Original Research Article

The Impact of Pumpkin Seed Extracts on the Histology of the Hypothalamus and Testosterone level of Alloxan Induced Diabetic Male Rats

ABSTRACT

Objective: Epidemiologic studies and randomized clinical trials demonstrate that type 2 diabetes can be substantially avoided by making dietary and lifestyle changes rather than relying heavily on medications. This study evaluates the ameliorating effect(s) of Telfairiaoccidentalis seed administration on the histology of the Hypothalamus and testosterone level in normal and Alloxan-induced diabetes in Wistar rats.

Methods: Twenty male Wistar rats, weighing between 120-180g, were divided into five groups of 4 rats each. Groups 1 and 2 representing Normal Control (NC) and Diabetic Control (DC) received only feed and water. Group 3 normal rats received 300mg/kg b.wTelfairiaoccidentalis seed extract; groups 4 and 5 received 50mg/kg b.w of Metformin and 300mg/kg b.w of Telfairiaoccidentalis seed extract respectively.

Results: The result revealed that the administration of Telfairiaoccidentalis seed extract moderately increased the body weight (16.76±1.75%) significantly at ($P<0.05$) when compared to normal control (14.06±2.93%) and diabetes significant decrease (-27.68±1.92%) body weight when compared to metformin-treated (-6.11±1.85%) and extract (-5.33±2.18%). The levels of FBG with the extract (60.70±4.88%) and metformin (61.24±6.19%) significantly decreased at ($p<0.05$) when compared to diabetic control (- 64.78±12.51%).The FSH and LH level significantly increased at($p<0.05$). Histological changes were also seen in animals of the treated groups.

Conclusion: it is evident that Telfairiaoccidentalis seed extract contains anti-hyperglycemic agents capable of lowering blood glucose levels.

Keywords: Pumpkin seed, hypothalamus, testosterone, diabetic

INTRODUCTION

Diabetes mellitus (DM) is a complex and multifarious group of disorders that disturb the metabolism of carbohydrates, fat, and protein. It results from a shortage or lack of insulin secretion or reduced sensitivity of the tissue to insulin (1).

The Type 1 diabetes mellitus is an autoimmune disease caused by the destruction of pancreatic beta cells and characterized by a defect in insulin secretion while Type 2 diabetes mellitus results from abnormalities in insulin secretion and or insulin action or both (2).Dyslipidemia, often presents in diabetic patients and is the main risk factor for cardiovascular and cerebrovascular diseases (3).

Diabetes mellitus is currently one of the most costly and burdensome chronic diseases and is a condition that is increasing in epidemic proportions throughout the world. It is a serious illness with multiple complications and premature mortality, accounting for at least 10% of total health care expenditure in many countries (4). The prevalence of diabetes in all age groups worldwide is projected to rise from 171 million in 2000 to 366 million in 2030 (5).Reasons for this increase include: an increase in sedentary lifestyle, consumption of energy-rich diet, obesity, and higher life span (6).

One of the most commonly used diabetogenic substances in experimental diabetes research is alloxan. It's a cyclic urea analog made up of 2,4,5,6-tetraoxo-hexahydro pyrimidine in its chemical makeup (7). Alloxan induces diabetes in animals and inhibits glucose-induced insulin production by $_{B}$-cells in the pancreas' Islets of Langerhans. Alloxan accumulates rapidly and selectively in $_{B}$-cells compared to non $_{B}$-cells, according to research.

Many traditional plant treatments for diabetes have also been in use but most of the evidence for their beneficial effects has been anecdotal (8).Traditional anti-diabetic herbs may give new oral hypoglycemic chemicals, which could help rural communities in underdeveloped nations overcome the high cost and limited availability of current medications.

This study,thereforeseeks to evaluate the ameliorating effect(s) of Telfairiaoccidentalis seed administration on the histology of the Hypothalamus and testosterone level in normal and Alloxan-induced diabetes in Wistar rats.

METHODS

Permission for the use of the animals and animal protocol was obtained from the Animal Ethics Committee of Delta State University, Abraka before the commencement of the experiments.*Telfairiaoccidentalis* seeds were purchased from fruit vendors in Abraka, Ethiope East Local Government Area, Delta State, Nigeria, and was authenticated and identified to the species level by Dr. (Mrs.) EdemaNoyo E. of the Department of Botany, Faculty of Science, Delta State University, Abraka. A voucher specimen of *Telfairiaoccidentalis* was kept in the laboratory. The powder was weighed (300g) and soaked in 1500ml of ethanol respectively, for 72hrs.An electrical evaporator extraction unit was used

to obtain the extract (rotary evaporator). The solvent was extracted at 45 degrees Celsius and 60 cm of water pressure. A paste-like extract was obtained and oven-dried to a solid state before being milled into a fine powder.

Metformin (500mg) as the standard drug was obtained from Omena Pharmacy, Abraka, Ethiope-East Local Government Area, Delta State. The powdered metformin was weighed (0.05g) in 1000ml of distilled water and the extract of seed was weighed (3g) in 10ml of distilled water to get the appropriate concentrations that were used to administer to the matured healthy adult male rats.

For the diabetic study, hyperglycaemia was induced using Alloxan-monohydrate was dissolved in sodium chloride buffer. Before induction, their fasting blood glucose of the animals was checked after an overnight fast. Alloxan was prepared by dissolving 2g of Sodium Chloride in 50ml of water to yield 0.9% of chloride buffer; 3g of Alloxan was dissolved in 10ml of chloride buffer to yield 40mg/kg of Alloxan. 1ml of the resultant solution was injected into the animals through the intraperitoneal and their fasting blood glucose was assessed using ACCU-CHEK active blood glucometer, 72hours after induction. A 200mg/dl increase in pre-induction fasting Blood glucose level was considered to be diabetic. Diabetes was not induced in animals for the normoglycaemic study.

The research was divided into two phases; the normoglycaemic experimental study with 10 animals divided into 2 groups (n=5).

Group 1 which served as Control,

Group 2 was treated with 300mg/kg of Crude *Telfairiaoccidentalis*,

The diabetic study which comprised of 15 animals divided into three (3) groups (n=5).

Group 3 (negative control) induced diabetes but untreated,

Group 4 was induced diabetes and treated with 50mg/kg of Metformin,

Group 5 was induced diabetes with 40mg of Alloxan and treated with 300mg/kg of Crude *Telfairiaoccidentalis*

The results were expressed as Mean ± SEM (Standard Error of the Mean). Data obtained were statistically analyzed using the Student's *t*-Test statistics, one-way analysis of variance (ANOVA),

followed by post-hoc Fisher's test for multiple comparisons, using the software, Statistical Package for Social Science (SPSS) version 20 windows software. Significance level was at p values < 0.05, while p values > 0.05 was considered to be statistically non-significant.

RESULTS

From table 1: The level of body weight in normal and diabetic rats after treatment was reported to be significantly lower in the diabetic group as compared to normal rats treated with 300mg/kg Telfairiaoccidentalis seed and control rats. When diabetic rats were given Telfairiaoccidentalis seed extract (300 mg/kg) and Metformin (50 mg/kg), their bodyweight was considerably higher (P<0.05) than diabetic control rats.

The diabetic group had a significantly higher blood glucose level than the normal control rats, as seen in Tables 2 and 3. When diabetic rats were given Telfairiaoccidentalis seed extract (300 mg/kg) and Metformin (50 mg/kg), blood glucose levels were considerably lower (P<0.05) than in diabetic control rats. When compared to normal control rats, the diabetic group had significantly lower FSH and LH levels. In diabetic rats, Telfairiaoccidentalis seed extract (300 mg/kg) plus Metformin (50 mg/kg) supplementation significantly increased FSH and LH levels (P<0.05) as compared to diabetic control rats. Plate I-IIIrevealed vacuolization of dark cells and neuropil in the hypothalamus of diabetic rats, hypothalamus further revealed lesser vacuolization of dark cells and neuropil in the granular layer and molecular layer. Hypothalamus with *Telfairiaoccidentalis seed*revealed lesser vacuolization of dark cells and neuropil in the granular layer and molecular layer.

Table 1: The effect of *Telfairiaoccidentalis* extract on the body of the experimental rat

Groups	Pre-treatment Weight	After-treatment Weight	% change in body weight
Control	122.77±4.84	142.77±0.77	14.06±2.93a
Diabetic control	197.00±4.26	142.25±1.80	-27.68±1.92b

Diabetic+Metformin	172.75±8.44	162.00±7.07	-6.11±1.85c
TOS extract alone	120.60±3.44	144.85±2.40	16.76±1.75a
Diabetic+TOS extract	160.50±3.28	151.75±1.60	-5.33±2.18c

Values are expressed as mean±SEM. n=4.

Table 2: The effect of *Telfairiaoccidentalis* extract on fasting blood glucose level of experimental rat

Groups	Post-induction Glucose level	Post-treatment Final Glucose level	% change in fasting blood glucose
Diabetic control	247.50±22.72	4400.25±16.82	-64.78±12.51a
Diabetic+Metformin	348.00±31.02	129.50±10.69	-61.24±.6.19b
Diabetic+TOS extract	357.75±22.36	139.25±17.71	60.70±4.88b

Values are expressed as mean±SEM. n=4.

Table 3: The effect of *Telfairiaoccidentalis seed* extract on reproductive hormones of experimental rat

S/N	Testosterone
Control	2.33±0.79
Diabetic control	0.60±0.00
TOS extract alone	2.80±0.74
Diabetic+Metformin	1.35±0.72
Diabetic+TOS extract	3.97±0.16

Histopathological Findings of the Hypothalamus Neuropil Dark cells

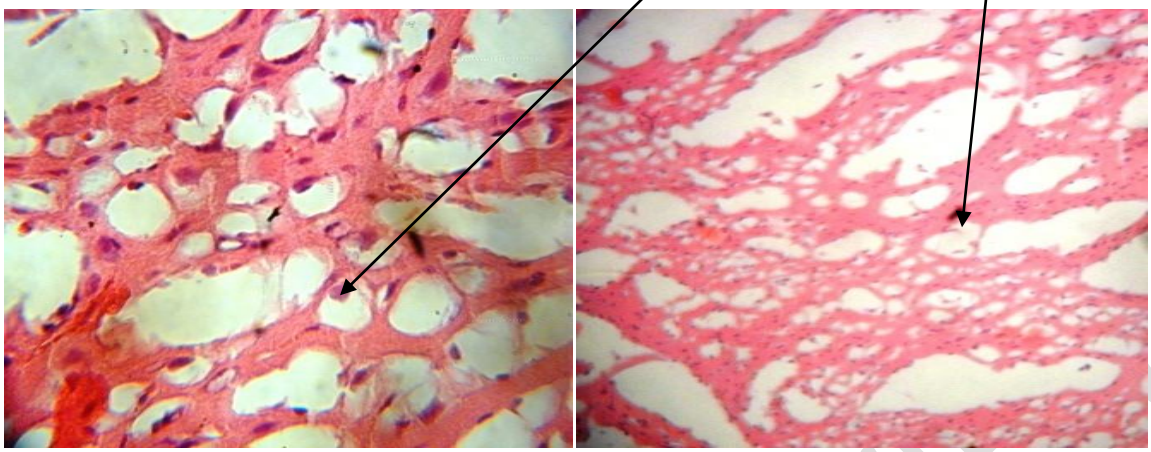

Plate 1. Negative control

Dark cell Neuropil

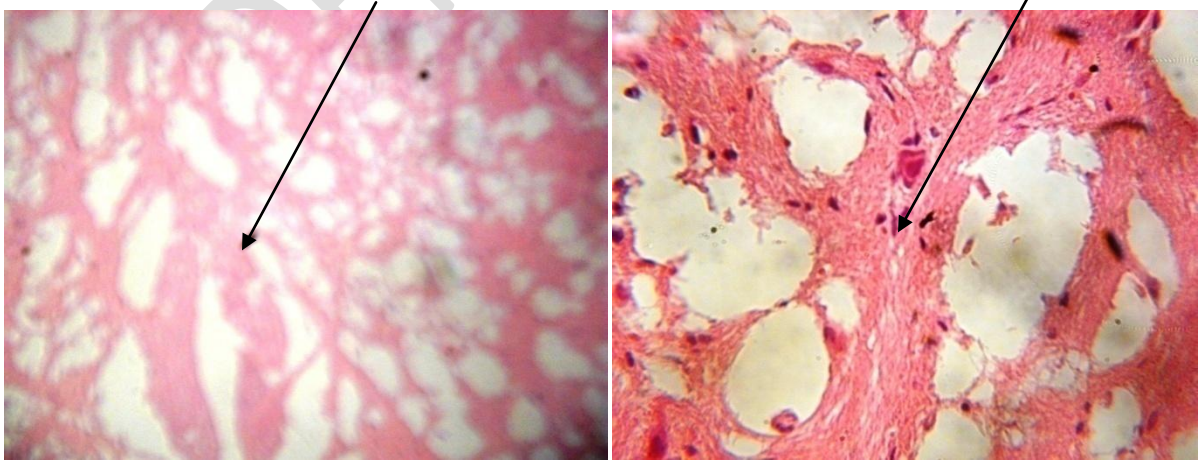

Plate 2. Metformin Dark cell

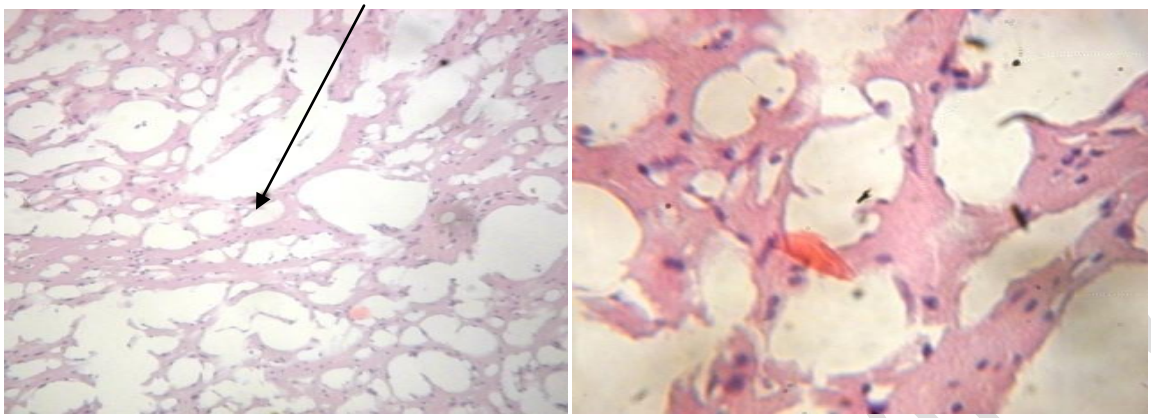

Plate 3.*Telfairiaoccidentalis seed*

DISCUSSION

In this study, we discovered a significant reduction in body weight of the diabetic rat agrees with the fact that diabetes causes alteration in the metabolism of macromolecules resulting changes in body composition and body weight. Significant weight loss is usually evident with the progression of the disease. In this study, *Telfairiaoccidentalis*seed extract treatment lead to a significant improvement in body weight of diabetic rats compared to the control groups. This is inconsistent with the findings of Ignacimuthu*et al*., (9) who observed an increase in the body weight of alloxan-induced diabetes in rats treated with *Telfairiaoccidentalis*seed extract.

The findings from the present investigation showed that 300 mg/kg of the *Telfairiaoccidentalis*seed extract revealed a significant ($p<0.005$) decrease in the blood glucose level after 14 days compared to diabetic control. Similar to *Telfairiaoccidentalis*seed extract, Metformin (50mg/kg) showed a significant ($p<0.005$) decrease in the blood glucose level after 14days compared to diabetic control, as diabetic rats' glucose levels progressively increase throughout the experiment. The extract produced 60.70±4.88% glucose reduction while metformin had 61.24±6.19% showing that *Telfairiaoccidentalis*seed extract reduced glucose levels as the standard drug (metformin) (10). This result

agreed with the finding of Kochhar*et al*.,(10) which stated that pumpkin seed extracts had a high content of total phenolics and antioxidant activity coupled with moderate to high alpha-glucosidase and angiotensin converting enzyme inhibitory activities and has the potential to reduce hyperglycemia-induced pathogenesis and associated complications linked to cellular oxidative stress (10,11, 12).

Another important finding from the histology of the hypothalamus in this study is that with the *Telfairiaoccidentalis*seed extract (300mg/kg BW) used in the study, there was a resurgence in the Hypothalamus which revealed lesser vacuolization of dark cells and neuropil in the hypothalamus in the granular layer and molecular layer.This might explain the poorer blood glucose control observed for diabetic rats treated with that dose of *Telfairiaoccidentalis*seed extract. Though it is not clear by which way *Telfairiaoccidentalis*seed extract at the dose of 300mg/kg BW produced a suboptimal control of blood glucose level, the observation from the study will suggest that beyond a certain dose of *Telfairiaoccidentalis*seed extract, the control of blood glucose level in the Alloxan-induced diabetic rats will be suboptimal.

CONCLUSION

It is evident that *Telfairiaoccidentalis*seed extract contains antihyperglycemic agents capable of lowering blood glucose level and enhancing testosterone level. The *Telfairiaoccidentalis*seed extract showed its ability to restore and reverse the already damaged tissues of alloxan-induced rats.

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