East African Scholars Journal of Medical Sciences
Abbreviated Key Title: East African Scholars J Med Sci
ISSN 2617-4421 (Print) | ISSN 2617-7188 (Online) |
Published By East African Scholars Publisher, Kenya

OPEN ACCESS

Volume-2 | Issue-9| Sept -2019 |

Research Article

Effect of Aqueous Root Extract of Moringa Oleifera Lam. on the Histology of the Testis in Alloxan-Induced Diabetic Rats

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Abstract: This study was conducted to investigate the effect of aqueous root extract of *Moringa oleifera* on the histology of the testis in Alloxan-induced diabetic rats. A total of twenty Albino Wistar rats were used for the experiment. The animals were divided into four groups of five rats each (Groups I, II, II and IV). Diabetes was induced by administering a single dose of 150mg/kg alloxan monohydrate. Glucose levels were taken on the onset of the experiment and a confirmation of diabetes after three days of administration of alloxan. The diabetic animals were treated with aqueous extract of *Moringa oleifera* root for a period of twenty eight days at 50mg/kg on daily basis orally, while glucose levels and weight gain/loss were recorded weekly. Blood glucose levels were also measured a day before sacrifice. At the end of the experiment, the animals were sacrificed and the testes of the rats in each group were processed for routing light microscopic analyses. Weight of animals and glucose levels obtained from the test animals were compared with the control. The results showed that the extract of Moringa root water extract was able to caused mild regeneration of the damaged seminiferous tubules and cells of the spermatogenic series damaged by Alloxan monohydrate. There was reduced hyperglyceamic activity in the *Moringa* treated animals when compared with the diabetic-non treated group. Several related literatures were consulted in the process of this investigation to match with the standard research procedures, ethical clearance was obtained from the ethical committee of the university. The performance of Moringa root in treating diabetes in rats might be due to its free radicals scavenging activity using its phenolic content or other relevant properties.

Keywords: *Moringa oleifera*, alloxan, diabetes, testis.

INTRODUCTION

Diabetes mellitus, also known as diabetes, is a group of metabolic disorders as a result of high blood sugar levels for a prolonged period and it is characterized by polyuria, polydipsia and polyphagia, weight loss and sometimes blurred vision (Report of the Expert Committee on the diagnosis and Classification of diabetes Mellitus. 2003; World Health Organization. 2014).Without proper treatment, diabetes can lead to various acute and chronic complications. Acute complications include diabetic ketoacidosis and nonketotic hyper osmolar coma, while the chronic complications include cardiovascular disease, stroke, chronic kidney failure, foot ulcers, and retinopathy (World Health Organization. 2014; Kitabchi, A. E. *et al.*, 2009).

Diabetes mellitus is a metabolic disorder of global concern (Yusuf, S. *et al.*, 2001). In developing countries, it is on its way to becoming a major cause of morbidity and mortality as infectious diseases, due to the progressive transition in these countries to a lifestyle characterized, among other aspects, by greater access to dietary calories and less demand for calorie expenditure (Hossain, P. *et al.*, 2007; Aje, T. O., & Miller, M. 2009).

Diabetes was one of the first diseases described, with an Egyptian manuscript in 1500 BCE meaning "too great emptying of the urine (Ripoll B. & Leutholtz I. 2011)". The first described cases were believed to be of type 1 diabetes (Leonid, P. 2009). Indian physicians around the same time identified the disease and classified it as madhumeha or "honey urine", noting the urine would attract ants. The term

Quick Response Code

Journal homepage:
http://www.easpublisher.com/easjms/

Article History
Received: 04.09.2019
Accepted: 12.09.2019
Published: 28.09.2019

"diabetes" or "to pass through" was first used in 230 BCE by the Greek Apollonius of Memphis (Leonid, P. 2009).

Moringa oliefera is an edible plant of wide variety of nutritional and medicinal values. Phytochemical analyses of this plant have shown that its leaves are particularly rich in potassium, calcium, phosphorous, iron, vitamins A and D, essential amino acids, as well as such known antioxidants such as β-carotene, vitamin C, and flavonoids (Bennett, R. N. *et al.,* 2003; Aslam, M.*et al.,* 2005).

The therapeutic use of *Moringa oleifera* parts in the Indian subcontinent dates back to Antiquity. In other parts of the world, in sub-Saharan Africa in particular, such a use appears not to have been known by the wide public, as it is being promoted by diverse organizations as an untapped opportunity (Thurber, M. D., & Fahey, J. W. 2009). In many regions of Africa, it is widely consumed for self-medication by patients affected by diabetes, hypertension, or HIV/AIDS (Dieye, A. M. *et al.,* 2008).

MATERIALS AND METHOD

This research was conducted in the Department of Human Anatomy University of Maiduguri, to observe the effect of aqueous root extract of *Moringa oleifera* on the histology of the testis in alloxan-induced diabetic rats.

Materials

Materials used during this investigation included 20 Wistar laboratory rats, Alloxan monohydrate, glucometer, glucose strips, rat cages, beddings, feeders, syringe, needle, feeding tubes, *Moringa oleifera* root aqueous extract, weighing balance device, specimen bottles, laboratory reagents for tissue processing, microscope, glass slides cover slips and dissecting set.

Animals

Mature male albino rats weighing between 150 to 220 grams were obtained from the laboratory animal holdings, Department of Human Anatomy, University of Maiduguri, Nigeria. The study was conducted on 20 albino rats. The animals were maintained on pellet diet and water *ad libitum*. The rats were housed 4 per cage and allowed to acclimatize to existing climatic condition in the animal house for the period of 14 days before the commencement of administration of alloxan monohydrate and *Moringa oleifera* aqueous solution. Animals were kept in well ventilated cages and housing with the average temperature of 27 ± 20C. The lighting consists of natural day light: darkness rhythm. Institutional animal ethical committee permission was obtained before carrying out the experiment.

Experimental Design

A total of 20 rats weighing between 120-250mg/kg were used for the study. After acclimatization of the animals for 7days, the rats were divided in to four groups of five animals each.

The Animals Were Grouped As Follows:

Group I was the control group in which the animals received only the vehicle (distilled water) at an equivalent dose in addition to normal diet. In the control group, diabetes was not induced and neither *Moringa* nor Alloxan was used for the period of twenty eight (28) days.

Group II contained five rats as the diabetic non- treated group; 150mg/kg of Alloxan monohydrate single dose was administered to the animals intraperitoneally. Afterwards, the effect of Alloxan was observed. Diabetes was confirmed using the glucometer. *Moringa eliferaroot water* extract was not administered to this group. Hence, they were not treated. Those with glucose level above 6mmol/l of blood levels were considered diabetic.

Group III, in which Alloxan monohydrate at 150mg/kg was used to induce diabetes and treated with *Moringa oleifera* aqueous root extract at 50mg/kg and the effect was observed in the test animals. The treatment commenced on the fourth day of the experiment.

Group IV, this group received *Moringa* aqueous root extract (50mg) alone without inducing diabetes mellitus.

Regular observations for weight loss or gain, was measured using weighing balance at the end of every week. Glucose level was checked at the end of every week, using glucometer and glucose test strips. At the end of 28 days, the rats were sacrificed.

Median incision was carried out on the scrotal sac to remove testis which were subsequently fixed in bouins fluid overnight. Afterwards, the organs were processed for paraffin section.

Plant Source and Authentication

Moringa oleifera roots were obtained during the dry season from home- grown garden in Maiduguri, Borno state, Nigeria. The family and species of *Moringa oleifera* were authenticated by a plant taxonomist, Professor S. S. Sanusi of the Department of Biological Sciences, University of Maiduguri Nigeria. Two kilograms of *Moringa oleifera* roots were washed and air-dried under the shade for one week.

Plant Extraction

The dried *Moringa oleifera* root was pulverized using pestle and mortar. The dried powder was then transferred into 5-litre capacity soxhlet reflux extractor apparatus to obtain a crude aqueous root

extract fitted with condenser for water to circulate. The crude aqueous extract was then removed, re-suspended in cool distilled water and filtered to remove debris. Thirty (30) grammes of the extract were obtained.

Body weight Determination

Body weights of animals were recorded with the aid of triple arm balance. Weights of animals were recorded weekly for the period that the experiment lasted.

Alloxination and Glucose level

The animals were fasted for 12 hours, but allowed access to water before and during the experiment. Diabetes was induced using slow intraperitoneal injection of 1% solution of Alloxan at 150mg/kg body weight dissolved in distilled water and administered within few minutes of its preparation. The diabetic state was determined on the fourth day using blood glucose determination device (glucometer/test strips). This was done by withdrawing blood from tip of the tail at the end of fasting, taken as the zero time (0 hour).

3.7Tissue Processing

At the end of the experiment the animals were sacrificed by cervical dislocation. The testes were removed by median incision on the scrotal sac. The tissues were trimmed, dehydrated in graded series of Alcohol in ascending order of 30%, 50%, 80%, 95% and 100% respectively. The tissues were cleared in Xylene, embedded in Paraffin wax then cleared in Xylene again. The slides were then covered with cover slips. Micrographs were taken using photomicroscope at x100, x200 and x400 respectively and interpreted.

3.8 Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by student "*t*" test. The values are mean ± S.D. for five rats in each group. *P*-values ≤0.05 were considered as significant.

RESULTS

Effects of *Moringa oleifera* root aqueous Extract in Alloxan-induced Diabetic rats on body weight

The results obtained showed that in the control group, the final mean body weight was 194.40g and the difference in body weight between the initial and final body weights was 1.80g. In the diabetic non-treated group, the final mean body weight was 177.40g and the difference between the initial and final body weight was -11.10g. In the diabetic treated group with *Moringa oleifera* root extract, 50mg/kg for 28days, the final mean body weight was 182.00g and the difference between the initial and final mean body weight was 2.00g. In the case of non-diabetic group but treated with *Moringa oleifera*, the mean body weight was 188.60g while the difference between the initial and final mean was 2.40g. All the results obtained showed minor variations in the mean body weights. In the control group (group 1), the final mean body weight was 194.40g. In the diabetic non-treated group (group11), the final mean body weight was 177.40g,which showed a decrease in body weight in this group when compared with the control group that was not significant (P>0.05). In the diabetic but treated group with *Moringa* root aqueous extract, the final mean body weight was 182.00g and this was considered a weight loss when compared to the control group but the value was not significant P>0.05. .In the non-diabetic but treated with *Moringa* root aqueous extract only (group 1V), the final mean body weight was 188.60g.compared with the control group. The values are shown in table 1.

Table 1 Effect of *Moringa oleifera* Aqueous Extract on Body Weight of Alloxan Diabetic Induced Albino Wister Rats

Groups (g)	Initial Body Weight (g)	Final Body Weight (g)	Differences (g)
Control	192.60 ± 9.48	194.40 ± 10.31	1.80
Diabetic Non -Treated	188.50 ± 10.53	177.40 ± 12.1	-11.10
Diabetic Treated *Moringa*	180.00 ± 4.28	182.00 ± 4.64	2.0
Non- Diabetic Treated	186.20 ± 13.31	188.6 ± 1.77	2.4

Values are Mean ± SD, p≤0.05 (Comparison Relative to Control). (n=5). ***= s**ignificant

Effect of *Moringa oleifera* root Aqueous Extract on Glucose Levels of Alloxan-induced Diabetic Rats

At the beginning of the experiment, the initial glucose levels of all the animals were determined. Subsequently, at the end of the experiment, the final glucose levels were taken. The initial and final mean glucose levels are shown in table 4.3. The results showed that the final mean glucose level of the control group (group 1) was 80.40mg/dl, while that of diabetic non-treated group (group11) was 278.20mg/dl which showed an increase in the glucose level compared to the control group and it was significant statistically at P-value. In diabetic but treated group, the mean glucose level was 167.60 mg/dl which was higher than that of the control group but it was not considered significant statistically. In the non-diabetic group (group1V) but treated with *Moringa* aqueous root extract the final mean glucose level was 71.60mg/dl which was insignificantly (p>0.05) slightly lower than the control value.

Table 2 Effect of *Moringa* Aqueous root Extract on Glucose Level of Alloxan-induced Diabetic Albino Wistar Rats

Groups	Initial mean Glucose Level mg/dl	Final mean Glucose Level mg/dl	Differences mg/dl
Control	72.00 ± 9.18	80.40 ± 13.24	8.40
Diabetic Non Treated	67.20 ± 12.14	278.20 ± 142.74**	211.00
Diabetic Treated *Moringa*	73.80 ± 11.05	167.60. ± 40.48	93.80
Non Diabetic Treated *Moringa*	78.40 ± 10.36	71.60 ± 8.14 **	-6.80

Values are Mean ± SD, p≤0.05 (Comparison Relative to Control). (n=5).
*= significant ***= highly significant

Effects of *Moringa oleifera* root aqueous extract on the histology of the testis

Photomicrograph of control rat testis showed normal seminiferous tubules, blood vessels and interstitial connective tissue (figure 1). Photomicrograph of the testis of a rat from the diabetic non-treated group (Alloxan 150mg/kg single dose) showed focal degeneration of seminiferous tubules, empty interstitial connective tissue space and degeneration of germ cells (figure 2). Photomicrographs of rat testis of diabetic treated group showed regeneration of some of seminiferous tubules, scanty interstitial connective tissue, mild edema and slight disruption of the basement membrane (figure 3). Photomicrograph of the testis of a rat from non-diabetic but treated group with *Moringa oleifera* root aqueous extract (50mg/kg) showed normal seminiferous tubules (ST) interstitial connective tissue and mild edema (figure 4).

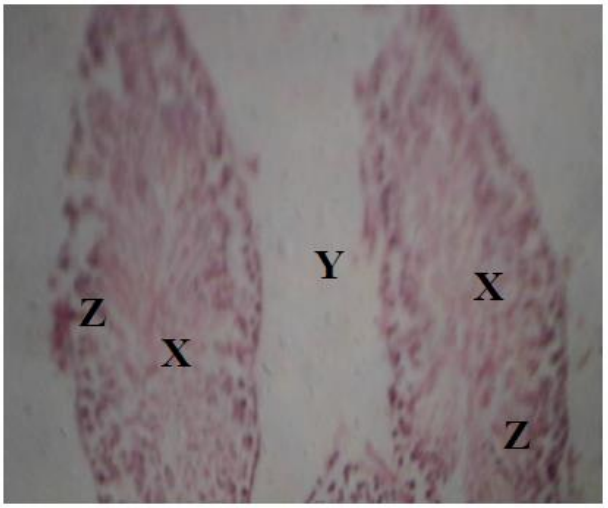

Figure 2. Photomicrograph of the testis of a rat from the diabetic non-treated group (Alloxan 150mg/kg single dose) showing focal degeneration of seminiferous tubules (x) empty interstitial connective tissue space (Y) degeneration of germ cells (z) x100(H&E stain).

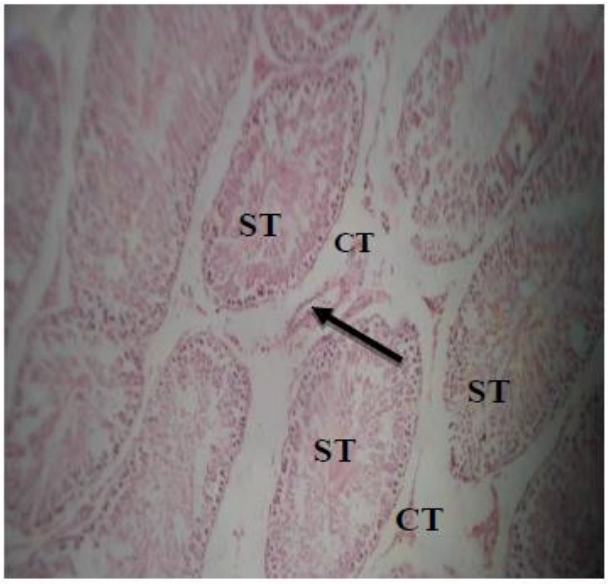

Figure1. Photomicrograph of control rat testis showing normal seminiferous tubules (ST), blood vessels (arrow) and interstitial connective tissue (CT) H&E x100.

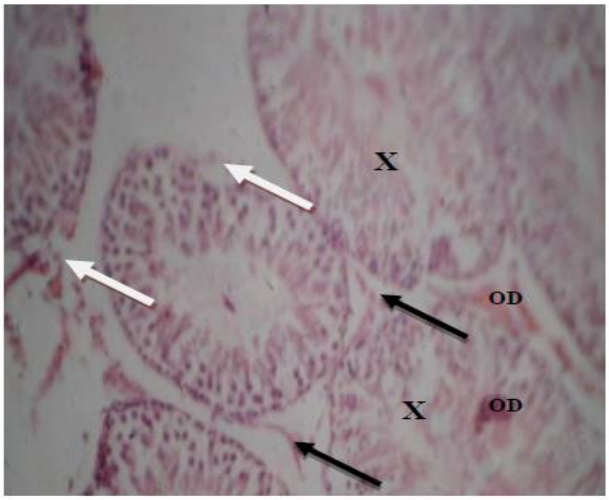

Figure 3.Photomicrographs of rat testis of diabetic treated group showing regeneration of some of seminiferous tubules (x) scanty interstitial connective tissue (black arrows) mild edema (OD) and slight disruption of the basement membrane (white arrows) x100 (H&E stain).

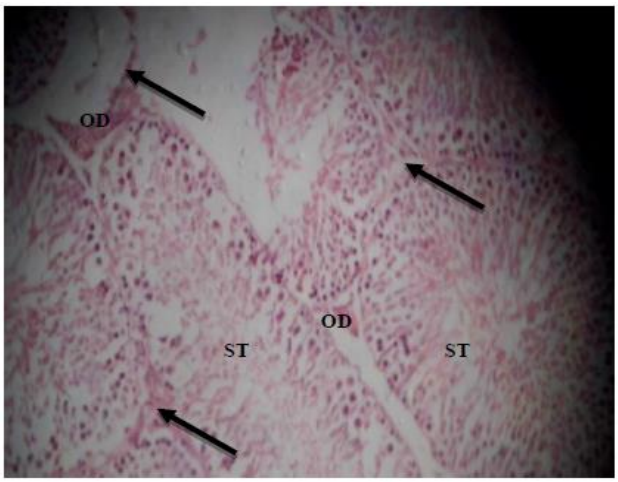

Figure4. Photomicrograph of the testis of a rat from non-diabetic treated group with *Moringa oleifera* root aqueous extract (50mg/kg) showing normal seminiferous tubules (ST) interstitial connective tissue (arrows) and mild edema (OD) x100 (H&E stain)

DISCUSSION

Diabetes mellitus is one of the leading causes of morbidity among the world population. Its prevalence has continuously increased globally over years. Alloxan-induced diabetes is one of the widely used methods of inducing type 1 diabetes mellitus in experimental animals. This is because alloxan has been found to be selectively toxic to the beta cells of the pancreas. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels (Trease, G. E., & Evan, W. C. 2002). Alloxan is hydrophilic and unstable chemical compound that resembles glucose molecule in shape and is responsible for its selective uptake and accumulation by the pancreatic beta cells. In addition, due to this similarity in shape, it is easily transported into the cytosol by the glucose transporter (GLUT 2) found in the plasma membrane of the beta cells (Elsner, M. *et al.*, 2002). The alloxan chemical caused oxidative damage to the pancreatic beta cells because alloxan is a well-known diabetogenic agent among the biomedical scientists that has been used to induce type I diabetes worldwide. In this current study, alloxan selectively destroys the insulin-producing beta-cells found in the pancreas. The toxic action of alloxan that facilitates the destruction of the beta- cells involved the oxidation of essential sulphydryl groups (S-H groups), inhibition of glucokinase enzyme, generation of free radicals and disturbances of intracellular calcium homeostasis. The alloxan action is normally preceded by its rapid uptake by pancreatic beta cells that have been proposed to be one of the important features that determine the diabetogenicity of alloxan chemical. In addition, in pancreatic beta cells, the reduction process occurred in the presence of different reducing agents such as reduced glutathione (GSPH), cysteine, ascorbate and protein-bound sulphydryl groups. The alloxan reacts with two S-H groups in the sugar binding site of the glucokinase resulting in the reduction of the alloxan and subsequent formation of the disulphide bond that led to inactivation of the enzyme. As a result of this reduction of alloxan, dialuric acid was formed and then re-oxidized back to alloxan which established a redox cycle for the generation of reactive oxygen species (ROS) and superoxide radicals. The above statement was in agreement with similar experiments conducted by (Gorus, F. K. *et al.*, 1982; Munday, R. 1988). The superoxide radicals then liberated ferric ions from ferritin and reduce them to ferrous and ferric ions. The superoxide radicals further underwent dismutation to yield hydrogen peroxide (H2O2) in the presence of superoxide dismutase. This resulted in to release of highly reactive hydroxyl radicals. In a related development, another mechanism of action of alloxan, demonstrated by (Das, J. *et al.*, 2012; Szkudelski, T. 2001) was the effect of reactive oxygen species on the DNA of pancreatic cells. According to them, fragmentation of DNA took place in the beta cells exposed to the alloxan which causes DNA damage and subsequently stimulation of poly ADP- ribosylation, a step in DNA repair. Antioxidants such as superoxide dismutase, catalase and non-enzymatic scavengers of poly hydroxyl radicals, have been demonstrated to give substantial protection against alloxan toxicity. Disturbances in intracellular calcium homeostasis have also been reported to constitute an essential step in determining the diabetogenic effect of alloxan. It was also observed that alloxan raises the cytosolic concentration of free calcium ions in the beta cells of the pancreas by stimulating an increased influx of calcium ions due to the action of alloxan of depolarizing the beta cells of the pancreas which further opens the dependent calcium channels and facilitates the entry of calcium ions in to the pancreatic beta cells. Such increment in the level of calcium causes high increase in the physiological activity of the cells thereby enhancing the degranulation of insulin stores and consequently enhanced insulin release that together with the reactive oxygen species (ROS) cause serious damage to the beta cells of the Islets of Langerhans.

The roots, of *Moringa oleifera* have been found to possess several distinct pharmacological properties and root preparations are used for the cure of bronchitis, stomatitis, common cold, fever, diarrhea, dental caries, flatulence, edema, analgesic, anti-inflammatory, anti-diabetic and many others (Mittal, M. *et al.*, 2007).

In line with the current investigation on the root of aqueous extract of *Moringa oleifera*, determining its antidiabetic effect (Al-Awwadi, N. *et al.*, 2004), in their research indicated that leaves aqueous extract of *Moringa oleifera* significantly decreased blood glucose concentration in Wistar rats and Goto-Kakizaki rats,. Another study conducted by

(Ghasi, S. *et al.*, 2000) indicated that the extract from *Moringa* leaf was effective in lowering blood sugar levels within 3 h after ingestion. Similarly, (Lenzen, S. 2008; Rohilla, A., & Ali, S. 2012) indicated that dark chocolate polyphenols and other polyphenols are responsible for hypoglycemic activity of *Moringa* leaves extract. Therefore, the potential antidiabetic activity of *Moringa oleifera* can be commercialized pharmaceutically through the development of suitable technology that will match the internationally standard recognized antidiabetic drugs.

Reduced mean body weight was observed among the test groups when compared to the control group. There was no weight loss in the other groups, including the control group. This will not be unconnected with the fact that in most cases of type I Diabetes mellitus, abnormalities associated with improper delivery of glucose, fats and proteins to the body tissues results in to reduction of the body weight. The basis for these abnormalities in carbohydrate, fat and protein metabolism in type I diabetes mellitus is deficient action of insulin on target tissues of the body as a result of inadequate secretion or diminished tissue responses to insulin at one or more points in the complex pathways of the hormone action (Rohilla, A., & Ali, S. 2012). In group III, the diabetic but treated; there was slight weight gain in both group 111 and 1V in final mean body weight as compared to the control (group I) which might have occurred as a result of the treatment with *Moringa oleifera* aqueous extract that was able to reverse the effect of the diabetes mellitus induced by the Alloxan monohydrate substance. The slight gain in mean body weight observed in group IV might have happened by chance or the *Moringa* roots aqueous extract has some contents that can mildly increase body weight when consumed. This is because there was no induction of diabetes mellitus in group IV but only treated with the aqueous extract of Moringa oleifera.

The study on the impact of the *Moringa* aqueous extract on glucose levels in the test animals revealed that the final glucose levels in the experimental animals were compared in relation to the control group. The final mean glucose observed in the control group was 80.40 ±13.24mgdl. In the diabetic non-treated group (group II), the final mean glucose level was 278.20 ±142.74mg/dl and that was highly significant statistically at $P<0.05$ as compared to the control group. This significant increase in glucose concentration in the blood circulation was due to the effect of alloxan monohydrate (150mg/kg) on the beta cells of Islets of Langerhans which led to the toxic damage of the beta cells by the alloxan and subsequent inhibition of Insulin secretion by the beta cells as explained earlier. Consequently, there was hyperglyceamia in group II indicating that diabetes had been induced. The development of diabetes mellitus was confirmed using the glucometer and test strips on the blood of the Wistar rats. In addition, some cardinal signs of diabetes mellitus type I were observed, such as increased urination or polyuria, polydipsia or increased thirst, weight loss, fatigue and polyphagia. The method employed in the induction of diabetes mellitus type I in the animal models was in line with similar studies conducted by Lenzen (2008); Rohilla and Ali (2012). In group III, the diabetic but treated group with *Moringa* root aqueous extract, there was an increase in the final mean glucose level compared to the control group. The increase in glucose level in these animals despite the fact that they received the *Moringa* extract, was attributed to the possible presence of an element of diabetes in the animals, since at the beginning they were induced with diabetes mellitus via the administration of the alloxan chemical at 150mg/kg before the administration of the *Moringa oleifera* extract 50mg/kg. The increase in final mean glucose level in this group was far less than the diabetic non-treated group when compared. In group IV, the non-diabetic but treated with *Moringa* aqueous extract, the level of glucose when compared to the control, was less. This might have occurred by chance, or that the *Moringa* root aqueous extract has hypoglyceamic effect of lowering the glucose concentration in the blood via some mechanisms.

CONCLUSION

This study concludes that Alloxan substance was found to be effective in causing type I (diabetes mellitus) in the experimental animals as observed in group II and diabetes treated group. On the other hand alloxan causes some damages on the testicular tissues. Treatment with *Moringa oleifera* root aqueous extract in the diabetic rats was found to be relatively effective in reversing the damaging effect of the Alloxan chemical on the testis.

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