Effect of Aqueous Sweet Potato Leaf Extract and Metformin on Some Biochemical Parameters in Streptozotocin-Induced Diabetic Rats

Rafiu A. Aa*, Luka C. Db

aQuality Assurance Department, Nasco Foods Limited Jos, P.O.Box 716, Jos, Plateau State, Nigeria
bDepartment of Biochemistry, University of Jos, P.M.B 2084, Jos, Plateau State, Nigeria

Email: akeemrafiu@gmail.com
Email: carrll@yahoo.com

Abstract

Sweet potato [Ipomoea batatas (L.) Lam] is the sixth most important food crop in the world. The sweet potato roots are a good source of carbohydrates, while sweet potato top (leaves) contain additional nutritional components in much higher concentrations than in many other commercial vegetables. In this study, the effect of aqueous sweet potato leaf extract was carried out in streptozotocin-induced diabetic rats for 28 days. The phytochemical screening indicated the presence of flavonoids, tannins, terpenes, steroids, phenols, resins and absence of alkaloids, cardiac glycosides. Diabetic rats exhibited high blood glucose, cholesterol, Triglycerol (TG) and Low Density Lipoprotein (LDL) while High Density Lipoprotein (HDL) was very low. The continuous administration of extract at 400 mg/kg body wt. for 28 days significantly (P < 0.05) reverse these effects on cholesterol, TG, HDL and LDL while a similar result was also observe for metformin; Rat dose = human dose (500 mg) / Rat bodyweight x 7 treated group. The extract decreased significantly (P > 0.05) serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), total and direct bilirubin which was significantly increased (P < 0.05) when compared to the control. After Administration of the plant extract, high level of cholesterol, triglycerides, and low density lipoproteins(LDL) were decreased (P < 0.05) while the HDL (high density lipoproteins) , total protein, and albumin were significantly increased (P < 0.05). The present study revealed that aqueous sweet potato extract can effectively control some of the metabolic disorders that are associated with diabetics.

Keywords: Metformin; Sweet Potato; Phytochemicals; Metabolic Disorder; Diabetics.

* Corresponding author.
1. Introduction

Diabetes Mellitus is a complex disorder that affects metabolism in humans and other subjects affecting several organs in the system. The incidence of diabetes is gradually increasing and is projected to be the 7th (seventh) cause of death worldwide by 2030 [1]. With the greatest increase in prevalence expected to occur in Africa and Asia due to urbanization and lifestyle changes [2]. Management of diabetic is challenging, its treatments are often associated with side effects as scientists are increasingly demanding for natural products with antidiabetic activity and fewer side effects [3]. 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 [4]. In Nigeria, the history of traditional medicine shows case thousands of plant species which have been used for many years in the practice of healing traditionally [5]. Other methods of treatment have also been employed which include dietary modifications and surgical treatment of the disorder [6]. 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 [7]. 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 glibenclamide are the only two oral antidiabetics in the World Health Organization Model List of Essential Medicines [8]. Sweet potato leaves is a staple food vegetable and are cooked as such in many parts of the world. They are rich in vitamin B, β-carotene, iron, calcium, zinc and protein, and the crop is more tolerant of diseases, pests and high moisture than many other leafy vegetables grown in the tropics. Because sweet potato tops can be harvested several times a year, their annual yield is much higher than many other green vegetables [9].

2. Materials and Methods

2.1. Assay Kits and Chemicals

Streptozotocin was obtained from Sigma–Aldrich company, U.S.A. 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 reagents and chemicals used were of analytical grade, products of Sigma U.S.A. Aldrich; obtained from reputable Scientific and Chemical companies. All solutions were prepared in distilled water.

2.2. Experimental Animals

Adult male Wister strain albino rats weighing from 150-200g were used to carry out the study. A minimum of twenty (20) adult albino rats were divided into 4 groups with 5 rats each. The rats were identified as head, back, tail, right hand, and left hand throughout diabetogen induction and plant aqueous treatment. After randomization into various groups and before the start of the experiment, the rats were acclimatized to the animal house condition [10,11,12]. The rats were maintained on a standard rat feed consisting (70% Carbohydrate, 14.50% protein, 7.0% Fat, 7.20% fibre and 1.20% mineral) for 28 days and given water ad libitum. The animals were grouped as follows; A- Normal control. B- Diabetic control . C- Diabetic + Plant
extract (400mg/kg) body weight. D – Diabetic + Standard drug (Metformin; Rat dose = human dose (500mg) /
Rat average body weight x 7.

2.3. Induction of Diabetes

Diabetes was induced by Single intraperitoneal (ip) injection of streptozotocin (55 mg/kgwt) dissolved in 0.01M
sodium acetate buffer with pH 4.6. After 48 hours, blood was withdrawn from the animal’s tail for blood
-glucose estimation with a glucometer (On call-plus, Roche Diagnostics). The animals with blood glucose level ≥
126 mg/dl were considered diabetic and included in the research.

2.4. Preparation of Extracts

Ipomoea batatas leaves was bought from building materials vegetable market, Jos-south Local Government area
of Plateau state. The plant root and leaf was identified and verified with a voucher number (FHJ 232) at the,
Herbarium Department, Federal College of Forestry Jos, Plateau state. The plant leaf was collected and removed
from the stem and air dried at room temperature under shade. The dried plant leaf was pounded to powdery form
using pestle and mortar. It was then sieved into a fine powder using mesh size of 180 micron. The powder was
stored in an air-tight container until required for use. The preparation of the plant extract was carried out using
hot water.100g of the fine powder was boiled in one (1) Litre of distilled water for 15 minutes (to ensure
maximum extractions of phytochemicals) using hot plate. The mixture was allowed to stand for 30 minutes
before filtering using whatman filter paper No 1 to remove all unextractable matter. The filtrate was dried in
the autoclave at a temperature of 50 -60 °C for two weeks. The solid extract was kept in the refrigerator in an air
tight container to be reconstituted in distilled water before use for treatment of diabetic rats.

2.5. Phytochemicals

Phytochemical tests were carried out using standard procedures as by [13,14,15].

2.6. Serum Collection

On the 29th day, the rats were anesthetized 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 clot and the serum was clearly remove and used for the assays.

2.7. Assay of Biochemical Parameters

Activities of alkaline phosphatase (ALP) were determined by the method of [16] while the activities of alanine
aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed by the method of [17]. Total
cholesterol concentration in the serum was assayed by the method of [18], while serum HDL-cholesterol
concentration was determined using the method described by [19]. Serum triacylglycerol concentration was
determined by the method of [20]. Total proteins were assayed by [21] as modified by [22]. Albumin and
Bilirubin were estimated by the methods of [23,24] respectively and glucose determined by method of [25].
2.8. Statistical Analysis

Statistical analysis of data was performed using the computer, Mackintosh Performa 5200 CD Version 7.0. The statistical analysis of numerical data obtained from the study were expressed as mean ± standard deviation (SD) using analysis of variance (ANOVA) one-way. Differences among means of control and test groups were determined using Statistical Package for Social Scientists (SPSS) version 20. The P < 0.05 was considered significant.

3. Results

The phytochemical screening of the aqueous and ethanol of Sweet potato leaf extract indicated the presence of tannins, balsams and phenols in the two solvents. The total presence of alkaloids and cardiac glycosides were lacking in the solvents (Table 1). Aqueous Sweet potato leaf extract and metformin administered in this study showed varying effects on blood glucose of the rats. Metformin was able to compete slightly more favorably than the aqueous extract when compared with the control group in lowering blood glucose significantly (P<0.05) (Table 2). Extract administered alongside metformin caused significant decreased (P<0.05) in total cholesterol, triglycerides TG, and low density lipoprotein LDL when compared to the control and the initially low high density lipoprotein HDL were significantly (P<0.05) increased in the diabetic rats. (Table 3). Aqueous Sweet potato leaf extract showed significant elevation (P<0.05) in Total serum protein, 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, AST and ALP of Aqueous Sweet potato leaf extract treated rats and metformin administration showed significant decreased (P>0.05) in the streptozotocin-induced diabetic rats when compared with the control. (Table 5).

Table 1: Phytochemicals of Aqueous and ethanol extract of Sweet potato

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Aqueous Extract</th>
<th>Ethanol Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpenes and Steroids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Resins</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Balsam</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

NOTE: + = Detected; - = Not Detected
Table 2: Effects of Aqueous Sweet potato leaf Extract on Blood Glucose

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Blood Glucose (Mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Normal Control</td>
<td>4.05±0.08</td>
</tr>
<tr>
<td>B</td>
<td>Diabetic Control</td>
<td>18.77±0.32*</td>
</tr>
<tr>
<td>C</td>
<td>Diabetic + Extract</td>
<td>6.75±0.06b</td>
</tr>
<tr>
<td>D</td>
<td>Diabetic + Metformin</td>
<td>5.12±0.02ab</td>
</tr>
</tbody>
</table>

NOTE: Values are expressed as Means ± SD, n = 4; Values in Each Column with Different Letter Superscripts are Significantly Different (P<0.05)

Table 3: Effects of Aqueous Sweet potato leaf Extract on Serum Lipid Profile

<table>
<thead>
<tr>
<th>Lipid Profile (Mmol/L)</th>
<th>Groups</th>
<th>Treatment</th>
<th>TOT.CHOL</th>
<th>TG</th>
<th>HDL</th>
<th>LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>Normal Control</td>
<td>3.75±0.03</td>
<td>0.08±0.04</td>
<td>1.26±0.02</td>
<td>1.84±0.01</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>Diabetic Control</td>
<td>5.87±0.47a</td>
<td>2.59±0.07a</td>
<td>0.41±0.05</td>
<td>3.09±0.05a</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>Diabetic + Extract</td>
<td>4.73±0.01b</td>
<td>1.53±0.01</td>
<td>0.99±0.01</td>
<td>2.26±0.02</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>Diabetic + Metformin</td>
<td>4.40±0.03b</td>
<td>1.15±0.01</td>
<td>1.17±0.01b</td>
<td>2.14±0.01</td>
</tr>
</tbody>
</table>

NOTE: Values are Means ± SD, n = 4; Values in Each Column with Different Letter Superscripts are Significantly Different (P<0.05)

TOT.CHOL = Total Cholesterol; TG = Triacylglycerol; HDL = HDL-Cholesterol; LDL = LDL-Cholesterol

Table 4: Effects of Aqueous Sweet Potato leaf Extract on Some Serum Parameters.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Total Protein(g/L)</th>
<th>Albumin(g/L)</th>
<th>(umole/L)</th>
<th>Total Bilirubin</th>
<th>Direct Bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Normal Control</td>
<td>77.9±0.58</td>
<td>38.75±0.60</td>
<td>8.19±0.46</td>
<td>3.90±0.04</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Diabetic Control</td>
<td>59.08±0.47a</td>
<td>28.8±0.19a</td>
<td>29.73±0.45a</td>
<td>10.30±0.07a</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Diabetic + Extract</td>
<td>70.71±0.14b</td>
<td>34.50±0.05b</td>
<td>21.17±0.05ab</td>
<td>13.03±0.16b</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Diabetic + Metformin</td>
<td>75.08±0.30b</td>
<td>36.03±0.10</td>
<td>17.79±0.401b</td>
<td>8.26±0.174b</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Values are Means ± SD, n = 4. Values in Each Column with Different Letter Superscripts are Significantly Different (P<0.05)
4. Discussion

Medicinal plants are employed in the management of diabetes mellitus. In the present study, aqueous sweet potato leaf extract was investigated for its effects on biochemical parameters in Streptozotocin-induced diabetic rats. Several organs are normally implicated in diabetic conditions; these include the pancreas, kidney, liver, eye and other in long term complications. Hence treatment of multidimensional and multidisciplinary. Several botanicals extracts have proven to be effective in treatment of diabetics and others related complications associated with it [26]. Phytochemical screening of the aqueous sweet potato leaf extract indicated the presence of tannins, balsams, Flavonoids, phenols, saponins, terpenes, and steroids, While the resins are detected in ethanol extract. However, alkaloids and cardiac glycosides were not detected (Table 1). Flavonoids and phenolics have been associated with increase insulin secretion and scavenging free radicals generated during diabetic disorder [27].

Streptozotocin is a glucosamine-nitrosourea compound. As with other alkylation agents in the nitrosourea class, it is toxic to cells by causing damage to the DNA, though other mechanisms may also contribute. DNA damage induces activation of poly ADP-ribosylation, which is likely more important for diabetes induction than DNA damage itself. Flavonoids are also known to regenerate these damage beta cells in diabetic rats [28].

Diabetic mellitus is characterized by increase in serum glucose concentration [29], this make glucose a necessary marker in diabetes. In the study, glucose level in experimental rats where significantly decreased (P<0.05) throughout the experimental period. Administration of a reference drug metformin also reduced these high levels of glucose (Table 2). Reduction in glucose can be due to the presence of saponins in the phytochemicals screening conducted on aqueous extracts. Saponins have been attributed to reduce serum glucose levels [30]. Previous reports has indicated that plant extracts possess hypoglycemic properties, possible insulin release stimulatory effects and uptake of peripheral glucose, which in turn reversed streptozotocin induced hyperglycemia [31]. Hyperglycemia, a characteristic of diabetes leads to long-term tissue damages and complications, such as liver dysfunctions, often associated with serious diseases. Glucose circulates in the blood of animals as blood sugar. Glucose can be obtained by hydrolysis of carbohydrates such as milk, cane sugar, maltose, cellulose, glycogen etc. It is however, manufactured by hydrolysis of cornstarch by steaming and diluting acid [32].

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 sweet potato leaf extract reduced the serum total cholesterol, TG and LDL concentrations while it significantly increased (P<0.05) the concentration of HDL (good cholesterol). Aqueous sweet potato leaf extract reverse significantly (P<0.05) these abnormalities observed in lipid metabolism. Lipids no doubt play vital role in pathogenesis of diabetic mellitus. Sweet potato leaves are also rich in vitamin B, beta-carotene, iron, calcium, zinc and protein [33] which makes it highly recommended in disease conditions with protein disorder. Diabetes affects both glucose and lipid metabolism [34]. In the postprandial state elevated serum insulin increases lipoprotein lipase activity in adipose tissue and promotes fuel storage as triglycerides in normal metabolism [35]. The deficiency of insulin depletes the activity level of lipoprotein lipase, thus leading to deranged lipoprotein metabolism during diabetes [36]. The hypercholesterolemia observed in diabetic rats generally might be due to increased intestinal cholesterogenesis resulting from increased activity of β-hydroxy-β-methyglutaryl CoA (HMG-CoA) reductase in the intestine of Streptozotocin-induced diabetic rats, partly from the increased availability of acetyl-CoA as a result of increased oxidation of fatty acids in diabetes mellitus [37]. Total protein, albumin and bilirubin in metformin and aqueous sweet potato leaf 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. A significant increase (P<0.05) was observed when aqueous extract was administered, a condition which was reversed earlier. Low albumin was significantly increased with the plant extract and metformin (Table 4). Hyperglycemia increases gluconeogenesis and as such leads to excess protein breakdown as well as excess loss of nitrogen resulting in negative nitrogen balance [38]. Fructosamine testing determines the fraction of total serum proteins that have undergone glycation (the glycated serum proteins). Albumin is the most abundant protein in blood, fructosamine levels typically reflect albumin glycation. Some fructosamine tests specifically quantify the glycation of albumin, or glycated serum albumin instead of all proteins. Because albumin has a half-life of approximately 20 days, the plasma fructosamine concentration reflects relatively recent (1-2 week) changes in blood glucose [39]. Rat model studies of diabetes mellitus 2 has shown that increased expression of Heme Oxygenase-1, the enzyme responsible for the conversion of Hemoglobin to Bilirubin, is associated with enhanced insulin sensitivity and glucose metabolism resulting in greater rates of rat model euglycemia [40]. According to [41] higher levels of total bilirubin convey a protective effect with regards to cardiovascular risks. In this present study direct bilirubin became increased with the plant extract, while total bilirubin became lower. Also, higher maternal serum direct bilirubin during second trimester of pregnancy could be a protective factor for the development of Gestational Diabetes Mellitus later [42].

Enzyme level of transaminases increased in diabetic rats treated with streptozotocin (Table 5). Increase in their levels indicates their activeness in the absence of insulin. These result in increased availability of amino acids in diabetic as well as increase in glucogenesis and ketogenesis observed in diabetes. Administration of aqueous sweet potato leaf extract and metformin result in lowering the levels of ALT, AST, and ALP to levels comparable to the control groups. The liver plays an important role in maintenance of normal glucose levels during fasting as well as in the postprandial period and its role in the pathogenesis of type 2 diabetes has attracted much interest. Indeed, hepatic dysfunction resulting from insulin-resistance syndrome may lead to development of type 2 diabetes [43].
5. Conclusion

As evident from this study, Aqueous sweet potato leaf extract is rich in phytochemicals which justified the various activities observed in treated rats. Hyperlipidemia, Hyperglycosuria, and other conditions observed in diabetic rats were attenuated by the administration of aqueous sweet potato leaf extract. Also, the glucose level was significantly reduced during the period of administration, the extract effectively controls the metabolic disorder that is associated with diabetes mellitus.

6. Conflict of Interest

There is no conflict of interest encountered during the study.

Acknowledgements

The Nobel diagnostic laboratory, Ahmadu bello way Jos, Nigeria and the University of Jos, Nigeria animal house/biochemistry department laboratory are appreciated for their contribution towards their support and the production of this work.

References


2010, 24.


