International Journal of Innovation Sciences and Research
Vol.4, No, 3, pp. 124-128 March- 2015

Available online at
http://www.ijisr.com

RESEARCH ARTICLE

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

[*1]Jamilu, Y. M. I., [2]Alhassan, A. J., [3]Darma, A. I. and [4]Karfi, I. A.

[1,2]Department of Biochemistry, Bayero University, Kano, Nigeria
[3]Department of Biological Sciences, Bayero University, Kano, Nigeria
[4]Department of Biotechnology, SRM University Chennai, India

Accepted 23rd February, 2015; Published Online 31st March, 2015

ABSTRACT

Phasealus vulgaris is herbaceous annual plant grown worldwide for its edible bean.Serum glucose for Alloxan induced diabetes rats grouped into four was determined. It 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 3.0g/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 6.0g/kg) was 5.94 + 0.61which 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.

Key Words: Alloxan: Bean: Extract: Diabetic: Glucose.

INTRODUCTION

The common bean, *Phasealus vulgaris* is herbaceous annual plant grown worldwide for its edible bean. The leaf occasionally used as leaf vegetables, and the straw is used for fodder. The common bean is 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 100g of edible matter, 10g water, 22.6gprotein, 1.4g fat, 62g carbohydrate, 4.3g fiber, 3.7g ash,120mg calcium, 323mg phosphorous, 8.2mg iron 10µg β-Carotene, 0.37mg thiamine, 0.16mg Riboflavin. The compositions of green pods are 91g water, 1.8g protein, 0.2g fat, 6.6g CHO, 1g fabric, and 0.7g ash, 43mg calcium 48mg phosphorous, 1.4mg iron, 750µg β carotene, 0.8m thiamine, 0.12mg Riboflavin, 0.5mg Niacin, 2.7mg Ascorbic acid. The fresh leaves contain 3.6g protein 110mg 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. Diabetes is syndrome characterized by disordered metabolism and abnormally high blood sugar (hyperglycemia) resulting from in sufficient 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.

Corresponding author: Jamilu, Y. M. I.,
Department of Biochemistry, Bayero University, Kano, Nigeria

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) is 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 auto immune attack which usually begins before age of twenty. Type2 (Non-insulin dependent Diabetes mellitus); this type is characterized due to insulin resistance or reduced insulin sensitivity combined with reduced insulin secretion. The defective responsiveness of body tissue to insulin is almost certainly involves the insulin receptor in cell membrane.

MATERIALS AND METHODS

The research work was aim at evaluating the anti-diabetic property of aqueous extract of bean pod on chemically induced diabetes rats. The specific objectives are:-

- To prepared aqueous extract of bean pod
- Administer the dose of extract to a groups of diabetes rat
- To determine serum glucose level in control and test groups of diabetes rats.
- To compare serum glucose levels of the negative control and positive control.
- To compare serum glucose levels of the negative control with the test group.

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 100g of powdered beans pod in 1L (1000ml) of distilled water and kept for about 24 hours (w/v). The mixture was then filtered using whatmanfilter 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.

$$\text{Conc. g/ml} = \frac{\text{initial weight of sample- final weight of sample}}{\text{Final volume}}$$

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.

$$\text{Volume (ml)} = \frac{\text{Dose (mg) x weight of rat (g)}}{\text{Conc. of extract (g/ml) x 1000}}$$

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 1and II consisted of 3rats 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 100mg Alloxan/kg body weight. In which the Alloxan was dissolved in freshly prepared 10mmol/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.

Determination of Blood Glucose Level Using Accu-chek active Glucometer

Procedure

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

Procedure

Three test tubes were labeled standard Blank and sample (or test), to all the test tubes, 1000µl of reagent 1 were measured. To the test tube labeled standard, 10µl of standard reagent was measured, and to the test tube(s) labeled sample, 10µl of sample reagent was measured. All the test tubes were mixed and incubated for 10 minute at 37^0C and the absorbance at 500nm was measured for the standard (A standard) and the sample (A sample) against the reagent blank within 60 minutes.

Calculation

Glucose concentration mmol/L = sample A x 5.55⁄A standard
Glucose concentration mg/dl = sample A x 100⁄A standard

Phytochemical Screening

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

Test for Tannins

Extraction: - 3g of powdered sample was boiled in 50mls of distilled water on the heater mantle for 3minute. 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

2 drops of 10% ammonia solution was added to 1ml 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 5ml of extract in a test tube 2-5 ml of dilute H_2S0_4 was added and boiled for 5 minute, the mixture was cooled and neutralized with 10% NaOH 5mls 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

Into 0.5g of powdered sample in a test tube 10ml 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 10ml 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 Flavanoids

Into 5g of the powdered sample completely detanned 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 detanned water extract. A yellow solution indicated the presence of flavonoids (Sofowara 1993).

Test for Resins

Acetic anhydride was use to dissolve 0.5g of powdered sample and 1drop 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 the following;

Meyer's reagent (Potassium mercuric iodine solution)
Dragendroff's reagent (Potassium bismuth iodine solution)
Wagner's reagent (solution of iodine in potassium iodine)
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).

RESULTS AND DISCUSSION

A) Phytochemical screening of bean pod

The result for phytochemical screening of bean pod is presented in Table 1 in which alkaloid, Flavanoids, 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.

Table 1. Phytochemical of aqueous extract of bean pod

Compound	Result
Saponins	-
Alkaloid	+
Resin	-
Flavanoids	+
Tannins	+
Anthraquinone	-
Glycosides	+

Also as described by (Lindhorst, 2007) glycoside is found to be a molecule in which a sugar is bound to non-carbohydrate moiety usually small organic molecules, which hydrolyses to give sugar. Present of these two phytochemical in bean pod is likely to be the factor that results in the rise of serum glucose.

B) Levels of serum glucose in rats induced with diabetes using Alloxan

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 100mg /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 result 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

GROUPS	SERUM GLUCOSE (mmol/l)
Group 1 Normal control n=3	4.40 ± 0.10^{a}
Group II negative control 1 n=3	7.10 ± 1.04^{a}

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

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

Table 3 present the level of serum glucose in rats induced with diabetes and treated with 3.0g/kg, increases in serum glucose compared to negative control II, indicates hyperglycemic effect of bean pod extract. The albino rats that were treated with 3.0g/kg of aqueous extract of bean pod for 3 days after interperitoneal injection of 100mg/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 3.0g/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

GROUPS	SERUM GLUCOSE (mmol/l)
Group III (treated with 3.0g/kg of bean pod extract) n=5	5.22±0.41
Group IV negative control II (not treated with 3.0g/kg of bean pod extract). n=5	5.06±0.71

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

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

The level of serum glucose in rats induced with diabetes and treated with 6.0g/kg of bean pod extract is 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 6.0g/kg of bean pod extract after interperitoneal injection of 100mg/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 6.0g/kg) after interperitoneal injection of 100mg/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. Also as described by (Lyndhurst, 2007) glycoside is found to be a molecules in which a sugar is bound to a non-carbohydrate moiety usually small organic molecules, which hydrolyses to give sugar.

Present of these two Photochemical in bean pod is likely to be the factor that results in the rise of serum glucose in Albino rats that were treated with 3.0g/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 (6.0g/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 (6.0g/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 100mg/kg of Alloxan) is 4.40 ±0.10. This value shows significant decrease at $P < 0.05$ when compared to other groups (which induced with 100mg/kg of Alloxan) II, III and IV, which signify the extent of Alloxan in destruction of pancreatic beta cells. As explained by (EtukEU, N.J, 2010) that Alloxan is a urea derivatives 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

GROUPS	SERUM GLUCOSE (mmol/l)
Group III (treated with 6.0g/kg of bean pod extract) n=5	$5.940±0.61^b$
Group IV negative control III (not treated with 6.0g/kg of bean pod extract). n=5	$4.86±0.62^b$

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

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 (6.0g/kg) had high serum glucose then groups that received low dose of bean pod (3.0g/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.

REFERENCES

Acherya Depeak and Shrivastava Anshu, 2003. Indigenous Herbal Medicines Tirbal Formulations and Traditional Herbal Practices. Aavishkar Publisher Distributor PP 240

Anderson, J.R. 1986. Murs Text Book of Pathology. 12th Ed. Appleton Century Crofts Company PP 1036-1037.

Anderson, W.A.D. and John M. Kissane, 1977. Text Book of Pathology 7th Ed Volume 2. Cv Mosby Company PP. 904.

Arlan Rosen Bloom and Janat H. Silversterin, 2003. Types 2 Diabetes in Children and Adolescent. American Diabetes Association PP 540 542

Bate Smith and Swain, 1993. Flavanoids Compounds in Comparative. 5th Ed. Vol III. Academic Press New York. PP 260

Charles Alais and Cylinden, 1999. *Food Biochemistry*3rd Ed. Arsan Company PP 634-643.

David L. Nelson and Michel M. Cox, 2002. Lehninger Principle of Biochemistry 4th Ed W.H Freeman and Company PP. 710-714.

Eddouk, M., Maghram, M., Lemhadin, A., Ouahidi, ML. and Juad, H. 2002. Ethno Pharmacological Survey Of Medicinal Plant Used for Treatment of Diabetes Mellitus. *Journal of Ethno Phermaco* PP 147

Etuk EU, N. J. 2010. Animals models for studying diabetes mellitus. *Agric. Biol*. 2010; 1:130-4.

Ebelt, H., Peschke, D., Brömme, HJ., Mörke, W., Blume, R. and Peschke, E. 2000. Influence of melatonin on free radical-induced changes in rat pancreatic beta-cells in vitro. *Journal of pineal research* 2000; 28(2):65-72.

Farombi, E.O. 2003. African Indigenous Plant with Chemotherapeutic Potential, *African Journal*. Biotech PP. 22, 44

Fornworth, N.R. and Socjarto, D.D. 1991. Global Importance of Medicinal Plant. 3rd Ed. Cambridge University Press PP. 17

Genuth, S. 2006. Insight from Diabetes Control and Complication Trial Epidemiology of Diabetes Intervention and Complication. *Epidomolgy. Pract*. 12 PP 616-620, 810

Goldner, MG. and Gomori, G. 1994. Studies on the mechanism of Alloxan diabetes. Endocrinal 1; 35:2418.

Hamilton, 1995. Current Medical Diagnosis and Treatment. *Lange Medical Publication* PP 400

Hudson T Hantman, Antom M. Karfranck, and Williams J. Flocker, 2000. Plant Science Growth Development and Utilization of Cultivated Plants. 2nd Ed. Prentice Hall Career and Technology PP. 630-633.

Huffman, M.A. 2000. Animal Self Medication and Ethno-medicine Exploration and Exploitation of Medicinal Properties of Plant. *Knowledge House Publishers* PP 240-241.

Iranloye, BO., Arikawe, AP., Rotimi, G. and Sogbade, AO. 2011: Anti-diabetic and anti-oxidant effects of Zingiberofficinale on Alloxan-induced and insulin-resistant diabetic male rats. *Nigerian journal of physiological l sciences*: official publication of the Physiological Society of Nigeria 2011; 26(1):89-96.

Jacobs, HR. 1997. Hypoglycemic action of Alloxan. *Proc Soc Exp Biol Med* 1937; 37 :407-9.

Katz, S.R., Newman, R.A. and Lansky, G.P. 2007. Punica Grantum Hauristic Treatment for Diabetes Mellitus. *Journal for Medicinal Food* 10 (2) PP 213-214.

Kliber, A., Szkudelski, T. and Chichłowska, J. 1996. Alloxan stimulation and subsequent inhibition of insulin release from in situ perfused rat pancreas. *Journal of physiology and pharmacology*. An official journal of the Polish Physiological Society 1996; 47(2):321-8.

Lai, Pk. 2004. Antimicrobial and Chemopreventive Properties of Herbs and Spices. 2nd Ed. Heinaman Professional Publishing Ltd PP54.

Lawrence, J.M., Conters, R., Chen, W. and Sacks, DA. 2007. Trends in Prevalence of Preexisting Diabetes and Gestational Diabetes Mellitus. Diabetes Care Association USA PP 844-845.

Leach, M.J. 2007. *Gymnema Silvestre for Diabetes Mellitus*. *Journal of Alternative and Complementary Medicine* PP. 211.

Lenzen, S. 2008. The Mechanism of Alloxan and Streptozotocin Induced Diabetes. Unique Press Italy PP 382

Lindhorst, T.K. 2007. Essential of Carbohydrate Chemistry and Biochemistry. 2nd Ed. VCH Company Press PP 535

Lubert Stryer, Jeremy M. Berg and John L. Tymmoczko, 2007. Biochemistry 6th Edition. W.H Freeman and Company PP 660-662,810-811

Lenzen, S. and Munday, R. 1991. Thiol-group reactivity, hydrophilicity and stability of Alloxan, its reduction products and its N-methyl derivatives and a comparison with Ninhydrin. *Biochemical pharmacology*; 42(7):1385-91

Mailloux and Lionel, 2007. Up to Date Dialysis in Diabetes Nephropathy. Retrieved on 3rd June 2009 http//patientuptodate.com.

Munday, R. 1988. Dialuric acid autoxidation. Effects of transition metals on the reaction rate and on the generation of "active oxygen" species, Biochemical pharmacology; 37(3):409-13.

Pamla C. Champe and Richard A. Hervery, 1994. LiPPoncotts Illustrated Reviews*;* Biochemistry. 2nd Ed. J.B LiPPoncott Company PP 649-653, 808-809.

Park, BH., Rho, HW., Park, JW., Cho, CG., Kim, JS., Chung, HT. et al., 1995. Protective mechanism of glucose against Alloxan-induced pancreatic beta-cell damage. *Biochemical and biophysical research communications* 210(1):1-6.

Peter Lea and Richard C. Leegood, 1993. Plant Biochemistry and Molecular Biology. *5th Ed PP 413-418.*

Romain H. Raemmackers, 2001. Crop Production in Tropical Africa. 6th Ed. Spectrum Books Ltd PP 437-440.

Satyanarayana, U. 2005. Biochemistry 2nd Ed Anumblia seen Books and Allied Ltd. PP 513-516, 906.

Shamley, P. and Luz, L. 2003. The Impact of Forest Degradation on Medicinal Plant Use and Implication for the Health Care. 8th Ed. Taylor and Frances Press Ltd PP 110.

Sofowara, A.A. 1993. Medicinal Plant and Traditional Medicine In Africa. Spectrum Books Ltd Ibadan Nigeria PP 281-283.

Tapsell, L.C. 2006. Health Benefit of Herbs and Spices; the Past the Present the Future. Knowledge House Publishers PP 413-416.

Thomas M. Devlin, 2006. Textbook of Biochemistry with Clinical Correlation.6th Ed. Willey Liss Press. PP 808-810.

Tierney, L.M., McPhac and Papadakis, M.A. 2002. Current Medical Diagnosis and Treatment. International Edition McGrawhill Ltd. PP 438-449.

Tindall, H.D. 1983. Vegetables in Tropics. 4th Ed. Tonad Publishers PP 93-98.

Trease and Evans, C.W. 1996. Tress and Evans Pharmacognosy. 14th Ed. W.B. Sunders Company Ltd London. PP 268-274.

Tyrberg, B., Andersson, A. and Borg, LA. 2001. Species differences in susceptibility of transplanted and cultured pancreatic islets to the beta-cell toxin Alloxan. General and comparative endocrinology; 122(3):238-51.

****