Original Research Article

Hyperglycaemia and Oxidative Stress in Wistar albino Rats: Effects of Aqueous Extract of *Moringa oleifera*(Lam) Leaf

Abstract

The hypoglycaemic and antioxidant properties of *Moringaoleifera*in alloxan induced Wistar albino rats were studied. The study was carried out on twelve male Wistar albino rats which were acclimatised for two weeks. At the end of one week after acclimatization, four rats were randomly selected with their weights and glucose concentration determined which were then sacrificed to determine the reduced glutathione (GSH) level, glutathione peroxidase (Gpx) activity and catalase activity of the rats which served as Stage I (positive control animal group). The remaining rats which served as Stage II (diabetic negative control group) were injected intra-peritoneally with 0.5mL of 40mg/Kg body weight alloxanand continued feeding with rat feed and water for another week after which the weights and glucose concentration of the rats were determined followed by sacrifice of four rats to determine the reduced glutathione (GSH) level, glutathione peroxidase (Gpx) activity and catalase activity. The remaining four rats which served as Stage III (treated animal group) were treated with 0.5mL of 30% aqueous extract of *Moringaoleifera* leaf for one week after which their weights and glucose concentration were determined followed by sacrifice of the four rats to determine the reduced glutathione (GSH) level, glutathione peroxidase (Gpx) activity and catalase activity. It was observed that induction with alloxan caused a decrease in the weight; GSH and GPx of the rats with significantlyincrease in glucose concentration. However, treatment with *Moringaoleifera* extract demonstrated remarkable hypoglycaemic effect and restoration of weight and improved antioxidant properties.

Keywords: Hypoglycaemic effect, Antioxidant capacity,Oxidative stress,Aqueous extract, *Moringaoleifera*

Introduction

Alloxan, besides Streptozotocin is commonlyemployed as an experimental model of insulin-dependent diabetes mellitus due to its selective destruction of the insulin-producing pancreatic beta-islets in animals (Rohillaand Ali, 2012).Consequently, hyperglycaemic condition of the body results due to oxidative stress in which oxidation exceeds the antioxidant systems in the body as a result of loss of the balance between them (Yoshikawa et al., 2002). Such antioxidant systems are necessary to protect body cells and biomolecules against constant attacks from reactive oxygen species (ROS) and other free radicals generated

from biochemical processes within the body. Diabetes has been complicated in macrovasculardisease conditionslike stroke, atherosclerosis and other microvascular diseases such as retinopathy, neuropathy, nephropathy etc (Forbes & Cooper, 2013). The global report on diabetes showed that it is steadily increasing especially among middle income countries. It has caused 1.5 million deaths in 2012 alone. And in 2014, 422 million people worldwide had diabetes. It is disheartening that people with diabetes who depend on life-saving insulin pay the ultimate price when access to affordable insulin is lacking (WHO, 2016).

*Moringaoleifera*is said to belong to the family Moringaceace. It is also reputed to contain a high amount of phytochemicals,proteins,vitamins A and C, calcium, potassium; iron and other minerals in quantities beyond those of most food sources (Kumar *et al*., 2016). This possibly explains why itis traditionally used by Africans and some Asian countries to treat malnutrition in children and to augment breast milk.Several studies have shown that *Moringaoleifera*can act as anti-diabetic agent. Yet, others suggested that it can also serve as anti-neoproliferative agent to prevent the growth of cancer cells (Kumar *et al*., 2016).Elangovan*et al.,* (2014) demonstrated potent anti-bacterial activity of *Moringaoleifera* against several gram negative and gram positive bacteria; specifically *Staphylococcusaureus*, *Enterococcusfaecalis*, *Bacillussubtilis*, *E. coli*, and *Salmonellatyphi*.Its anti-fungal effect was made evident by Torres-Castillo *et al*., (2013).Furthermore, Nadeem*et al.*(2013)showed in their studies that the leaf extract at the rate of 600 ppm may be used for the enhancement of storage stability of butter stored at refrigeration temperature for three months with acceptable sensory characteristics. The storage stability was attributed to the antioxidant properties of *Moringaoleifera*.However, this study focused on the hypoglycemic and antioxidant potentials of the aqueous extract of *Moringaoleifera*in the treatment of diabetic and other oxidative complications.

Materials and Methods

Sample collection and preparation

Plant material: The leaves of the *Moringaoleifera* plant were collected from a house near Federal University of Technology Akure (FUTA), Ondo State, Nigeria and were authenticated at Department of Plant Science, Ekiti State, University, Ado-Ekiti. The leaves were air-dried in the laboratory of Medical Biochemistry department of College of Medicine, Ekiti State University, Ado-Ekiti. Subsequently, the dry leaves were pulverised to powder using MarlexExcella laboratory electric blender.

Extract preparation:30g of pulverised leaves was weighed into a 100mL standard volumetric flask with distilled water, mixed continuously overnight and sieved to obtain the crude extract solution referred to as 30% aqueous extract of *Moringaoleifera*.

Experimental Procedure

The study was carried out on twelve male wistar albino rats, fed with standard rat pellets and acclimatised for two weeks in the Animal House of College of Medicine, Ekiti State University, Ado-Ekiti, Nigeria before administration of the drug. The animals with an average weight of 80g were selected at random. The Stage I served as positive control animal group without any treatment but fed on rats feed and water for a week after acclimatization,then four rats were selected and sacrificed. The remaining eight rats were injected intra-peritoneallywith 0.5mL of 40mg/Kg body weight alloxanwith continued feeding with rat feed and water for another one week before four rats were sacrifice to form Stage II (diabetic negative control group). The remaining four animals which were administered with 0.5mL of 30% leaf aqueous extract of *Moringaoleifera* for another one week which served as Stage III treated animals group. Animals were kept at optimum temperature with a 12 hour light/dark cycle and given rat feed and water.

Preparation of Plasma

At the end of each stage, four animals were selected to determine the weights and glucose concentration of the rats after which they were anaesthetised and sacrificed. Sterile syringes and needles were used to collect blood from the heart into EDTA bottles;the blood sample was centrifuged to obtain clear plasma at the end of each stage.

Biochemical Assay

ON-CALL plus Glucometer was used to obtain the glucose concentration in mg/dL when the tail ends of the rats were pricked to collect blood into the compatible glucose test stripes. This was done at the end of each stage. Subsequently, reduced glutathione (GSH) level was estimated using the method of Jollow*et al.,* (1974); glutathione peroxidase (Gpx) activity was measured using the method described by Paglia and Valentine (1967). While catalase activity was determined based on the method described by Sinha (1972).

Statistical Analysis

The data were evaluated using the statistical test of one-way analysis of variance (ANOVA). And the results were presented as mean ± standard deviation.

Results and Discussion

The results obtained are means of three determinations ± standard deviation. The results with same superscript letter show they are not significantly different from normal control group at ($p < 0.05$) while the results with different superscript letter show the results are significantly different from normal control at ($p<0.05$)

Table 1.0: Effect of aqueous extract of *Moringaoleifera* leaf on the weight of alloxan-induced diabetic rats

Parameters	Stage I (control)	Stage II (diabetic)	Stage III (Treatment)
Weight (g)	85.59±4.25[a]	80.47±4.04 [b]	85.00±3.61 [a]

Table 1.0 shows the effect of Moringa*oleifera* extract on the weight of alloxan-induced diabetic rats. There was a significant decrease in the weight of the animals after injected with alloxanwhen compared with the control stage 1. However, treatment with Moringa extract reversed the weight loss in weight. Similar results were obtained by Adeeyo*et al*. (2013) on streptozotocin-induced diabetics.

Table 2.0: Effect of aqueous extract of *Moringaoleifera* leaf on the glucose level of alloxan-induced diabetic rats

Parameters	Stage I (control)	Stage II (diabetic)	Stage III (Treatment)
Glucose (mg/dl)	41.00±3.60 [a]	104.00±5.29 [b]	57.89±2.17 [a]

The results from Table 2.0 present a significant rise in blood glucose level after administering alloxan. This is very high when observed against the control. A treatment with the leaf extract indicated a considerable drop in the level of blood glucose; demonstrating positive hypoglycaemic potential of the plant. The result is in agreement with those of El-Desouki*et al.,* (2015) which demonstrated visible restoration of pancreatic cells of high dose Moringa-treated diabetic rats. No doubt, Tuorkey (2016) stated that treating diabetic mice with Moringa significantly reduced hyperglycaemia.

Table 3.0: Effect of aqueous extract of *Moringaoleifera* leaf on the reduced glutathione (GSH) in (mg/mL)

Parameters	Stage I (control)	Stage II (diabetic)	Stage III (Treatment)
Glutathione (GSH)	0.34±0.01 [a]	0.18±0.02 [b]	0.28±0.02 [a]

From Table 3.0, reduced glutathione level decreases upon injection with alloxan against the

control stage one. When treated with Moringaoleifera extract, a significant increase is observed. This is consistent with the results obtained by Luqman*et al.*,(2012) in their work which higher antioxidant capacity was reported with increase in GSH level in a dose-dependent manner for the extract used. The ethanolic extract of the plant leaf reportedly showed highest phenolic content along with strong reducing power and free radical scavenging capacity.

Table 4.0: Effect of aqueous extract of *Moringaoleifera* leaf on glutathione peroxidase in µmol/min/mL

Parameters	Stage I (control)	Stage II (diabetic)	Stage III (Treatment)
Glutathione Peroxidase (Gpx)	0.19±0.01 [a]	0.11±0.02 [b]	0.35±0.01 [c]

Table 4.0 is the effect of *Moringaoleifera* extract on glutathione peroxidase (Gpx) in µmol/min,mL. There is a fairly decrease in its level compared to the control stage I. Treatment with the leaf extract showed significant increase in its level.

Table 5.0: Effect of aqueous extract of *Moringaoleifera* leaf on the catalase activity in µmol/min/mL

Parameters	Stage I (control)	Stage II (diabetic)	Stage III (Treatment)
Catalase	0.03±0.02 [a]	0.05±0.01 [b]	0.02±0.00 [a]

The catalase activity as presented in Table 5.0. When viewed with respect to the control stage I, there was an increase in its level when the animals received alloxan. On the contrary, a treatment with Moringa extract in stage III shows a fall slightly below the control level in stage I. The result obtained is contrary to that obtained on kidney by Oguntibeju*et al.* (2017) in which case administration of Moringa*oleifera* significantly increased the activity of CAT in diabetic rats.

DISCUSSION

The present study was undertaken to evaluate the antidiabeticand antioxidant properties of aqueous extract of *Moringaoleifera* in Alloxan induced diabetic rats.

The alloxan induced diabetic rats had a marked loss in the body weight (Table 1.0). This is expected as one of the effects of diabetics in the body is weight lost due to the destruction of the pancreatic cells in the system and the weight of the rats after treatment with *Moringaoleifera* aqueous extract was observed to be slightly higher (85.00±3.61) than (80.47±4.04) as observed in the stage II diabetic rat which was almost brought back to

normal weight of the control rat stage I (85.59±4.25). However, the treated rat with *Moringaoliefera* leaf had a remarkable gain in body weight (Table 1.0).

As observed in Table 2.0, rats induced with Alloxan were hyperglycemic. The concentration of fasting blood glucose was increased in the second stage of alloxan induced diabetic rats. It increased significantly over two times the glucose level in the control rats (41.00±3.60) to 104.00±5.09 in the diabetic stage but after treatment with *Moringaoleifera* aqueous extract, the glucose level almost reduced back to the glucose level of the control rats (57.89±2.17). Alloxan is known to destroy the cell of the islets of the pancreases that function in the regulation of insulin secretion and thus leads to the increase in the concentration of blood glucose. However the significant decrease in the *Moringaoleifera*treated rats stage II blood glucose shows the hypoglycemic action of the *Moringaoleifera* which was also observed in similar works of (Gomathy, *et al.,* 1990).

Our results in Tables 3.0 and 4.0 respectively show that reduced glutathione (GSH) and glutathione peroxidase reduced slightly in the diabetic stage II rats and increase almost two times of the control rat in the treatment stage III rats. Reduced glutathione (GSH) a very special peptide molecule and glutathione peroxidase possessed antioxidant protection and scavenge any oxidant in the system. The results thereforeshow that *Moringaoleifera*has a protective effect on antioxidant defencemechanism of the system to improve the glucose metabolism.

Table 5.0 also shows catalase increases in diabetic induced rats and depletes in *Moringaoleifera*aqueous extract from 0.05±0.01 (diabetic) to 0.02±0.01 (treated). The increase in blood catalase activities after injection of alloxan is another significance finding in this study which may be due to many metabolic processes in the system. The decrease in concentration of cell catalase is attributable in part to the reduced synthesis of this antioxidant enzyme whose concentration fell with the *Moringaoleifera*aqueous extract that was given to the rats. This study shows the ability of *Moringaoleifera*diet to restored altered antioxidant status of diabetic rats, though some studies have reported no alteration in the activity of red blood cell catalase in diabetic (Dohi*et al.,* 1992). However this study agrees with earlier work of Eleazu*et al.,* (2010) who observed an appreciable increase in catalase activity of alloxan induced diabetics in rabbits and decrease in the catalase activity after treated alloxan induced diabetic rabbits with unripe plantain.

To boost the body's response to such stress, *Moringaoleifera* leaves aqueous extract has been administered to diabetic rats. The results proved that *Moringaoleifera* possess considerable hypoglycaemic and antioxidant capacity. These findings corroborated the results of Pakade*et*

al. (2013) which concluded that Moringa has good antioxidant properties better than other common vegetables. Of all parts of the plant, the leaves possess the highest antioxidant based on the quantity of polyphenolic and flavonoid compounds recorded (Torres-Castillo *et al*., 2013) even though a previous study by Fakurazi*et al.* (2012) showed the flower extracts contain the highest total phenolic content and antioxidant capacity, followed by leaves extract.

Conclusion and Recommendation

In conclusion, alloxan is destructive to islets cells of the pancreas. As a result, it has become a means of inducing diabetes in experimental animals with a view to developing suitable drugs that can combat its worrisome effects. The induced hyperglycaemic state is being linked to oxidative stress due to insufficient antioxidants in the body.

It is therefore, recommended that diabetes can explore the hypoglycaemic potential of this plant while other people can be encouraged to include Moringa in their diets because of its protective and recuperative power against various diseases.Furthermore, researches can still becarried out to develop affordable Moringa-based drugs.

Consent

It is not applicable.

Ethical approval

All authors hereby declare that "Principles oflaboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well asspecific national laws where applicable. All experiments have been examined and approvedby the appropriate ethics committee”

Competing interests

Authors have declared that no competinginterests exist

References

Adeeyo, A. O., Adefule, A. K., Ofusori, D. A., Aderinola, A. A., and Caxton-Martins E. A (2013).Antihyperglycemic Effects of Aqueous Leaf Extracts of Mistletoe and *Moringaoleifera*in Streptozotocin-induced Diabetes Wistar Rats.DiabetologiaCroatica 42(3) 81-88

Parekh, J., Chanda, S. (2007). Antibacterial and phytochemical studies on twelve species of Indian medicinal plants.African Journal of Biomedical Research.Vol. 10: 175 - 181

Dohi, Y., Hahn, A.W.A., Boulanger, C.M., Buhler, F.R.,Luscher, T.F. (1992).Endothelin stimulated by angiotensin II augments contractility of spontaneously hypertensiverat resistance arteries.Hypertension19:131–137.

Eleazu, C.O., Okafor, P.N., Ahamefuna, I. (2010). Total Antioxidant Capacity, Nutritional Composition and Inhibitory Activity of Unripe Plantain (*Musa paradisiacae*) on Oxidative Stress inAlloxan Induced Diabetic Rabbits. Pakistan Journal of Nutrition, 9(11): 1052-1057.

El-Desouki, N.I., Basyony, M.A., Hegazi, M.M.A., El–Aama, M.S.I. (2015).Moringaoleiferaleaf extractameliorates glucose, insulin and pancreatic beta cellsdisorder in alloxan-induced diabetic rats. Research Journal ofPharmachological and Biological Chemistry Science, 6(3): 642-53.

Elangovan, M., Dhanarajan, M. S., Rajalakshmi, A. Jayachitra A., Mathi, P., Bhogireddy N.(2014). Analysis of Phytochemicals, Antibacterial and Antioxidant activities of *Moringaoleifera* Lam. Leaf extract- an in vitro study: International Journal of Drug Development& Research, 6 (4): pp.173-180

Fakurazi, S., Sharifudin S. A., and Arulselvan, P (2012) *Moringaoleifera*Hydroethanolic Extracts Effectively Alleviate Acetaminophen-Induced Hepatotoxicity in Experimental Rats through Their Antioxidant Nature. *Molecules*, *17*, 8334-8350;

Forbes, J. M., Cooper, M. E. (2013).Mechanisms of Diabetic Complications.Physiological Reviews 93(1).pp.137-188

Gomathy, R. Vijayalekshmi, N.R., Kurup, P.A. (1990). Hypoglycemic action of the pectin present in the juice of the inflorescence stalk of plantain (*Musa sapientum*) mechanism of action. Journal of Bioscience, 15, pp. 297-303

Jollow, D.J., Mitchell, J.R., Zampaglione, N., Gillette, J.R. (1974). Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology. 11: pp. 151-69.

Kumar, D. S., Doriya K., &Gopalakrishnan, L.(2016). *Moringaoleifera*: A review on nutritive importance and its medicinal application. Science direct: Food Science and Human Wellness 5.pp. 49–56

Luqman, S., Srivastava, S., Kumar, R., Maurya, A.K., Chanda, D. (2012).Experimental Assessment of Moringaoleifera Leaf and Fruit for Its Antistress, Antioxidant, and Scavenging Potential Using In-vitro and In-vivo Assays.Evidence-Based Complementary and Alternative Medicine. 32: 47-66.

Nadeem M., Abdullah M., Hussain I., Inayat S., Javid A., Zahoor Y. (2013): Antioxidant potential of *Moringaoleifera*leaf extract for the stabilisation of butter at refrigeration temperature. Czech Journal Food Science, 31: 332–339

Oguntibeju, O. O., Omodanisi, E. I. and Aboua, Y. G. (2017) Assessment of the Anti-Hyperglycaemic, Anti-Inflammatory and Antioxidant Activities of the Methanol Extract of *MoringaOleifera* in Diabetes-Induced Nephrotoxic Male Wistar Rats. Molecules 22, 439

Paglia, D.E., Valentine, W.N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 70:158-69.

Pakade, V., Cukrowska, E., Chimuka, L. (2013).Comparison of antioxidant activity of *Moringaoleifera*and selected vegetables in South Africa.SAfr.J. Sci.109(3/4)

Rohilla, A., Ali, S. (2012).Alloxan Induced Diabetes: Mechanisms and Effect.International Journal of Research in Pharmaceutical and Biomedical Sciences 3(2) 819-823.

Sinha, A.K. (1972). Colorimetric assay of catalase.Anal Biochem. 47: pp. 389-94.

Torres-Castillo, J. A., Sinagawa-García, S. R., Martínez-Ávila,G. C., López-Flores, A. B., Sánchez-González, E. I., Aguirre-Arzola, V. E., Gutiérrez-Díez, A.,Torres-Acosta, R. I., Olivares-Sáenz, E., and Osorio-Hernández, E (2013).*Moringaoleifera:* Phytochemical detection, antioxidants, enzymes and antifugal properties. International Journal of Experimental Botany (82) pp.193-202

Tuorkey, M. J (2016).Effects of Moringaoleifera aqueousLeaf Extract in Alloxan-Induced Diabetic Mice: Interventional Medicine & Applied Science, 8 (3), pp. 109–117

W.H.O (2016).World Health Organization. Global Report on Diabetes

Yoshikawa, T., Naito, Y. (2002). What is Oxidative Stress? Japan Medical Association Journal 45(7) pp. 271-276