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EFFECT OF AQUEOUS SOYABEAN EXTRACT AND METFORMIN ON SOME BIOCHEMICAL PARAMETERS IN ALLOXAN- INDUCED DIABETIC RATS

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ABSTRACT

Soya bean is a staple food with great nutritional value. In this study, the effect of aqueous soya bean extract was carried out in Alloxan induced diabetic rats for 7days. The phytochemical screening indicated the presence of alkaloids, flavonoids, tannins, cardiac glycosides, terpenes, steroids, phenols and resins. Diabetic rats exhibited high blood glucose, cholesterol, Triglycerol (TG) and Low Density Lipoprotein (LDL) while High Density Lipoprotein (HDL) wasvery low. The continuous administration of extract at150 mg/kg b.wt. for 7days significantly ($P<0.05$) reverse these effects on cholesterol, TG, HDL and LDL while a similar result was also observe for metformin (14.2 mg/kg b.wt) treated group. The extract had no significant effect ($P>0.05$) on serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) while serum alkaline phosphatase (ALP) was significantly increasing ($P<0.05$) when compared to the control. Although the extract could not significantly lower the blood glucose when compared to the control, a gradual reduction was observed on day7. The present study revealed that aqueous soya bean extract can effectively control some of the metabolic disorders that are associated with diabetics.

Keywords: Soya Bean, Metformin, Diabetics, Phytochemicals, Metabolic Disorder

INTRODUCTION

Diabetic is a complex disorder that affects metabolism in humans and other subjects, affecting several organs in the system. Diabetic is also one of the main threats to humans, as the global number of people with diabetic is estimated at 366 million in 2030 **[1]**. Management of diabetic is challenging, its treatments are often

associated with side effects as scientist are increasingly demanding for natural products with antidiabetic activity and fewer side effects **[2]**. Over 400 medicinal plants are present worldwide for the treatment of diabetic mellitus, only few of such have been subjected to scientific authentication as antidiabetic agents **[3]**. Other methods of treatment have also been employed which include dietary modifications and surgical treatment of the disorder **[4]**.

Metformin (Glucophage) is an oral antidiabetic in the biguanide class. It is a drug of choice for the treatment of type 2 diabetic, in particular, in overweight and obese people and those with normal kidney function **[5]**. Metformin is the only antidiabetic drug that has been conclusively shown to prevent the cardiovascular complications of diabetic. It helps reduce LDL cholesterol and triglyceride levels, and is not associated with weight gain.Metformin and glibenclamidearethe only two oral antidiabetics in the World Health Organization Model List of Essential Medicines **[6]**.

Soya bean isa staple food in Nigeria with great nutritional value. It is used in milk production, oil processing, livestock feeds, medical, industrial and human consumption **[7]**. Due to its affordability and high protein content, soya bean is important in household nutrition **[8]**. Studies have indicated that soya bean protein reduces cholesterol, triglycerol and low density lipoproteins in healthy persons, diabetic patient and in experimental rats **[9, 10, 11]**. Chloroform and alcohol extract of soya bean have been shown to possess antidiabetic activities **[12]**. However, his study evaluates the effects of aqueous soya bean extract using an extraction method which mimic a typical cooking process of lesser temperature.

MATERIALS AND METHODS

Assay Kits and Chemicals

Alloxan monohydrate used was products of Sigma Chemical Company, St. Louis, USA. Metformin Hydrochloride was a product of Jiangsu Ruinian Qianjin Pharmaceuticals Ltd, China. AST, ALT, bilirubin, cholesterol, triglycerides and HDL-C 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.

Experimental Animals

Twenty (20) adult Wistar rats with an average weight of 180±23 g, obtained from the Animal Holding Unit of the Department of Biochemistry, University of Jos, Jos, Nigeria were used for this study. The animals were housed in standard plastic cages and acclimatized for a period of two weeks. They were fed with rat pellet (Bendel Feeds Ltd, Ewu, Nigeria) and given

water *ad libitum*. The animals were randomly distributed into four groups of five animals each; Group I were control non –diabetic, non-treated rats which receive 0.2 ml of distilled water, Group II were Alloxaninduced diabetic rats which also receive 0.2 ml of distilled water, Group III were diabetic rats treated with aqueous Soya bean extract (150 mg/kg b. wt) and Group IV were diabetic rats treated with reference drug Metformin (14.2mg/kg b.wt).

Induction of Diabetics

Diabetics was induced by singleintraperitoneal (ip) injection of alloxan monohydrate (150 mg/kgwt) dissolved in normal saline. After 48 hours, blood was withdrawn from the animal's tail for blood glucose estimation with a glucometer (ACCU-CHEK, Roche Diagnostics). The animals with blood glucose level ≥ 250 mg/dl were considered diabetic and included in the research.

Preparation of Extracts

The Soya bean used was purchased from new market, Jos, Plateau state. It was dried and grinded to powdered form using a blender. 180 g of the powdered soya bean was then weighed and dissolved in 300ml of distilled water for 24 hours, the filtrate obtained with white handkerchief and allowed to dry in a hot air oven (40^{o}C). The extract was stored in an air tight container and was later reconstituted in distilled water to give the required dose of 150 mg kg^{-1}b.wtwhich was administered for 7 days.

Phytochemicals

Phytochemical tests were carried out using standard procedures as by Harborne, 1973, Trease and Evans, 1989 and Sofowora, 1993, as described by Njoku and Obi, 2009, **[13]**.

Serum Collection

On the eight day, the rats were anesthetised with ethyl 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. The blood sample was allowed to cloth and the serum was clearly remove and used for the assays.

Assay of Biochemical Parameters

Activities of alkaline phosphatase (ALP) were determined by the method of Wright *et al*., 1972, **[14]** while the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed by the method of Reitman and Frankel, 1957, **[15]**. Total cholesterol concentration in the serum was assayed by the method of Fredrickson *et al*., 1978, **[16]** while serum HDL-cholesterol concentration was determined using the method described by Albers *et al.*, 1980, **[17]**. Serum triacylglycerol concentration was determined by the method of Hainline, *et al*., 1980, **[18]**. Total proteins were assayed by Gornell, *et al*., 1949 as modified

by Plummer, 1978, **[19]**. Albumin and Bilirubin were estimated by the methods of Doumas, *et al.*, 1971, **[20]** and Jendrassik and Grof, 1938, **[21]** respectively.

Statistical Analysis

Data were presented as Mean ± SEM of 5 replicates andwere analyzed using Duncan multiple range test following one-way analysis of variance (ANOVA) using SPSS 16.0 computer software package (SPSS Inc., Chicago, U.S.A). Differences at $P<0.05$ were considered significant.

RESULTS

The phytochemical screening of the aqueous Soya bean extract indicated the presence of alkaloids, flavonoids, tannins, cardiac glycosides, terpenes, steroids, phenols and resins. The presence of saponins and balsams were not detected **(Table 1)**.

Aqueous Soya bean extract and metformin administered in this study showed varying effects on blood glucose of the rats. Metformin was able to compete more favourably in a similar pattern with the control group in lowering blood glucose level on Day 4 and Day 7. On like the metformin treated groups, the extract showed a difference at Day 7 of administration. This value however was significantly higher from both control and metformin treated groups **(Table 2)**.

Extract administered cause no significant alterations ($P>0.05$) in total cholesterol when compared to the control although metformin treated groups were significantly ($P<0.05$) altered from the diabetic rats. Extract also showed a comparable difference in Triglyceride levels in control and metformin group. HDL and LDL showed a significant difference ($P<0.05$) when compared to the Diabetic rats **(Table 3)**.

Aqueous Soya bean extract showed no significant difference ($P>0.05$) in Total cholesterol, Albumin and Direct Bilirubin when compared to the control and metformin treated groups. However, in Total Bilirubin, a significant decrease ($P<0.05$) was observed when compared with metformin treated group **(Table 4)**.

Activities of ALT and AST of Aqueous Soya bean extract treated rats showed no significant difference ($P>0.05$) when compared with the control. However, significant alterations were observed in AST and ALP assayed when the metformin treated groups where compared to diabetic rats **(Table 5)**.

Table 1: Phytochemicals of Aqueous Soya bean Extract

Phytochemicals	Aqueous Extract
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	-
Cardiac glycosides	+
Terpenes and Steroids	+
Phenols	+
Resins	+
Balsam	-

NOTE: + = Detected; - = Not Detected

Table 2: Effects of Aqueous Soya bean Extract on Blood Glucose

Blood Glucose (mg/dl)				
Groups	DAY 0	DAY 1	DAY 4	DAY 7
Control	80.23±3.23	84.45±5.48^{a}	82.00±6.37^{a}	83.34±5.43^{a}
Diabetic	310.18±5.23	507.37±9.13^{c}	510.00±9.03^{c}	515.01±9.10^{d}
Diabetic + Extract	302.04±4.24	540.83±43.06^{d}	538.75±45.53^{d}	457.50±31.75^{c}
Diabetic + Metformin	309.63±4.23	433.25±60.29^{b}	254.25±62.63^{b}	294.75±60.70^{b}

NOTE: Values are Means ± SEM, n = 5; Values in Each Column with Different Letter Superscripts are Significantly Different (P<0.05)

Table 3: Effects of Aqueous Soya Bean Extract on Serum Lipid Profile

Lipid Profile (mmol/L)				
Groups	CHOL	TG	HDL	LDL
Control	4.17±2.17^{ab}	1.11±0.17^{b}	0.57±0.07^{a}	1.14±0.15^{a}
Diabetic	4.53±0.27^{b}	1.42±0.09^{c}	0.41±0.13^{b}	1.21±0.10^{b}
Diabetic + Extract	4.17±0.35^{ab}	1.08±0.35^{ab}	0.53±0.06^{a}	1.04±0.10^{a}
Diabetic + Metformin	4.07±0.61^{a}	1.05±0.37^{a}	0.52±0.06^{a}	1.10±0.12^{a}

NOTE: Values are Means ± SEM, n = 5; Values in Each Column with Different Letter Superscripts are Significantly Different (P<0.05)[CHOL = Total Cholesterol; TG = Triacylglycerol; HDL = HDL-Cholesterol; LDL = LDL- Cholesterol

Table 4: Effects of Aqueous Soya Bean Extract on Some Serum Parameters

Serum Parameters				
Groups	Total Protein(g/L)	Albumin (µmole/L)	Total Bilirubin (µmole/L)	Direct Bilirubin (µmole/L)
Control	75.00±0.82^{a}	43.60±0.95^{a}	14.00±0.91^{a}	3.83±0.33^{a}
Diabetic	74.00±4.08^{a}	35.00±1.68^{a}	15.40±0.88^{a}	4.11±0.25^{a}
Diabetic + Extract	71.00±5.16^{a}	34.50±2.65^{a}	14.25±0.91^{a}	3.95±0.38^{a}
Diabetic + Metformin	72.25±3.25^{a}	37.25±4.11^{a}	11.25±0.96^{b}	3.50±0.2^{a}

NOTE: Values are Means ± SEM, n = 5. Values in Each Column with Different Letter Superscripts are Significantly Different (P<0.05)

Table 5: Effects of Aqueous Soya Bean Extract on Serum Marker Enzymes

Serum Marker Enzymes (U/L)			
Groups	ALT	AST	ALP
Control	11.00±1.68^{ab}	16.01±1.83^{ab}	20.10±1.68^{a}
Diabetic	12.00±1.68^{ab}	17.01±0.41^{b}	32.00±5.16^{b}
Diabetic + Extract	13.75±1.71^{b}	17.75±1.71^{ab}	30.25±2.98^{b}
Diabetic + Metformin	9.25±0.65^{a}	14.50±1.08^{a}	15.75±5.74^{a}

NOTE: Values are Means ± SEM, n = 5. Values in Each Column with Different Letter Superscripts are Significantly Different (P<0.05)

DISCUSSION

Several organs are implicated in diabetic condition; these include the pancreas, kidney, liver, eye and other in long time complications. Making its treatment multidisciplinary. Several botanicals extracts have proven to be effective in treatment of diabetics and others related complications associated with it **[22, 23]**. Dietary modifications are also being employed in its management. In the present study, aqueous soya bean extract was investigated for its effects on biochemical parameters in Alloxan induced diabetic rats. Phytochemical screening of the aqueous soya bean extract indicated the presence of alkaloids, flavonoids, tannins, cardiac glycosides, terpens, steroids, phenols and resins. However, saponnins and basams were not detected **(Table 1)**. Flavonoids and phenolics have been associated with increase insulin secretion and scavenging free radicals generated during diabetic disorder **[24]**. Alloxan, a toxic glucose analogue, is known to act as a cytotoxin for beta cells of the islet of Langerhans, causing diabetes cell necrosis **[25]**. Flavonoids are also known to regenerate these damage beta cells in diabetic rats **[26]**. Cardiac glycosides present in the aqueous extracts may also be responsible for reduction of the effects of diabetic complications **[27]**.

Diabetic mellitus is characterize by increase in serum glucose concentration **[28]**, this make glucose a necessary marker in diabetes. In the study, glucose level in experimental rats where significantly increased ($P<0.05$) throughout the experimental period. Administration of a reference drug metformin remarkably reduced these high levels of glucose. However, aqueous soya bean extract administered during the period of the experimental could not significantly lower the glucose level to the control or metformin treated groups. This could be due to the absence of saponins in the phytochemicals screening conducted on aqueous extracts. Saponins have been attributed to reduce serum glucose levels **[29]**.

Total cholesterol in diabetic rats increased when compared to the control and metformin treated rats (significantly) as shown in Table 3. This is possibly due to increase in mobilization of free fatty acids from peripheral fat deposited. Administration of aqueous soya bean extract reduced the serum total cholesterol, TG and LDL concentrations while itsignificantly increase ($P<0.05$) the concentration of HDL (good cholesterol). Lipids no doubt play vital role in pathogenesis of diabetic mellitus, aqueoussoya bean extract reverse significantly ($P<0.05$) these abnormalities observed in lipid metabolism.

Soya bean contain essential amino acids **[30]** which makes it highly recommended in disease conditions with protein disorder.Total protein, albumin and bilirubin in metformin and aqueous soya bean extract were comparable to the control rats. Albumin is an essential component of blood, transporting essential fatty acids and other substances such as drug and hormones.

Enzyme level of transaminases increased in diabetic rats treated with alloxan **(Table 5)**. Increase in their levels indicates their activeness in the absence of insulin. These result inincreasedavailability of amino acids in diabetic as well as increase in glucogenesis and ketogenesis observed in diabetes **[31]**. Administration of aqueous soya bean extract and metformin result in lowering the levels of ALT, and AST to levels comparable to the control groups. Restoration of these enzymes including ALP to near normal may be due to the presence of flavonoids in the aqueous soya bean extract which have shown hepatoprotective properties **[32]** and reversing the enzymes leakages from hepatocytes.

CONCLUSION

From this study, it is evident that aqueoussoya bean extract is rich in phytochemicals which justified the various activities observed in treated rats. Hypercholesterolemia, hypertriglyceridemia and other conditions observed in diabetic rats were attenuated by the administration of aqueous soya bean extract. Although the glucose level was not significantly reduced during the period of administration, the extract effectively controls some of the metabolic disorder that is associated with diabetic.

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