetes. After seven days of induction, blood glucose level of rats in Group I and II was determined. After which diabetes was confirmed. Rats in Group III were treated with extract in addition to food and water. While animal in Group IV were allowed for free access to food and water. The extract was orally given once a day for about six days. After three days five rats in each Group III and IV were taken and level of glucose was determined. In the next three days, the remaining five rats in each Group III and IV were also taken and blood glucose was determined using Accu-Chek Active Glucometer and using enzymatic method.

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Determination of Blood Glucose Level Using Accu-chek active Glucometer

Accu-Chek active test strip was inserted with the orange fad facing up and arrows pointing towards the Accu-chek active meter until it click into place. The meter turns on automatically, and then a drop of whole blood was applied to the center of the squire orange pad and allowed to stand for 2 - 3 seconds, the meter read automatically.

Determination of Blood Glucose Level Using Enzymatic Method

Three test tubes were labeled standard Blank and sample (or test), to all the test tubes, 1000 µl of reagent 1 were measured. To the test tube labeled standard, 10 µl of standard reagent was measured, and to the test tubes labeled sample, 10 µl of sample reagent was measured. All the test tubes were mixed and incubated for 10 minute at 37 °C and the absorbance at 500 nm was measured for the standard (A standard) and the sample (A sample) against the reagent blank within 60 minutes. The blood glucose level is calculated by:

\[
\text{Glucose concentration (mmol/L)} = \frac{\text{Sample A} \times 5.55}{\text{A standard}}
\]

\[
\text{Glucose concentration (mg/dL)} = \frac{\text{Sample A} \times 100}{\text{A standard}}
\]

Phytochemical Screening

A fresh powder of bean pod was subjected to the following tests.

Test for Tannins

Three g of powdered sample was boiled in 50 ml of distilled water on the heater mantle for 3 minute. The mixture was filtered and a portion of water extract was diluted with distilled water in a ratio of 1:1. A few drops of 10 % ferric chloride were added. Formation of blue or green color indicated the presence of tannins (Trease and Evans, 1993).

Test for Chlorogenic Acid

Two drops of 10 % ammonia solution was added to 1 ml of water extract, the mixture was heated over a flame and then exposed to air. A green color indicated the presence of Chlorogenic acid (Trease and Evans,1993).

Test for Glycoside

To 5 ml of extract in a test tube 2 - 5 ml of dilute H₂SO₄ was added and boiled for 5 minute, the mixture was cooled and neutralized with 10 % NaOH 5 ml of Fehling solution A and B was added. A brick red precipitate indicated the presence of glycosides (Trease and Evans, 1993).

Test for Free Anthraquinone

An amount of 0.5 g of powdered sample in a test tube 10 ml of chloroform was added and shaken for 5 minutes. The extract was filtered and equal volume of ammonia was added and shaken. A bright pink colour in the upper aqueous layer indicated the presence of free anthraquinones (Trease and Evans, 1993).

Test for Saponins

To small quantity of powdered sample 95 % ethanol was added and boiled. The mixture was filtered and 2.5 ml of the filtrate was added to 10 ml of distilled water in a test tube. The test tube was stopped and shaken vigorously for about 30 seconds. It was allowed to stand for over 30 minutes. Appearance of honey comb froth indicated the presence of saponins (Sofowora, 1993).

Test for Flavonoids

Five g of the powdered sample completely detainted with acetone warm water was used for extraction after evaporating the acetone on a water bath the mixture was filtered, 5 ml of 10 % sodium hydroxide was added to an equal volume of the detainted water extract. A yellow solution indicated the presence of flavonoids (Sofowara 1993).

Test for Resins

Acetic anhydride was use to dissolve 0.5 g of powdered sample and one drop of concentrated sulphuric acid was added. A purple or violet color indicated the presence of resins (Sofowara, 1993).
Test for Alkaloids

A set of five test tube containing sample were taken to each test tube little amount of ethanol solution was added, then a few drop of Meyer’s reagent (Potassium mercuric iodine solution), Dragendorff’s reagent (Potassium bismuth iodine solution), Wagner’s reagent (solution of iodine in potassium iodine) and Hager’s reagent (saturated solution of picric acid). Presence of precipitate in at least 3 or all the reagents indicated the presence of alkaloids (Trease and Evans, 1996).

3. Result and Discussion

Phytochemical screening of bean pod

The result for phytochemical screening of bean pod is presented in Table - 1 in which alkaloid, flavonoids, tannins and glycoside were found to be present while saponins, resin and anthraquinone were absent. As described by Smith and Swain (1993), tannic acid is a polymer of garlic acid molecules and glucose which hydrolyze to glucose and garlic acid or ellagic acid, when hydrolyze. As per the description by Lindhorst (2007), glycoside was found to be a molecule in which a sugar was bound to non-carbohydrate moiety usually small organic molecules, which hydrolyses to give sugar. Presence of these two phytochemical in bean pod was likely to be the factor that results in the rise of serum glucose.

Table - 1: Phytochemical of aqueous extract of bean pod

<table>
<thead>
<tr>
<th>Compound</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>+</td>
</tr>
<tr>
<td>Resin</td>
<td>-</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
</tbody>
</table>

Levels of serum glucose in rats induced with diabetes using Alloxan

The Table - 2 present levels of serum glucose in rats induced with diabetes using Alloxan, increases in serum glucose compared to normal usually indicates diabetes. It was found from the experiment the normal control albino rats had a serum glucose 4.40 ± 0.10, while the serum glucose for albino rat that had injected (interperitoneal) of 100 mg/kg of Alloxan for one week (Group II) had a serum glucose 7.10 ± 1.04 this values shows a significant increase when compared with normal at P < 0.05. As reported by Hamilton (2005), certain factors such as food, whether physical or emotional activity may raise blood glucose but in this case is not to be the factor that results in the rise of blood glucose, as explain by Lenzen (2008) which describe the toxicity cause by Alloxan which selectively destroy the pancreatic beta cells.

Table - 2: Serum Glucose of Alloxan diabetes induced rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum Glucose (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1: Normal control (n = 3)</td>
<td>4.40 ± 0.10^a</td>
</tr>
<tr>
<td>Group II: Negative control (n = 3)</td>
<td>7.10 ± 1.04^a</td>
</tr>
</tbody>
</table>

Values are mean ± SD, values bearing similar super script are significant at p < 0.05.n=number of albino rat

Level of serum glucose in rats induced with diabetes and treated with 3.0g/kg, of bean pod extract

The Table - 3 present the level of serum glucose in rats induced with diabetes and treated with 3.0 g/kg, increases in serum glucose compared to Negative control II, indicates hyperglycemic effect of bean pod extract. The albino rats that were treated with 3.0 g/kg of aqueous extract of bean pod for 3 days after interperitoneal injection of 100 mg/kg of Alloxan had a serum glucose of 5.22 ± 0.41 this values shows an increase when compared with Negative control II (not treated with 3.0 g/kg aqueous extract of bean pod), despite that no significant difference at p<0.05. The rise in serum glucose level was due to the presence of tannin and glycosides as found to be present in the bean pod extract.
Table - 3: Serum glucose level of Alloxan diabetes induced rat and treated with bean pod extract for three days

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum Glucose (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group III (treated with 3.0 g/kg of bean pod extract) (n = 5)</td>
<td>5.22 ± 0.41</td>
</tr>
<tr>
<td>Group IV Negative control II (not treated with 3.0 g/kg of bean pod extract) (n = 5)</td>
<td>5.06 ± 0.71</td>
</tr>
</tbody>
</table>

n= number albino rats: Values are mean±standard deviation: values on the same column are not significant at p<0.05

**Level of serum glucose in rats induced with diabetes and treated with 6.0 g/kg of bean pod extract**

The level of serum glucose in rats induced with diabetes and treated with 6.0 g/kg of bean pod extract was presented in Table - 4. The increase in serum glucose compared to negative control III usually indicates hyperglycemic effect of bean pod extract. Albino rats that were treated with 6.0 g/kg of bean pod extract after interperitoneal injection of 100 mg/kg of Alloxan had serum glucose of 5.94 ± 0.61. This value has shown significant increase at p < 0.05 when compared to Negative control III (not treated with 6.0 g/kg) after interperitoneal injection of 100 mg/kg of Alloxan, this was likely due to the present of Tannic acid and Glycosides, as described by Smith and Swain (1993), tannic acid is a polymer of garlic acid molecules and glucose which hydrolyze to glucose and garlic acid or pelagic acid, when hydrolyze. As per the description by Lyndhurst (2007), glycoside was found to be molecules in which a sugar was bound to a non -carbohydrate moiety usually small organic molecules, which hydrolyses to give sugar. Present of these two photochemical in bean pod was likely to be the factor that results in the rise of serum glucose in Albino rats that were treated with 3.0 g/kg of bean pod extract after interperitoneal injection has a serum glucose of 5.22 ± 0.41 this values shows significant decrease at P<0.05 when compared with group that treated with higher dose of bean pod extract (6.0 g/kg) after interperitoneal injection of 100mg/kg of Alloxan. The rise in serum glucose in group that treated with high dose of bean pod extract is due to the fact that they received higher amount of extract (6.0 g/kg), which in turn high tannins and glycosides which lead to rise of serum glucose level. The value of serum glucose of normal control albino rat (which not induced with 100 mg/kg of Alloxan) was 4.40 ± 0.10. This value shows significant decrease at P < 0.05 when compared to other groups (which induced with 100 mg/kg of Alloxan) II, III and IV, which signify the extent of Alloxan in destruction of pancreatic beta cells. As explained by Etuk (2010), Alloxan is a urea derivative which causes selective necrosis of beta cells pancreatic islets.

Table - 4: Serum glucose level of Alloxan diabetes induced rat and treated with beans pod extract for six days

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum Glucose (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group III (treated with 6.0 g/kg of bean pod extract) (n = 5)</td>
<td>5.940±0.61</td>
</tr>
<tr>
<td>Group IV Negative control III (not treated with 6.0 g/kg of bean pod extract) (n = 5)</td>
<td>4.86±0.62</td>
</tr>
</tbody>
</table>

n = number of albino rat. Values are expresses as mean ±standards deviation. Values on the same column bearing similar superscript are statistically significant at p<0.05

4. Conclusion

It can be concluded that Experimental groups that were treated with bean pod extract had higher serum glucose compared to groups which were not treated with bean pod extract indicating that bean pod has hyperglycemic property, which may likely be not effective in cure or management of diabetes. Experimental groups that received higher dose of bean pod (6.0 g/kg) had high serum glucose then groups that received low dose of bean pod (3.0 g/kg), which shows that phytochemical (tannins and glycoside present in the extract) had contributed a lot in the rise of serum glucose. Serum glucose of experimental groups that were injected with Alloxan was significantly higher compared to group which was not injected with Alloxan.
5. References


15) Hamilton. 1995. Current Medical Diagnosis and Treatment. Lange Medical Publication: 400


methyl derivatives and a comparison with Ninhydrin. Biochemical Pharmacology, 42 (7): 1385 – 1391.


