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The Effect of *Moringa Oleifera* Methanol Leaves Extracts on Sodium/Potassium (Na^+/K^+) Atpase in Streptozotocin-Induced Experimental Diabetic Albino Male Rat Models

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: An evaluation of the effect of *Moringa oleifera* methanol leaves extracts on Sodium/Potassium ATPase in streptozotocin-induced experimental diabetic albino male rats' model.

Methods: Qualitative phytochemical analysis was carried out on the methanol extracts of *M. oleifera* leaves using acceptable chemical methods. The LD50 of the plant extract was conducted and was non-lethal at 5000mg/kg. Thirty, albino male rats weighing 120g – 180g were arranged into five groups, comprising six rats per group. Parameters such as sodium/potassium ATPase (Na+/K+ ATPase), glycosylated hemoglobin (HbA1c), and fasting blood glucose levels were assayed.

Results: *M. oleifera* methanol leaves extract (at 500mg/kg and 1000mg/kg) increased sodium/potassium -ATPase activities in an albino male rat induced with hyperglycemia. It was also observed that the extract at doses of 500mg/kg (132.67 + 8.14) and1000mg/kg (114.00 + 15.38) for fasting blood glucose, 500mg/kg (6.29 + 0.26) and1000mg/kg (6.08 + 0.26) for glycosylated haemoglobin (HbA1c) were effective in ameliorating diabetes induced by streptozotocin.

Conclusions: This study established that the decrease in the activity of sodium/potassium -ATPase in streptozotocin-induced type II diabetes in albino male rats can be increased to normal functionality by oral administration of *M. oleifera* methanol leaves extracts at doses of 500mg/kg and 1000mg/kg.

Keywords: Sodium/Potassium Adenosinetriphosphatase (Na^+/K^+ ATPase), Streptozotocin (STZ), Diabetes mellitus (DM), *Moringa oleifera*, Glycosylated haemoglobin (HbA1c)

INTRODUCTION

Diabetes mellitus (DM), particularly type II DM, has been growing speedily (Roglic *et al*, 2005). Hence, there is a continuous rise in the number of diabetic cases, as about 463 million individuals worldwide live with the disease as of 2019. By estimation, it has been said that by the year 2045, about 700, 000,000 individuals world over will have diabetes. The devastating effects of diabetes can be seen in individuals, societies, and countries and lead to over 4 million deaths a year (IDF, 2019). In Nigeria, there has been a surge in diabetic cases from 2.2% in 1997 to 5.0% by 2013. Nigeria occupies the first position in Africa concerning her diabetes cases (3.9 million cases), closely followed by South Africa, with about 2.6 million cases. Rural communities are more affected than in urban communities. Many of the diabetic cases in Nigeria are undiagnosed. Because of the many undiagnosed cases in Nigeria, death-related diabetes cases are on the rise, as 105,091 deaths were recorded in 2013 (Oputa and Chinenye, 2015).

Hyperglycemia is a basic property of diabetes mellitus, which occurs when there is impairment of insulin secretion and/or action. It may result in macro- and micro-vascular complications, examples of which are; hypertriglyceridemia, nephropathy, and neuropathy (Villarruel-López *et al*, 2018). The gene that codes for sodium/potassium -ATPase is richly expressed in erythrocytes and peripheral nerves. Thus, a decrease in sodium-potassium - ATPase activity is critical to the manifestation of neuropathy (Vague *et al*, 1997). Therefore, because of the critical role of Na^+/K^+- ATPase in many transports and membrane potentials, changing its activity by diabetes would have serious consequences in the tissues where it is found – intestine, erythrocytes, peripheral nerves etc (Unachukwu *et al*, 2008).

There are proven and effective actions that countries or territories have urgently been taking to improve the management and prevention of diabetes. Locally in Nigeria, one such method is the use of natural products such as plants. *M. oleifera* (drum stick tree) leaves have been consumed just like fluted pumpkin, bitter leave due to rich content of macronutrients and antioxidants to make up for nutritional deficiencies. It has also been used to control glucose levels because of its anti-hyperglycemic properties (Asare *et al*, 2012). A study has shown that *M. oleifera* ethanol leaf extract has the potential to reduce hyperglycemic state and improve beta-cell function in diabetic rats (Anyanwu *et al*, 2014). In another study, *M. oleifera* seeds oil reportedly served as a source of potential antidiabetic agents that can be an alternative to oral hypoglycemic agents or adjuvant (Busari *et al*, 2014).

Diabetic-induced animal models with either streptozotocin or alloxan have been used and are being used to evaluate the metabolic as well as the physiological changes induced by diabetic conditions. One of the changes in metabolism that the researcher has looked at in the past is the disturbances in sodium/potassium - ATPase activity (Sima and Sugimoto, 1999). Hence, this study is aimed at evaluating the effect of *M. oleifera* methanol leaves extract in Sodium/Potassium -ATPase activity in streptozotocin -induced experimental diabetic albino male rats

METHODOLOGY

Plant and Animal materials

Fresh *M. oleifera* leaves were harvested from the Nsukka Town, South- Eastern Nigeria. It was given to a taxonomist Mr. Felix Nwafor of the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka, for authentication. White albino male rats were used for this research. The animals were acclimatized for seven days to the environment and were fed as often as necessary prior to the start of the experiment.

Preparation of methanol extract

Washed *M. oleifera* leaves were air-dried and then milled with an electric blender (Kenwood). The milled leaves (500mg) were macerated with 2.0L of 99% methanol in an air-tight container for three days with occasional stirring. The mixture of ground *M. oleifera* leaves and methanol was sieved, and the filtrate was freeze-dried. The concentrated solid residue obtained as the extract was stored in a bottle tightly corked and stored in a refrigerator until needed for analysis.

Acute Toxicity (LD_{50}) tests.

The median lethal dose test (LD_{50}) of *M. oleifera* methanol leaves extract was determined in mice via oral administration by way of Lorke's method (Lorke, 1983).

Phytochemical tests.

Standard procedures were used to determine the qualitative phytochemical contents of the freshly prepared *M. oleifera* methanol leaves extract (Trease *et al*., 1983).

Induction of diabetes

A single intraperitoneal administration of streptozotocin at a dosage of 65mg/kg body weight was used to induce diabetes in the test rats. Streptozotocin was dissolved in freshly prepared citrate buffer (0.1M, pH 4.5). Control rats were intraperitoneally administered citrate buffer only. At the end of the administration, 5% glucose solution was given to the rats for two days and they were then given drinking water. After the induction of diabetes, rats with a fasting blood sugar $\geq$ 200mg/dl were grouped as diabetic (Shetty *et al*, 2004).

Experimental design

Initially, there were two groups, namely the un-induced group (n = 6), which served as normal control, and the diabetic induced group (n = 24). When diabetes had developed in the second group, they were later classed into four subgroups. Thus, the groups include:

Group I: Control rats receiving vehicle (0.5ml distilled water /rat/day) orally for 28 days + feed and water.
Group II: Diabetic rats receiving *M. oleifera* methanol leaves extract (500mg/kg/rat/day) orally for 28 days + feed and water.
Group III: Diabetic rats receiving *M. oleifera* methanol leaves extract (1000mg/kg/rat/day) orally for 28 days + feed and water.
Group IV: Diabetic rats receiving glibenclamide standard drug (5mg/kg/rat./day) orally for 28 days + feed and water.
Group V: Diabetic rats receiving vehicle (0.5ml distilled water /rat/day) orally for 28 days + feed and water.

Animal sacrifice

After 28 days, the animals were starved overnight. Before sacrifice through cervical dislocation, the animals were anaesthetized according to their groups. Blood samples were collected using 2ml syringes and stored in clean plain sample bottles and EDTA bottles. The intestine was harvested and used for biochemical analysis.

Measurement of Na^+/K^+ ATPase activity

The measurement of activity was carried out by determining phosphate via spectrophotometry at 690 nm using the Sigma Diagnostics kit (catalog no. 670). Na^+/K^+ ATPase activity was stated as μmol Pi mg^{-1}protein hr^{-1} and was calculated as the difference between total ATPase activity and ouabain-insensitive ATPase activity (Del-Castillo and Robinson 1985; Gal-Garber O et al, 2003).

Determination of HbA1c

The Roche Tina-quant II assay method was performed to determine HbA1c values in samples according to the manufacturer's instructions on the Hitachi 917 auto-analyzer. The following calculation was used on the Roche Tina-quant immunoassay to convert the GHb and THb results into %HbA1c (NGSP/ DCCT): %HbA1c = (91.5 * [GHb/THb]) + 2.15 (Roche, 2003).

Determination of fasting blood sugar

To ascertain the blood glucose level of the experimental rat, a sterile disposable needle was used to draw blood from the ear of the rat. Then using a glucometer (acute check), the blood sugar level was determined/read before inducing the animals with diabetes and after induction to ascertain diabetic animals every week until the end of the animal experiment (Ohiri, 2003).

STATISTICAL ANALYSIS

Data obtained were expressed as Mean ± Standard deviation, and statistically significant difference were determined at $P<0.05$ using one-way analysis of variance (ANOVA). Turkey Post hoc test was used to further evaluate differences between groups. All analysis was performed using SPSS version 22.

RESULTS AND DISCUSSION

Phytochemical Test

The phytochemical tests of *M. oleifera* methanol leave extract, tested positive to the following phytochemicals: alkaloids, reducing sugars, flavonoids, resins, glycosides, steroids, saponins, tannins, and terpenoids.

Acute Toxicity Test

The oral LD_{50} of *M. oleifera* methanol leaves extract (MOME) in mice was greater than 5000 mg/kg.

Na^+/K^+ ATPase

Figure 1 presents the result of *M. oleifera* methanol leaves extract of Na^+/K^+ ATPase of controls and treatment groups. After the statistical analysis, a significant ($p<0.05$) difference between groups was determined by one-way ANOVA.

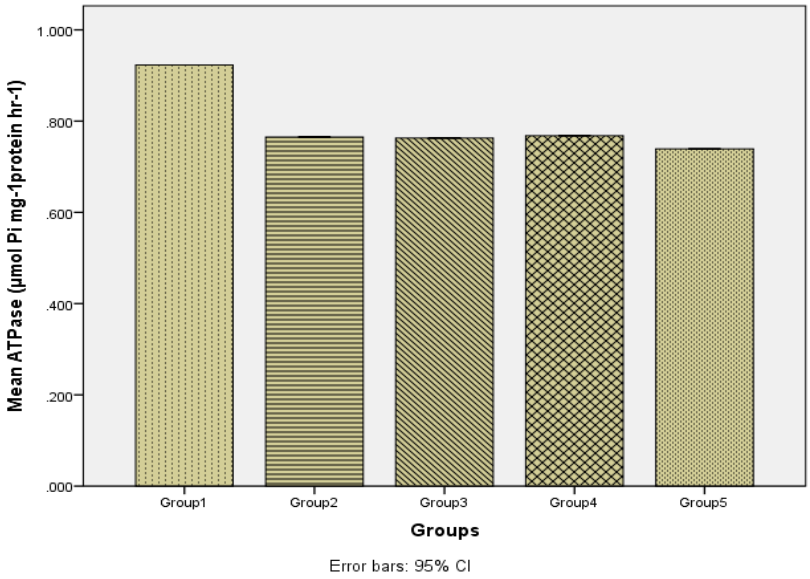

Figure 1: Moringa oleifera methanol leaves extract effect on Na^+/K^+ ATPase of control and diabetic albino male rats

HBA1c

Figure 2 shows the result of *M. oleifera* methanol leaves extract on HBA1c of controls and treatment groups. After statistical analysis, the one-way ANOVA revealed a significant ($p<0.05$) difference between groups.

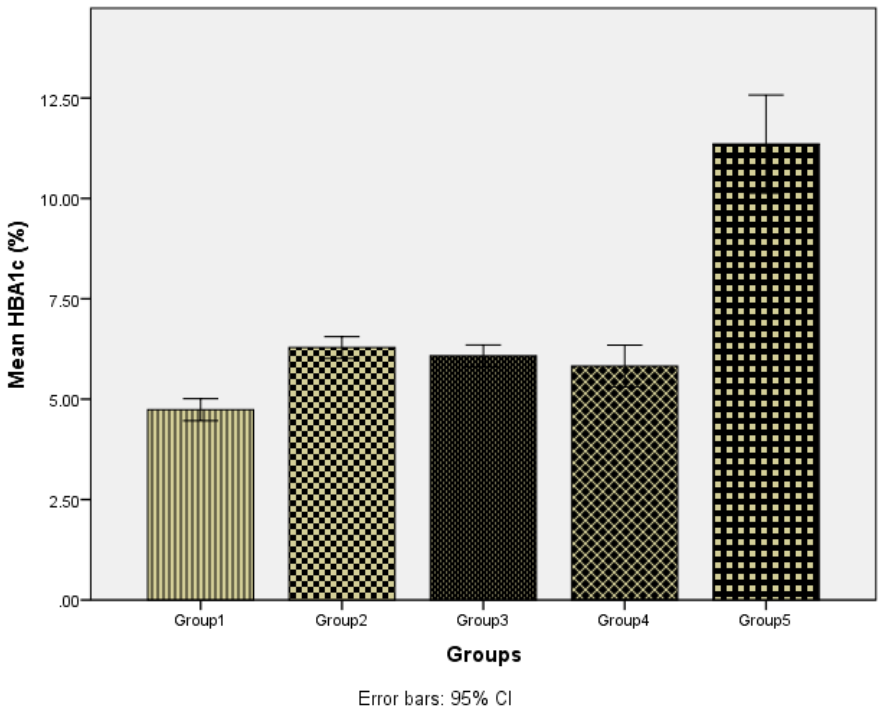

Figure 2: Moringa oleifera methanol leaves extract effect on HBA1c of control and diabetic albino male rats

Table 1 presents the result of *M. oleifera* methanol leaves extract of fasting blood glucose of controls and treatment groups. After the statistical analysis, a significant ($p<0.05$) difference between groups was determined by one-way ANOVA.

Table 1. Effects of M. oleifera methanol extracts on fasting blood glucose

WEEK / GROUP	ZERO(Mg/dl)	ONE(Mg/dl)	TWO(Mg/dl)	THREE(Mg/dl)	FOUR(Mg/dl)
1	84.33 ± 7.39	83.33 ± 3.45	82.17 ± 6.46	82.67 ± 1.63	88.00 ± 7.85
2	81.50 ± 6.72	298.83 ± 54.29	254.83 ± 28.53	160.50 ± 22.56*	132.67 ± 8.14*
3	83.33 ± 5.20	324.17 ± 64.96	256.67 ± 44.92	144.50 ± 31.99*	114.00 ± 15.38*
4	82.50 ± 8.29	246.83 ± 42.03	160.50 ± 37.53*	126.50 ± 35.84*	97.83 ± 13.20*
5	84.50 ± 11.11	316.33 ± 78.66	300.67 ± 25.62	304.00 ± 44.79	300.67 ± 46.33

Values are mean ± SD (n=6). *shows statistically significant ($p<0.05$) decrease in fasting blood glucose of treatment groups compared to group 5 (Diabetic control).

DISCUSSION

The phytochemical examination of *M. oleifera* methanol leaves extracts revealed that it contains tannins, carbohydrates, saponins, alkaloids, steroids, phenols, flavonoids, which are important secondary metabolites. A study had reported the presence of the same secondary metabolites in the extract (Kasolo *et al*, 2010). Flavonoids, one of the phytochemicals of *M. oleifera* methanol leaves extracts possess the potentials to inhibit α-amylase activity and thus regulate serum glucose. Hence the contribution of the phytochemical properties of *M. oleifera* methanol leaves extracts towards its anti-diabetic property (Farooq *et al*, 2007). Saponins and tannins possess the property of precipitating and coagulating red blood cells, bind cations and other biomolecules, and stabilize the erythrocyte membrane (Oyedapo and Araba, 2001; Ladan *et al*, 2014). Phenols serve as preservatives and also offer some health benefits as the extract is consumed (Ali, 2012).

Acute toxicity (LD_{50}) test on *M. oleifera* methanol leaves extract administered on male albino mice, showed no mortality and noticeable behavioral changes in all the groups tested. The extract was safe up to a dose of 5000mg/kg body weight.

Sodium/Potassium –ATPase concentration of the diabetic groups were decreased by hyperglycemia. This study shows that the reduced level of Na^+/K^+ -ATPase in intestinal membranes accounted for reduced activity, resulting in changes in membrane lipid fluidity or protein content. The impairment in sodium/potassium –ATPase activity is responsible for the progression of diabetic complications such as neuropathy (Souad *et al*, 2000; Totan and Greaby 2002). Thus, figure 1 show a decrease in sodium/potassium concentration -ATPase in intestinal membranes of group V (diabetic control) and an increase in groups II and III (treatment groups). Thus, in STZ induced diabetic albino male rats, treatment with *M. oleifera* methanol leaves extract, statistically significantly ($p<0.05$) increased the activity of sodium/potassium -ATPase of diabetic albino male rats in groups II and III as compared to group V. This agrees with a study which reported that erythrocyte sodium/potassium -ATPase activity was decreased in type II diabetes than in normal controls (Humayoun *et al*, 2016). A comparison of normal control (group1) with the diabetic control (group V), shows a statistically significant ($p<0.05$) decrease in sodium/potassium -ATPase activity of group V. This indicates that the *M. oleifera* methanol leaves extract (at 500mg/kg and 1000mg/kg) can restore to a normal and/or near normal level the activities of sodium/potassium-ATPase in an albino male rat induced with hyperglycemia.

The glycosylated haemoglobin (HbA1c) of groups II and III was statistically significantly ($p<0.05$) decreased when compared to group V. *M. oleifera* methanol leaves extract, indicated a dose-dependent decrease in HbA1c. Thus, the administration of *M. oleifera* methanol leaves extract, at 500mg/kg, and 1000mg/kg reduced glycosylated haemoglobin and with that of 1000mg/kg being more effective against STZ induced diabetes. This result is supported by the findings of a study that also reported a significant fall in HbA1c level in STZ induced diabetes after administration with 70% ethanol extract of *M. oleifera*

leaves (Soliman, 2013). Similarly, another study also reported amelioration by the tablets of *M. oleifera* leaves in HbA1c of type II diabetic patients (Giridhari *et al*, 2011).

A statistically significant ($p<0.05$) decrease in fasting blood sugar in treatment groups II and III was recorded when compared to group V. This is as *M. oleifera* methanol leaves extract was administered to evaluate the fasting blood glucose of diabetic albino male rats induced with STZ in the third and the final week of the study. Thus, *M. oleifera* methanol leaves extract, at doses, 500mg/kg, and 1000mg/kg are effective in ameliorating diabetes induced by STZ. A dose-dependent decrease in fasting blood glucose was observed. This result obtained is in agreement with that of a study that reported a decreased fasting blood sugar level of albino male rats induced with type II diabetes and administered with *M. oleifera* leaves extract (Omeodu *et al*, 2017). Furthermore, another independent research established that *M. oleifera* leaves contain many effective chemicals, mainly flavonoid, effective in protecting against streptozotocin-induced diabetes and exhibits hypoglycemic properties in diabetic rats (Shetty *et al*, 2004; Coskun *et al*, 2005).

Thus, *M. oleifera* methanol leaves extracts are very effective hypoglycemic agents, which may be as a result of the fact that the leaves might have had a direct effect on glucose reduction. Thus improving/increasing the rate at which glucose is used by the tissues leading to the absence of gluconeogenesis in the liver or assimilation of sugar into fat cells and muscles (Gray *et al*, 2000; Omeodu *et al*, 2017).

CONCLUSION

This study shows that the decrease in sodium/potassium -ATPase activity in type II diabetes mellitus induced albino male rats can be increased by oral administration of 500mg/kg and 1000mg/kg of *M. oleifera* methanol leaves extract. The findings of this research also supported that *M. oleifera* methanol leaves extract, have potential antidiabetic effects on STZ-induced type II diabetes on albino male rats. Furthermore, *M. oleifera* methanol leaves extract, at a dose of 500 mg/kg and 1000mg/kg, can increase the activity of the decreased sodium/potassium -ATPase in STZ-induced diabetes in albino male rats within four weeks. The outcome/findings of this research also show that *M. oleifera* methanol leaves extracts are better consumed at a higher safe, tolerable dose than at a lower safe tolerable dose for maximum activity.

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