VOL. 8, NO. 4, APRIL 2013 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science

www.arpnjournals.com

BIOCHEMICAL EVALUATION OF LIMA BEANS (*Phaseolus lunatus*) IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

This study was conducted to determine the effect of heat- treated lima beans (*Phaseolus lunatus*) on the serum glucose level and some biochemical parameters in alloxan-induced diabetic rats. Albino rats of wistar strain weighing between 125g to 150g were induced with single freshly prepared alloxan monohydrate (150 mg/kg body weight). Diabetes was confirmed after seven-two hours in alloxan-induced rats showing fasting blood glucose levels ≥ 200mg/dl. The rats were randomly divided into four (4) experimental groups (n = 4). Group I (Normal control fed with normal rat chow), Group II (Control diabetic group fed with normal rat chow), Group III (Diabetic rats treated with test diet: 70% heat treated lima beans + 30% rat chow) and Group IV, (Diabetic rats fed with normal rat chow and 0.5g/Kg body weight of glucophage). After 21 days the animals were sacrificed and blood samples were collected for serum glucose and other biochemical parameters evaluation. Changes in the animal body weights were also measured within the period. From the results it was observed that treatment of rats with Lima beans compensates for the reduction of body weight, and caused an increase in the body weight of the treated rats (+11.5%) in contrast to 24.8% reduction observed in diabetic control. In the same order, serum glucose significantly decreased ($p<0.05$) after the 21-day treatment compared to diabetic control. The extent of reversal of hyperglycemia in the lima beans treated animals compared well with the glucophage treated group. The results therefore showed that heat treated Lima beans has a significant ($p<0.05$) hypoglycaemic effect in diabetic rats and moreover, elevations in the measured biochemical parameters were significantly ($p<0.05$) attenuated in rats fed with heat treated lima beans. It was concluded that the consumption of heat treated lima beans produced a significant hypoglycaemic and hypolipidemic effects in diabetic rats. In addition heat treated lima beans is capable of protecting the liver and the kidney functions in alloxan-induced diabetic rats as shown in the activities of serum enzymes and other biochemical parameters examined.

Keywords: lima beans, diabetic rats, alloxan, heat-treated, glucophage.

INTRODUCTION

It has been estimated that there are 135 million people in the world with diabetes and that this would rise to 380 million by 2025, this report also highlighted the fact that low and middle income countries will bear the brunt of the increase with Africa contributing significantly to this rise (King *et al*., 1998; WHO, 1999). In Nigeria more than 1.71 million citizens above 15 years are diabetic, 70, 000 children under 15 years develop insulin dependent diabetes each year (Winifred, 2008). Diabetes mellitus and other numerous pathological events such as atherosclerosis and inflammatory processes are associated with the generation of Reactive Oxygen Species (ROS) and consequently the induction of several chain reactions among them, lipid peroxidation (Grober, 2010). Evidence suggests that oxidative cellular injury caused by free radicals contributes to the complications of diabetes mellitus (Baimbolkar and Sainani, 1995). Some of these radicals are extremely reactive and therefore interact with some vital macromolecules including lipids, nucleic acids and protein (Nia *et al*., 2003). Chronic hyperglycaemia during diabetes causes glycation of body protein that in turn leads to secondary complications affecting eyes, kidney, nerves and artery (Sharma, 1993).

Despite considerable progress in the treatment of diabetes by oral hypoglycaemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. Insulin therapy for example affords glycaemic control in diabetes, yet its shortcomings such as ineffectiveness on oral administration, short shelf life, the requirement of constant refrigeration, fatal hypoglycaemia in event of excess dosage, reluctance of patients to take insulin injection and above all the resistance due to prolonged administration limits its usage (Kasiviswanath *et al*., 2005).

Diet has been named as one of the major causative factors for the prevalence of the disease (Omorogiuwa *et al*., 2010); therefore, dietary modification is probably the simplest and cheapest form of diabetes treatment and is even the clinically recommended primary therapy in type 2 diabetes (Mshelia *et al*., 2005; Atangwho *et al*., 2012). For this reason, Patients with diabetes need dietary recommendations that are not just supported by scientific evidence, but that can be easily understood and translated into everyday life (Atangwho *et al*., 2012). To achieve the goals and objectives of dietary therapy, it is important that diabetic patients are provided with dietary guidelines appropriate to their cultural situations, since Africa and other developing countries have no standard dietary therapy in diabetes management (Mshelia *et al*., 2005; Atangwho *et al*., 2012). Also availability and affordability of the foods must be considered to prevent poor compliance with the diabetic diet.

Grain legumes are a major source of cheap proteins for humans in West Africa; they are typically recommended foods because of their content of dietary fiber, protein, vitamins, and minerals. In Nigeria lima beans, pigeon peas, African yam beans and jackbeans are popular legumes consumed in many Communities (Aletor and Aladetimi, 1989; Edem *et al*., 1990). Chemical composition of these grain legumes were shown to contain high quantities of proteins, amino acids, fiber and minerals (Apata and Ologhobo, 1994a, b). Their high intake has been associated with reducing the risk of developing diabetes, hypertension, animal's cancer and hypercholesterolemia (Gardner *et al*., 2005; Anderson, 2005). The aim of this study was to investigate the efficacy of heat treated Lima beans in prevention of elevated serum glucose levels; and on some vital biochemical parameters in the alloxan-induced diabetic rats.

MATERIALS AND METHODS

Plant material

Lima beans (*Phaseolus lunatus*) used for the experiment were purchased from Sabo market, Kaduna, Kaduna state, Nigeria. The beans were sorted out to obtain injury/disease free beans and identified to species level at the Department of Biology, Bingham University, Karu, Nasarawa state, Nigeria.

Animal model

Adult white wistar rats (*R. norvegicus*) weighing 125g to 150 g bred in the animal house of College of Medicine, Bingham University, Karu, Nasarawa state,Nigeria, were used for the study. They were fed ad labitum with water and feed (Guinea feed). They were allowed to acclimatize under standard photoperiodic condition in a clean rat cage for 21 days in the animal house of College of Medicine, Bingham University Karu, Nasarawa state. All animals were maintained under the standard laboratory condition for temperature (26 ± 2^{o}C) and light (12 hours day length) and were allowed free access to feed and water.

Preparation of experimental diet

The whole seeds that were free from injury and insects' invasion were sorted out and washed in distilled water and were added to boiling distilled water (1:5w/v) at 100°C and cooked on an electric hot plate for 3h. The cooked seeds were drained to remove the water and dried at 70°C in an oven for 16 h. The dried seeds were cooled in a dessicator and milled to powder in a warring blender. The bean flour was stored in an air - tight container at -10°C until used as feed for the rats.

Experimental design and induction of diabetes mellitus

The study was carried out for six weeks in the Department of Biochemistry, Bingham University Karu Nasarawa state, Nigeria, between July and August, 2011: The rats were allowed to acclimatize under standard photoperiodic condition in a clean rat cage for 21 days in the animal house of College of Medicine, Bingham University Karu, Nasarawa state (All animals were maintained under the standard laboratory condition for temperature (26 ± 2^{o}C) and light (12 hours day length) and were allowed free access to feed and water).

Two grams of crystalline powdered alloxan monohydrate (sigma) was dissolved in 50mls of normal saline to yield a concentration of 40mg/ml. 150 mg/kg body weight of alloxan per rat was administered intraperitoneally after overnight fast (access to water only) to the rats except the rats in the normal group. The serum glucose levels were determined after 72 hours; diabetes was confirmed in alloxan-treated rats showing fasting blood glucose levels ≥ 200mg/dl. The experimental animals were then divided into groups and treated accordingly:

a) Normal rats fed with normal rat chow (normal control)
b) Diabetic rats fed with normal rat chow only (diabetic control)
c) Diabetic rats fed with test diet (70% lima beans + 30% rat chow)
d) Diabetic rats fed with normal rat chow and 0.5g/Kg body weight of glucophage

Body weights of all the rats were taken before and after the treatment by electronic balance. At the end of the experiment, the rats were sacrificed under sodium pentobarbitone anaesthesia (Nafisa, 2007). Whole blood was collected via cardiac puncture using sterile syringes and needles and emptied into plain tubes, allowed to clot for about two hours. The clotted blood was thereafter centrifuged (Homef, FC 06) at 3, 500 rpm for 30 minutes to recover serum from clotted blood. Serum was separated with sterile syringes and needles and stored frozen until used for biochemical analysis.

Assays

The blood glucose in a protein free serum was determined as described by Sood (Sood, 1999). Urea, creatinine and total bilirubin concentrations were determined by the methods of Patton and Crouch (1977); Henry *et al*. (1974) and Pearlman and Lee (1974), respectively. Total cholesterol was measured by the procedure described by Allain *et al*., (1974). Serum aspartate amino transferase (AST) and alanine amino transferase (ALT) activities were estimated with the Randox reagent kit using 2, 4-dinitrophenylhydrazine as substrate according to the method described by Reitman and Frankel (1957). Protein content was determined by the method of Lowry *et al*. (1951). All the assays were carried out at the Department of Biochemistry and Chemistry, Bingham University Karu Nasarawa state, Nigeria.

VOL. 8, NO. 4, APRIL 2013 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science

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Statistical analysis

The data are expressed as mean ± SD. Readings within a group were compared using the one-way ANOVA analysis and readings between groups were compared using the Independent sample t- test. Statistical analysis was performed using SPSS (Version 17). A level of $p < 0.05$ was considered to be significant results.

RESULT

Table-1. Effect of Lima beans and Glucophage on serum glucose levels in diabetic rats.

Groups	Treatment	**Day 3** (72 hours after induction) (mg/dl)	**Day 21** (mg/dl)
1	Normal control	82.50 ±5.00	85.25 ± 2.97[d]
2	Control diabetic group	208.0± 2.16	238.25± 4.19[b,c]
3	Test diet (70% lima beans + 30% rat chow).	235.75 + 4.87	97.75.0 ± 3.78[a,d]
4	Glucophage (0.5g/Kg)	247.25±5.74.3	105.75 ±3.30[a,d]

Values are expressed as Mean ± SD, n = 4, a = significant decrease at $p<0.05$ compare with Day 3; b = significant increase at $p<0.05$ compare with Day 3; c = significant difference at $p<0.05$ compare to Normal Control on day 21, d = significant difference at $p<0.05$ compare to Diabetic Control on day 21.

The administration of heat treated Lima beans to the diabetic rats significantly ($p< 0.05$) reduced plasma glucose level when compared with diabetic group.This reduction was not enough to reach normal rats, but it was significantly lower when compared with the diabetic control.

Table-2. Effect of Lima beans and Glucophage on serum Urea, Creatinine, Bilirubin and Cholesterol levels in diabetic rats.

Groups	Treatment	**Urea** (mg/dl)	**Creatinine** (mg/dl)	**Bilirubin** (mg/dl)	**Cholesterol** (mg/dl)
1	the Normal control	29.35 ±0.74[a]	0.74± 0.02[a]	0.71 +0.01[a]	84.18 ± 0.89[b]
2	control diabetic group	85.23±1.67[b]	1.72±0.03[b]	2.77 ±0.03[b]	145.92 ±2.96[c]
3	Test diet (70% lima beans + 30% rat chow).	32.15±1.58[a]	0.86± 0.02[a]	0.74±0.04[a]	52.92 ± 1.96[a]
4	Glucophage(0.5g/Kg)	32.69± 1.81[a]	0.96 ± 0.06[a]	0.72 ±0.01[a]	101.45 ± 1.24[b]

Given values represent the Mean ± SD of 4 observations
Values with different superscript in the same column are significantly different at $p< 0.05$

As shown in Table-2, alloxan produced significant hyperlipidemic action, where a significant increase was recorded in the levels of serum cholesterol when compared with normal group. Post-treatment with heat treated lima beans to diabetic rats produced significant reduction in the levels of serum cholesterol when compared with diabetic group, also, the alloxan produced significant increase in the levels of serum creatinine; bilirubin and urea when compared with normal group, while, post administration of heat treated lima beans to the diabetic rats significantly reduced the levels of serum creatinine; bilirubin and urea when compared with the diabetic group, but no significant changes were observed when compared with the normal rats. This indicates that, post-treatment with lima beans can normalise the plasma creatinine, urea and bilirubin.

VOL. 8, NO. 4, APRIL 2013 ISSN 1990-6145
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Table-3. Effect of Lima beans and Glucophage on serum ALT, AST and Total protein levels in diabetic rats.

Groups	Treatment	**ALT** (IU/L)	**AST** (IU/L)	**Total protein** (mg/dl)
1	the Normal control	12.75 ±8.554^{a}	12.50± 3.32^{a}	6.75 ±0.06^{b}
2	control diabetic group	45.50 ±13.43^{b}	35.75 ±8.46^{b}	4.07 ± 0.04^{a}
3	Test diet (70% lima beans + 30 % rat chow)	16.50 ±10.41^{a}	12.75± 6.02^{a}	7.45 ±0.04^{b}
4	Glucophage (0.5g/Kg)	21.50 ± 6.45^{a}	16.25 ± 4.79^{a}	6.45 ± 0.05^{b}

Given values represent the Mean ± SD of 4 observations
Values with different superscript in the same column are significantly different at $p< 0.05$

As shown in Table-3, alloxan produced significant decrease in total protein. Post-treatment with heat treated lima beans to diabetic rats produced significant increase in the levels of total protein when compared with diabetic group; also, the alloxan produced significant increase in the levels of plasma AST and ALT when compared with normal group, while, post administration of heat treated lima beans to the diabetic rats significantly reduced the levels of plasma ALT and AST when compared with the diabetic group, but no significant changes were observed when compared with the normal rats. This indicates that, post-treatment with lima beans can normalize the serum AST and ALT

DISCUSSIONS

Dietary modification is probably the simplest and cheapest form of diabetes treatment. For this reason, patients with diabetes need dietary recommendations that can be easily understood and translated into everyday life (Atangwho *et al*., 2012). To achieve the goals and objectives of dietary therapy, it is important that diabetic patients are provided with dietary guidelines appropriate to their cultural situations. (Mshelia *et al*., 2005).

In this study, oral administration of lima beans, a popular legume consumed in many Communities in Nigeria (Aletor and Aladetimi, 1989; Edem *et al*., 1990) leads to a decrease in glucose level. It was observed that oral administration of lima beans was as effective as glucophage as hypoglycaemic agent though the drug had a better effect (Table-1). A statistically significant decrease in serum glucose concentrations after 3 weeks was observed with the lima beans treatment in contrast to the diabetic control, although this reduction was not enough to reach normal rats glucose levels, but it was not significantly higher when compared with the normal group, as shown in Table-1. This in line with some findings which show that higher legume intakes were associated with lower incidence of diabetes mellitus, lower body mass index (BMI), blood pressure, and serum total cholesterol (TC) compared with lower legume intakes (Darmadi-Blackberry, 2004). Also, beans has been shown to contain Phenol compounds which have been reported to reduce the risk of diabetes (Yang *et al*., 2011); Villegas *et al*. (2008) examined the association between the consumption of legume and diabetes over an average follow-up of approximately five years in the Shanghai Women and concluded that total legume consumption was associated with a decrease in risk of Type 2 diabetes (Villegas *et al*., 2008).

Injection of alloxan caused an increase of serum cholesterol; the marked hyperlipidemia that characterizes the diabetic state may therefore be as a result of the uninhibited actions of lipolytic hormones on the fat depots due to the absence of insulin (Goodman and Gilman, 1985). The treatment of the induced-diabetic rats with Lima beans caused reduction in the levels of serum cholesterol (Table-2), the same results have been observed by others in experimental rats, when heat-treated legumes, were fed to hypercholesterolemic rats (Zulet and Martinez, 1995; Singh *et al*., 2002; Oboh and Omofoma, 2008).

These results may be due to the presence of saponins in Lima beans. Saponins form strong insoluble complexes with cholesterol and bile making them unavailable for absorption, this mixture is then removed from the body through the normal elimination process, increased bile acid excretion may cause compensatory increase in bile acid synthesis from cholesterol in the liver. As the body needs more cholesterol for bile acid production used for digestion, the liver removes cholesterol from the blood stream through increase hepatic LDL-receptor levels, increase hepatic uptake of LDL-cholesterol and aid its catabolism to bile acids, thus lowers serum cholesterol (Oboh and Omofoma, 2008). Also, saponins are known to inhibit pancreatic lipase, leading to greater fat excretion due to reduced intestinal absorption of dietary fats (Jadeja *et al*., 2010). The saponin content of raw and processed lima beans has been determined and found to be 1.26g/Kg for the raw and 732.3g/Kg for the heat treated samples respectively (Oboh *et al*., 1998; Oboh and Osagie, 2003).

The lima beans used as test diet were cooked without dehulling. The fiber content of the legume could have a contributory role in the reduction of cholesterol levels (Oshodin and Adeladun, 1993); as the role of dietary fiber in the reduction of cholesterol has been reported (Glore *et al*., 1994). Therefore, the presence of saponins and fiber in the heat treated lima beans is

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probably responsible for the cholesterol lowering ability of the beans.

The effect of Lima beans on the kidney functions was assessed by the determination of the levels of serum creatinine, and urea, as level of serum urea and creatinines are often regarded as reliable markers of renal function (Adelman *et al.*, 1981; Davis and Berdt, 1994). Thus, elevations in the serum concentrations of these markers in diabetic rats which are significantly ($p < 0.05$) higher compare to the normal control group are indication of renal injury (Adelman *et al.*, 1981); that is, diabetes could lead to renal dysfunction. The treatment of alloxan induced diabetic rats with Lima beans, significantly reduced urea ($p < 0.05$) in serum compared to the mean value of diabetic group (Table-2). Similarly, the elevation of creatinine level caused by diabetes was declined after administration of Lima beans ($p < 0.05$), compared with the diabetic group (Table-2). From the data obtained, it can be concluded that treatment with Lima beans produced a significant improvement of the impaired kidney functions in alloxan induced- diabetic rats.

The increase in the activities of serum AST and ALT (Table-3) in diabetic rats indicated that diabetes may induce hepatic dysfunction. In support of this finding, Larcan *et al*. (1979) had observed that liver was necrotized in diabetic patients. This increase is an indicative of cellular leakage and loss of functional integrity in liver (Sallie *et al*., 1991). In particular, the increase in the serum level of ALT is indicative of liver damage (Lin and Wang, 1986; Ngaha *et al*., 1989). These enzymes are located in the cell cytoplasm and are emptied into the circulation once the cellular membrane is damaged (Lin and Huang, 2000). Therefore, the increment of the activities of AS T and ALT, in serum may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (Navarro *et al*., 1993), which gives an indication on the hepatotoxic effect of alloxan. On the other hand, treatment of the diabetic rats with Lima beans caused reduction in the activity of these enzymes in the serum (Table-3) compared to the mean values of diabetic group. The reduction in the levels of marker enzymes, ALT and AST in rats treated with heat treated lima beans as reported in this study is also in agreement with the commonly accepted view that serum levels of transaminases return to normal with healing of hepatic parenchyma and the regeneration of hepatocytes. This is also similar to the findings of Thabrew *et al*., (1987) who found that serum transaminases returned to normal activities with the healing of hepatic parenchyma and regeneration of hepatocytes. A possible explanation for the differential effects of Lima beans on the activities of AST and ALT in serum is that these treatments may heal the liver damage induced by alloxan.

Furthermore, the improvement of the liver damage by oral administration of Lima beans could be confirmed by studying their effects on the level of serum bilirubin. The results in Table-3 showed that the experimentally induced diabetes increased ($p < 0.05$) the level of serum bilirubin compare to the control. Also, Lima beans intake produced significant ($p < 0.05$) decrease in serum bilirubin of alloxan-diabetic rats compared to the diabetic rats. Rana *et al*. (1996) reported that the increase in serum bilirubin (hyper-bilirubenimia) may resulted from the decrease in liver uptake, conjugation or increase bilirubin production from haemolysis, which has been shown to result in decrease in total erythrocyte counts (El-Demerdash *et al*., 2005). Also, the elevation in serum bilirubin indicates liver damage as confirmed by the changes in the activities of liver enzymes (AST and ALT) (Table-3). Thus, the increased level of bilirubin observed in rats in diabetic control group could be attributed to liver damage. However, the decrease in bilirubin levels in treated rats is indicative of reversal of liver damage by lima beans.

The decrease in total protein concentrations in the serum of diabetic rats may be ascribed to (i) a decreased amino acid uptake (Garber 1980) (ii) a greatly decreased concentration of a variety of essential amino acids (Brosnan and Man, 1984) and (iii) an increased conversion rate of glycogenic amino acid to CO_2 and H_2O (Mortimore and Manton, 1970). Administration of Lima beans maintained the protein level near normal.

As shown in Table-4, Induction of diabetes, prevented increase in body weight in diabetic rats compared to the weight gain found in the control rats. This is in line with the observation of Oduye and Adadevoh (1976) that high blood urea levels obtained are associated with excessive tissue catabolism. Treatment of rats with Lima beans compensates for the reduction of body weight, and caused a significant increase in the body weight of treated rats. The loss in weight of the diabetic rats has also been supported by a study which shows that during diabetes mellitus, the blood sugar increases and results in lack of sugar in the cells; forcing, the cells to use amino acids and fatty acids as a source of energy which eventually leads to the reduction of proteins and fats in the body which causes body weight loss, that is, the animals will fall back on the stored energy when there is insufficient glucose(Esonu *et al*. 2001).

Table-4. Percentage change in the body weight of the treated rats.

Group	Treatment	Day 0 (g)	Day 21 (g)	% Change in body weight
1	Normal control	140.80±2.05	173.24± 2.19	+23.04
2	Diabetic control group	134.06± 3.86	100.80 ± 1.97	- 24.80
3	Test diet (70% lima beans + 30% rat chow)	119.63± 3.44	133.39±2.33	+11.50
4	Glucophage(0.5g/Kg)	132.27±2.21	148.01 ± 2.23	+ 11.90

Values given represent the Mean± SD of 4 observations

CONCLUSION

From the observations above it can be concluded that the consumption of heat treated lima beans produced a significant hypoglycaemic and hypolipidemic effects in diabetic rats. In addition heat treated lima beans is capable of protecting the liver and the kidney functions in alloxan-induced diabetic rats as shown in the activities of serum enzymes and other biochemical parameters examined.

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