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 *Moringa oleifera* in allozan 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-peritonially with 0.5mL of 40mg/Kg body weight alloxan with 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 *Moringa oleifera* 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 significantly increase in glucose concentration. However, treatment with Moringa oleifera extract demonstrated remarkable hypoglycaemic effects and restorations of weight and improved antioxidant properties.

Keywords: Hypoglycaemic effect; Antioxidant capacity; Oxidative stress; Aqueous extract, Moringa oleifera

Introduction

Alloxan, besides Streptozotocin is commonly employed as an experimental model of insulin-dependent diabetes mellitus due to its selective destruction of the insulin-producing pancreatic beta-islets in animals (Rohilla and Ali, 2012). Consequent hyperglycaemic condition of the body results to oxidative stress in which oxidation exceeds the antioxidant systems in the body secondary to a 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 macrovascular disease conditions like 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).

Moringa oleifera 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 it is traditionally used by Africans and some Asian countries to treat malnutrition in children and to augment breast milk. Several studies have shown that *Moringa oleifera* 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 *Moringa oleifera* against several gram negative and gram positive bacteria; specifically *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis*, E. *coli*, and Salmonella *typhi*. 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 *Moringa oleifera*. Hence this study focused on the hypoglycemic and antioxidant potentials of the aqueous extract of *Moringa oleifera*.

Materials and Methods

Sample collection and preparation

Plant material: The leaves of the *Moringa oleifera* 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 Marlex Excella laboratory electric blender.

Extract preparation: A known weight of the pulverised leaves was mixed with known volume of distilled water to obtain 30% aqueous extract of *Moringa oleifera*.

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 drinking water for a week after acclimatization after which four rats were selected and sacrificed. The remaining eight rats were injected intra-peritonially with 0.5mL of 40mg/Kg body weight alloxan with 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 *Moringa oleifera* for another one week which served as Stage III treated animals group. Animals were kept at optimum temperature with a 12 h 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 their hearts 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). Lastly, the 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

Table 1.0: Effect of aqueous extract of *Moringa oleifera* 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]

Each value is a mean of 3 determinations ± SEM

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

Table 2.0 Effect of aqueous extract of *Moringa oleifera* 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]

Each value is a mean of 3 determinations ± SEM

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 *Moringa oleifera* 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]

Each value is a mean of 3 determinations ± SEM

From Table 3.0, reduced glutathione level decreases upon injection with alloxan against the control stage one. When treated with Moringa oleifera extract, a significant increase is observed. This is consistent with the results of Luqman *et al.* (2012) in which higher antioxidant capacity was reported with increase in GSH level in a dose-dependent manner. The ethanolic extract of the plant reportedly showed highest phenolic content along with strong reducing power and free radical scavenging capacity.

Table 4.0: Effect of aqueous extract of *Moringa oleifera* 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]

Each value is a mean of 3 determinations ± SEM

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

Table 5.0 Effect of aqueous extract of *Moringa oleifera* 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]

Each value is a mean of 3 determinations ± SEM

The catalase activity is presented in Table 5.0. When viewed with respect to the control stage one, there was a increase in its level when the animals received alloxan. On the contrary, a treatment with Moringa extracts in stage three shows a fall to italmost the control level. The result is the reversal of 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 antidiabetic and antioxidant properties of aqueous extract of *Moringa oleifera* 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 pancreases cell in the system and the weight of the rats after treatment with *Moringa oleifera* 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 *Moringa oliefera* leaf had a remarkable gain in body weight (Table 1.0).

As observed in Table 2.0, rats treated 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 *Moringa oleifera* 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 *Moringa oleifera* treated rats' stage II blood glucose shows the hypoglycemic action of the *Moringa oleifera* which was also observed in similar works of (Gomathy, *et al.,* 1990).

Our results in Table 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 therefore show that *Moringa oleifera* has a protective effect on antioxidant defence mechanism of the system to improve the glucose metabolism.

Table 5.0 also shows catalase increases in diabetic induced rats and depletes in *Moringa oleifera* 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 *Moringa oleifera* aqueous extract that was given to the rats. This study shows the ability of *Moringa oleifera* 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 ratbbits with unripe plantain.

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 spread. The induced hyperglycaemic state is being linked to oxidative stress due to insufficient antioxidants in the body.

To boost the body's response to such stress, *Moringa oleifera* leaves aqueous extract has been administered to diabetic rats. The results proved that *Moringa oleifera* 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.

It is therefore, suggested that people be encouraged to include Moringa in their diets because of its protective and recuperative power against various diseases. Again, further research can be done to develop affordable Moringa-based drugs.

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