

MORINGA LEAF EXTRACT AMELIORATES CEREBRAL CORTEX TISSUE DAMAGE IN STZ-INDUCED DIABETES CONDITION

Author details: Omotoso OD1, 3, Olawuyi VO2, Owolabi JO1, Dare BJ3

1 Anatomy Department, Ben Carson Sr. School of medicine, Babcock University, Nigeria.

2 Histopathology Department, LAUTECH Teaching Hospital, PMB 4000, Ogbomoso, Nigeria.

3 Anatomy Department, Bingham University, PMB 005, Karu, Nigeria.

Correspondence: salemyfatty13@gmail.com

olaowolabi001@yahoo.com

Abstract

Moringa oleifera is a plant that has huge nutritional and phytomedicinal values particularly due to its rich nutrients, antioxidants and medicinal phytochemicals. This investigation examined the effect of moringa oleifera leaf extract on the histology of the prefrontal cortex of STZ induced diabetic adult Wistar rat; and the accompanying effects on diabetes-related disorders associated with lipid peroxidation (MDA) and the blood sugar of STZ induced diabetic Wistar rat. Thirty Wistar rats [n=30] were divided into three groups of ten animals each. Group 1 animals served as the control group (non-diabetic) and were simply fed ad libitum; Group 2 animals were administered 65mg/kg of streptozotocin to induce diabetes and 200mg/kg of moringa leaf extract to observe the effects of the latter; Group 3 were administered 65mg/kg of streptozotocin to serve as the untreated diabetic group. Experiment lasted six weeks and the blood sugar level and animal health were duly monitored. The animals were euthanized at the end of six weeks and the brain tissues excised and processed using the haematoxylin and eosin technique. Diabetic animals were hyperglycemic and had cortical neuronal morphological distortions and histoarchitectural disruptions indicating brain tissue damage. Moringa leaf extract was potent in controlling the blood glucose level in STZ-induced diabetes murine models; it also ameliorated the damaging effects or consequences of the diabetic conditions on the neurons morphology and relative volume and distribution in the cortical tissue.

Key Words: Diabetes, Prefrontal Cortex, Moringa, Streptozotocin, Neurons

Introduction

Moringa is the sole genus in the flowering plant family Moringaceae. The name is derived from the Tamil word murungai or the Malayalam word muringa, both of which refer to *M. oleifera* [4]. It contains thirteen species; the most widely cultivated species is *Moringa oleifera*, a multipurpose tree native to the foothills of the Himalayas in northwestern India and cultivated throughout the tropics [9]. Nutritional analysis indicates that Moringa leaves contain numerous disease preventing nutrients [2].

The leaves of the *Moringa oleifera* [moringa] tree contain a substantial amount of oleic acid [7] and it is readily available for immediate absorption by the body. Also, *Moringa oleifera* leaf is rich in other important nutrients that could aid in combating diabetes [7]. Vitamin C is important for proper production and regulation of insulin; deficiency in vitamin C has been

shown to adversely affect the ability of the pancreas to secrete insulin, which contributes to higher levels of blood sugar. Moringa, however, contains high levels of vitamin C which could help the pancreas secrete insulin at a normal level. Other vitamins and minerals have also been shown to help in the production and regulation of insulin, both in the pancreas and elsewhere around the body. For example, vitamin E has been shown in several studies to decrease the risk of developing diabetes [7]. A powerful antioxidant, vitamin E makes it easier for the body to transport and manage insulin by improving the integrity of cell membranes.

Streptozotocin ($C_8H_{15}N_3O_7$, STZ, and Zanosar) is a naturally occurring chemical which has a molecular mass of 265.221g/mol and toxic to the insulin-producing beta cells of the pancreas in mammals. It is used in medicine for treating certain cancers of the Islets of Langerhans and used in medical research to produce an animal model for Type 1 diabetes. 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 [11]. Streptozotocin is similar enough to glucose to be transported into the cell by the glucose transport protein GLUT2, but is not recognized by the other glucose transporters. This explains its relative toxicity to beta cells, since these cells have relatively high levels of GLUT2 [5]. Streptozotocin was originally identified in the late 1950s as an antibiotic [10]. Thus, streptozotocin is selectively toxic to the beta cells of the pancreatic islets that normally regulate blood glucose levels by producing the hormone insulin. This explains why it is often used as an animal model of diabetes [6] and in the treatment of cancers of the beta cells.

The brain prefrontal cortex is of the neocortex, the cells are arranged as a 6-layered structure. The most superficial is the cell-poor molecular layer and the deepest is the multiform (polymorphic) layer, which is populated largely by fusiform cells. Between these two layers are 4 layers that are alternatively mostly populated by stellate or pyramidal cells. The prefrontal cortex is primarily concerned with executive functions. The executive functions of the frontal cortex involve the ability to recognize future consequences resulting from current actions, to choose between good and bad actions (or better and best), override and suppress unacceptable social responses, and determine similarities and differences between things or events. Therefore, it is involved in higher mental functions. The frontal cortex also plays an important part in retaining longer term memories which are not task-based. These are often memories associated with emotions derived from input from the brain's limbic system. The frontal cortex modifies those emotions to generally fit socially acceptable norms. Psychological tests that measure frontal cortex function include finger tapping, Wisconsin Card Sorting Task, and measures of verbal and figural fluency [1].

Many investigations on experimentally induced diabetes conditions have focused on testing substances effects on the directly affected structures and systems, with the pancreas being an organ of a major concern. This investigation however shows that the cerebral cortex is also affected in STZ-induced diabetic conditions. Attempt is being made to study the effect of moringa leaf extract on the histology of the prefrontal cortex of STZ-induced diabetic adult Wistar rats; and the accompanying effects on diabetes-related disorders associated with lipid peroxidation (MDA) and the blood sugar of STZ induced diabetic Wistar rat. Results could point to the complications of diabetes that could affect the nervous tissues structures negatively. It would also provide information on more possible uses of moringa leaf as a natural source of antioxidants and medicinally active phytochemicals. Results would be useful in various fields of biomedical sciences, especially phytomedicine. It will also guide the regular users of moringa for nutritional and trado-medicinal purposes especially in the developing world.

Materials and Method

A total of thirty Adult Wistar rats [n=30] were divided into three groups of ten animals each. Group 1 animals serve as the control group (non-diabetic) and were simply fed ad libitum; Group 2 animals were administered 65mg/kg of streptozotocin to induce diabetes and 200mg/kg of moringa leaf extract to observe the effects of the latter; Group 3 were administered 65mg/kg of streptozotocin to serve as the untreated diabetic group. Ethical approval for the use of animals and adopted methods was obtained from the ethical committee of the Department of Anatomy, Bingham University, Nigeria.

Fresh moringa leaves were collected from the host tree were washed, air dried at room temperature and milled using a grinder. Aqueous extract was obtained from the powder. Diabetes was induced in the animals by administering STZ (intra-peritoneal)- using 65mg/kg single dosage which is in the range of the dosage regularly used [12] and diabetes was confirmed by analysing the blood glucose levels, particularly relative to the controls. The blood sugar measurement was carried out with the aid of the Accucheck® glucometer. The fasting blood sugar was checked every week to monitor their progress and they were also being weighed daily. The food and fluid intake were also checked daily. MDA assay was done with the Lipid Peroxidation (MDA) Assay Kit (Sigma-Aldrich®). Quantitative results were collated and statistical analysis was done using the SPSS software (SPSS version: SPSS 22.0).

The animals were euthanised at the end of six weeks. The brain tissues excised and processed. A portion of the sample [for each group] was homogenized with 5% sucrose buffer at pH 7.4 for biochemical analysis and the other was put in fixative 10% formal calcium- for histological procedures. Tissues were processed for histological analysis using the Haematoxylin and Eosin technique [13] and histo-morphological analyses were done following the principles of Garman [14].

Results

Table 1: Lipid Peroxidation Levels at the 6th Week.

There is a significant decrease in the lipid peroxidation of the Group 2 animals [moringa-treated diabetic group] and a significant increase in the plasma lipid peroxidation of Group 3 animals [untreated diabetic group] relative to the control Group 1.

GROUPS	Group 1	Group 2	Group 3
MDA	3.17±0.03	2.39 ±0.18*	3.53±0.12*

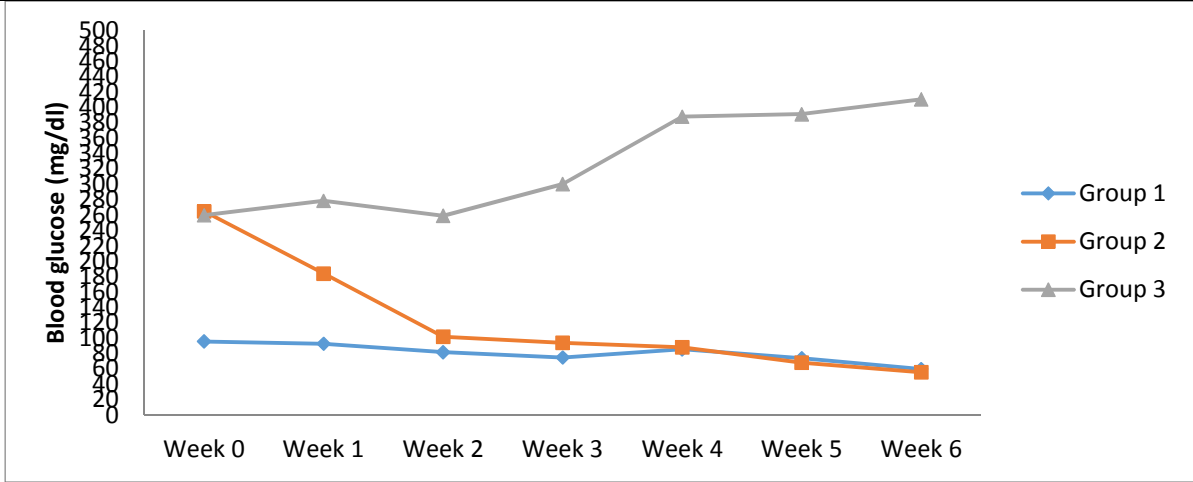
Table 2: Blood Sugar Levels of the Animals

Weeks/Groups	Group 1	Group 2	Group 3
Initial week	96.00	265.00	260.00
Week 1	93.00	184.22	278.44
Week 2	82.00	102.11	259.44
Week 3	75.11	94.33	300.67
Week 4	85.63	88.27	388.14
Week 5	74.24	68.34	391.46
Week 6	60.16	56.02	410.69

Table 3: The Mean ± Standard Error of values for blood sugar of animals per group throughout the duration of treatment. Statistical analyses [T-test and Anova] with p<0.05 show significant difference in the Group 3 animals [untreated diabetic animals].

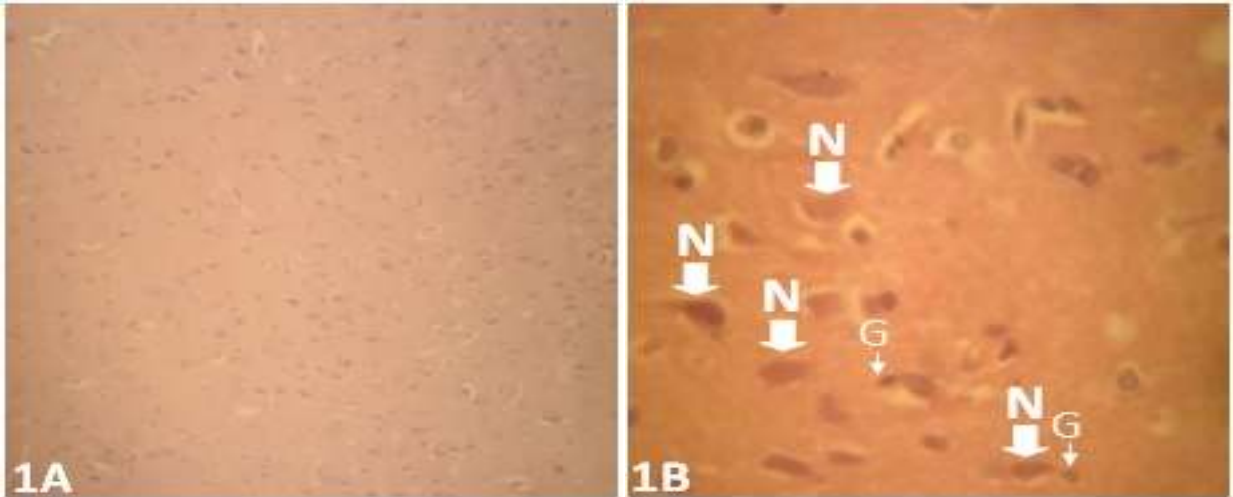
125

Groups	Weeks	Mean	S. deviation	±SEM	P- value
1	7	80.88	12.29	4.65	
2	7	122.60	75.11	28.39	0.17
3	7	327.00	67.08	25.40	0.00 *



126
127

128 **Chart 1: The graph of the blood sugar of the animals showing weekly changes and trends**
129 **in blood sugar levels.** Group 1 [normal control group] was normoglycemic from initial Week 0
130 to the Week 6. The blood sugar of the Group 2 animals [treated diabetic group] became
131 relatively normoglycemic as from the 2nd week while the blood sugar of the Group 3 [untreated
132 diabetic] was quite high and increased throughout the 6 weeks.



133
134
135

Figure 1: Photomicrographs of cerebral cortex (Frontal cortex) of the Control Group 1 [H & E, A- X40 and B- X100]; neurons and glia appear morphologically normal, so also the neuropil [N= Neuron; G= Glia].

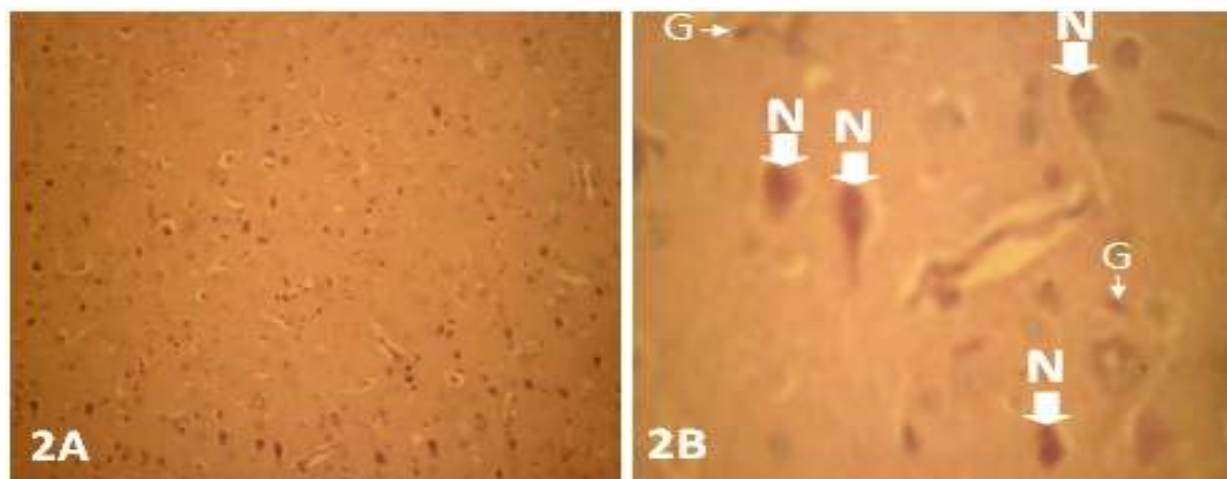


Figure 2: Photomicrograph of cerebral cortex (Frontal cortex) of the Group 2 diabetic animals administered moringa leaf extract [H & E, A- X40 and B- X100]; neurons and glia are relatively normally distributed and morphology show no marked anomaly or distortions [N= Neuron; G= Glia].

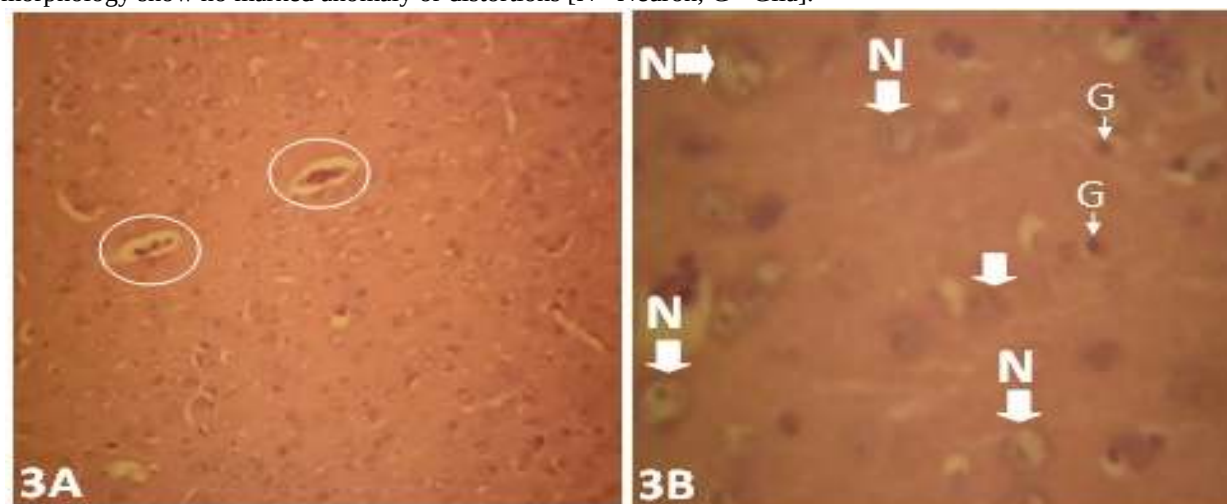


Figure 3: Photomicrographs of cerebral cortex (Frontal cortex) of the untreated diabetic group [H & E, A- X40 and B- X100]; cortex has areas of localized damage [lower magnification] and individual neurons show signs of morphological distortions and karyorrhexis [N= Neuron; G= Glia].

Discussion

The lipid peroxidation levels as observed at the 6th week of treatment showed that the level in Group 2 is statistically low and Group 3 is statistically higher relative to the Control Group 1. Blood sugar level measurements over the course of the experiment durations show that the Group 3 animals were diabetic as a result of the administered STZ and the condition persisted throughout the experiment- the animals were hyperglycemic all through. Also, on the average, the blood glucose level in the Group 3 animals was significantly higher than the other groups during the duration of treatment. However, Group 2 animals that were administered *Moringa oleifera* leaf extract [aqueous], after being induced to be diabetic had rapid declination in the level of blood sugar and it was brought to a relatively low level by week 2 of treatment which shows potent ability of moringa leaves extract to mop reactive oxygen radical that has implicated the nervous tissue in group 3 animals [7].

. The blood sugar was relatively normoglycemic for the rest of the treatment duration. These observations show that moringa leaf extract was potent in lowering the blood sugar level and could restore it to a relatively normal level within two weeks of treatment. This is also suggests that the accompanying anomalies of diabetes as induced could be taken care of by the administered moringa extract [8].

Photomicrographs [H&E] at the lower magnification of X40 and the higher magnification of X100 show the general histoarchitecture cross section of the prefrontal cortex and the cell morphology respectively. Group 1 served as the control, and the photomicrographs [Figure 1A and 1B] show normal cortical histoarchitecture, cell distribution and neuronal morphology as well as glia presence. However, photomicrographs illustrating the cortex of the untreated diabetic group of animals show signs of localized tissue damage [see white circles in Figure 3 A]. At the higher magnification, there are evidences of neuronal morphological distortions as neurons have largely lost their normal pyramidal or stellate forms, but rather enlarged and deformed [5]. Neuronal nuclei appear swollen, with an appearance that suggests impending degeneration. The observed anomalies especially of the nuclei of neurons suggest karyorrhexis. In Group 2 [Figure 2 A and 2B], neurons appear relatively normal and there are no localized areas of tissue damage; a few neurons however still appear heterogeneous.

The stated observations show that the brain is also affected in diabetes condition especially as induced by the STZ and the effects include deleterious changes on the cells especially the neurons. Very importantly, moringa leaf extract when administered after the diabetes condition is being induced could ameliorate the deleterious consequences of STZ-induced diabetes on the brain tissues. Thus, diabetes as a condition could be controlled by the administration of moringa leaf extract. Consequently, the damaging consequence on the brain could also be significantly ameliorated. It is however important to note that it could not be established whether moringa oleifera acted as a prophylaxis by preventing extensive damage to the tissue or in a therapeutic capacity by producing continuous healing or amelioration of the severity of the consequence of the diabetes condition. What is observable however is that the extract has the potential to prevent brain extensive tissue damage in diabetes condition and could effectively control the level of blood sugar as well [3].

Conclusion

Moringa leaf extract was potent in controlling the blood glucose level in STZ-induced diabetes murine models; it also ameliorated the damaging effects or consequences of the diabetic conditions on the neurons morphology and relative volume and distribution in the cortical tissue.

References

1. Anderson, SW., Bechara, A., Damasio, H., Tranel, D., and Damasio, A.R. (1999). Impairment of social and moral behavior related to early damage in human prefrontal cortex. *Nature Neuroscience* 2 (11): 1032–7.
2. Dolcas Biotech LLC, 2006-2008. "Nutritional value of moringa".
3. Fridl, N., Makkar, H.P.S., and Becker, K. (2001). The potential of *Moringa oleifera* for agricultural and industrial uses." In: L.J. Fuglie (Ed.). *The Miracle Tree: The Multiple Attributes of Moringa* (pp. 45-76). Dakar, Senegal: Church World Service, 2001.
4. Fuglie L.J (1999). *Moringa: Natural Nutrition for the Tropics*. Dakar: Church World Service.
5. Kumar, Vinay; Fausto, Nelson; Abbas, Abul K.; Cotran, Ramzi S., Robbins, Stanley L. (2005). Robbins and Cotran Pathologic Basis of Disease (7th ed.). Philadelphia, Pa.: Saunders. pp. 1194–1195.
6. Mansford KR, Opie L (1968). "Comparison of metabolic abnormalities in diabetes mellitus induced by streptozotocin or by alloxan". *Lancet* 1 (7544): 670–1.
7. Moringa Source LLC, 2008-2012 "Sources and importance of moringa".
8. National Research Council (2006-10-27). "Moringa". *Lost Crops of Africa: Volume II: Vegetables*. *Lost Crops of Africa*. 2. National Academies Press.
9. Robert E Paul (2008). "Description of moringa plant".
10. Schnedl WJ, Ferber S, Johnson JH, Newgard CB (1994). "STZ transport and cytotoxicity. Specific enhancement in GLUT2-expressing cells". *Diabetes* 43 (11): 1326–33..
11. Szkudelski T (2001). "The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas." *Physiol Res* 50 (6): 537–46.
12. Akbarzadeh A, Norouzian D, Mehrabi MR, Jamshidi Sh, Farhangi A, Allah Verdi A, Mofidian SMA and Lame Rad B (2007). Induction of Diabetes by Streptozotocin in Rat. *Indian Journal of Clinical Biochemistry*, 22 (2) 60-64.
13. Sheehan D, Hrapchak B (1980). Nuclear and cytoplasmic stains. *Theory and Practice of Histotechnology*. Batelle. 2nd ed; 8:143:144.
14. Garman RH (2011). *Histology of the Central Nervous System*. *Toxicol Pathol* 39 (1) 22-

221

222