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Moringa oleifera Leaf Extract Ameliorates Glucose, Insulin and Pancreatic Beta Cells Disorder in Alloxan-Induced Diabetic Rats.

Nabila Ibrahim El-Desouki*, Mohamed Aboulfotouh Basyony, Mona M Abdelmonaim Hegazi, and Mohamed Samir I El –Aama.

Department of Zoology, Faculty of Science, Tanta University, Tanta 31527, Egypt.

ABSTRACT

The purpose of this study is to evaluate the effect of aqueous leaf extract of *Moringa oleifera* (orally administered) on blood glucose level, insulin and pancreatic β- cells in alloxan- induced diabetic rats. Experimental method was adopted. Tanta University, Egypt between September 2013 and January, 2015. Sixty adult male albino rats of the Wistar strain weighing 99 ± 1.03 g were used for this study. These were randomly assigned into 6 groups of 10 animals per group as; Group I: Normal control rats, Group II: Normal rats administered with low dose of moringa (200 mg/kg/d for 30 days), Group III: Normal rats given high dose of moringa (400 mg/kg/d for 30 days), Group IV: Diabetic rats, animals received only alloxan intraperitoneally and were not treated (served as Hyperglycemic group), Group V: Diabetic rats administered with low dose of moringa as in group II, Group VI: Diabetic rats administered with high dose of moringa as in Group III. At the end of the experiment, rats were sacrificed, and pancreatic specimens and blood samples were collected after 14 hours fast. Changes in the rats' blood levels of glucose and insulin were determined in all animal groups. Pancreatic histopathology and the IHC expression of insulin producing cells (β-cells) were also examined. A significant increase in blood glucose and a significant decrease in insulin of diabetic rats were recorded when compared with the normal control group. Treated diabetic rats with high dose of *Moringa oleifera* extract recorded a significant decrease in blood glucose level and a significant increase in insulin. Low dose of moringa treatment to diabetic rats recorded slight decrease in plasma glucose and a slight increase in insulin. Histopathologically, diabetic rats treated with low dose of moringa showed no improvement of pancreatic islets and acinar cells, while the pancreatic tissues of diabetic rats treated with a high dose of moringa illustrated an obvious recovery of pancreatic islets and acinar cells to approximately normal status. By using IHC, the expression of insulin producing cells (β- cells) that, were minimized in diabetic rats, restored in the rats group treated with high dose of moringa. Oral administration of *Moringa oleifera* extract at a high dose diabetic rats is considered as a strong anti-hyperglycemic, reduced the blood glucose and increased the insulin level as well as the histological improvement of the pancreatic tissues, minimized the degeneration of pancreatic β-cells immunoreactivity and recovery the insulin producing cells (β- cells).

Keywords: Diabetes, *Moringa oleifera*, Glucose, insulin, Alloxan, Histology, β- cells IHC, Rats

Corresponding author

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INTRODUCTIN

Diabetes mellitus or simply diabetes is a group of metabolic diseases in which a person has high blood sugar. This high blood sugar produces the symptoms of frequent urination, increased thirst, and increased hunger. Untreated diabetes can cause many complications. Acute complications include diabetic ketoacidosis and nonketotic hyperosmolar coma. Serious long-term complications include heart disease, kidney failure and damage to the eyes *(*Alberti *et al.,* 1998; WHO, 2014). Diabetes is due to either the pancreas does not producing enough insulin or because cells of the body do not respond properly to the insulin that is produced (Gardner and Dolores *et al*., 2011).

Globally, as of 2013, an estimated 382 million people have diabetes worldwide, with type 2 diabetes making up about 90% of the cases. This is equal to 3.3% of the population, with equal rates in both women and men (Vos *et al.,* 2012). In 2011 diabetes resulted in 1.4 million deaths worldwide, making it the 8th leading cause of death (WHO, 2013).The number of people with diabetes is expected to rise to 592 million by 2035.

Oxygen free radicals and other "reactive oxygen species" are constantly produced in the human body. Multiple studies have shown that the type 2 diabetes is accompanied by increased oxidative damage to all biomolecules in body. Diabetes produces disturbances of lipid profiles, especially an increased susceptibility to lipid peroxidation. An increased oxidative stress has been observed in diabetic patients as indicated by high free radical production (Giugliano *et al.,* 1996). Oxidative damage due to free radicals was associated with vascular disease in people with diabetes (Oberley, 1988; Giridhari *et al.,* 2011).

There are several potential resources of free radical production in diabetics including autoxidation of plasma glucose, activation of leucocytes, and increased transition metal bioavailability. The total antioxidant status in diabetes was lower than that of age-matched controls, and this might be attributed to lower levels of vitamin C, vitamin E in blood or other factors including micronutrients (Giridhari *et al.,* 2011) and (Rohilla and Ali, 2012).

The increased susceptibility of tissues such as the liver and kidney of diabetic animals to diabetic complications may be due to increased lipid peroxidation and to excessive oxidative stress. From this view point, prevention of oxidative damage was considered to play a crucial role in diabetes and / or its complications resulting from lipid peroxidation (Stanely and Menon, 2001).

Moringa oleifera belongs to the family of Moringacaea, a fast growing drought resistant tree but now distributed worldwide in the tropic and sub tropics and is cultivated extensively in central and South America, Africa, Indonesia, Mexico, Malaysia, the Philippines, and India (Fuglie *et al.,* 1999). *Moringa oleifera* is an edible plant. Different parts of moringa plant contain important minerals as K, Ca, P, Fe, and are a good source of protein, vitamins, beta-carotene, amino acids and various phenolics as zeatin, quercetin, β-sitosterol, caffeoylquinic acid and kaempferol (Anwar *et al.*, 2007) and high concentrations of natural dietary antioxidants: Vitamins A, C and E. Moringa provides high concentrations of four natural dietary antioxidants: Vitamins A, C, E and phenolics (Gowrishankar *et al.,* 2010).

Moringa is a rich source of ascorbic acid helps in insulin secretion. It is interesting to note that certain nutrients like vitamins B1, B2, B12, pantothenic acid, vitamin C, protein and potassium - along with small frequent meals containing some carbohydrate - can actually stimulate production of insulin within the body (Quisumbing, 1978). Researchers recently reported that vitamin D is essential for the pancreas to be able to secrete insulin properly. The studies have shown that individuals with the lowest vitamin D levels experienced the worst blood sugar-handling problems and had a greater risk of developing diabetes (Talaei *et al.,* 2013).

Additionally, moringa contains 46 antioxidants which help cells to neutralize free radicals. It is traditionally used for relieving spasm, for treatment of diarrhea, diuretic and stimulant in paralytic affliction, epilepsy and hysteria *(*Quisumbing, 1978*)* and treatment of diabetes mellitus (Babu and Chaudhuri, 2005); hepatotoxicity *(*Ruckmani *et al.,* 1998), rheumatism, venomous bites and also for cardiac stimulation (Chaudhary and Chopra, 1996). Moringa oleifera is very useful in regulating the thyroid hormone status in adult Swiss rats (Tahiliani and Kar, 2000). Its leaves are also used as nutritional supplement and growth promoters (Lakshminarayana *et al.,* 2005 and Sanchez *et al.,* 2006).

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The present investigation was designed to study the effect of the leaf extract of *Moringa oleifera* on the blood glucose and insulin levels of the experimentally-induced diabetes in adult albino rats as well as the histopathological and IHC observations of the pancreatic tissues.

MATERIALS AND METHODS

Animals

Sixty adult male albino rats weighing 99 ±1.03 g were used in the present investigation and were supplied from Vacsera 51 Wezaret El Zeraa St. Agouza, Giza, Egypt. All rats were kept under the same environmental conditions for two weeks before study. The animals were fed *ad Libitum* with a standard diet and allowed free access of water and they were housed in metal cages in a well-ventilated animal room. All Care and procedures adopted for the present investigation were in accordance with the approval of the Institutional Animal Ethics Committee of National Research Center and in accordance with recommendation of the proper care and use of laboratory animals**.**

Chemicals

Alloxan monohydrate was received from Sigma Chemical Company (st. Louis. Mo.USA) and used in the induction of diabetes. To induce expermental diabetes , alloxan was dissolved in acetate buffer and was injected into fasting rats at 150 mg/kg as recommended by Ajibola *et al.* (2014), then the rats were injected with 100mg/kg of alloxan (2^{nd} injection) after two days . Lastly, 3rd injection of alloxan (100 mg/kg) was applied two days after the 2^{nd} one. Note, the 2nd and 3^{rd} injections of alloxan were used to ensure the insult of diabetes through the experimental duration. Blood glucose levels were measured, and the glucose level >250 mg/dl was accepted to be diabetic.

Moringa oleifera was received from Vacsera 51 Wezaret El Zeraa St. Agouza, Giza, Egypt which used in treatment of diabetic rats. Fresh leaves of *Moringa oleifera* were collected and were air-dried and reduced to powdered form. The powdered leaves were percolated in distilled water for 12 h and filtered; the filtrate was subsequently evaporated to dryness and yielded a concentrate. Then rats were taken orally low and high doses (200 & 400 mg/kg/bw/d) of moringa (Sharifudin *et al*., 2013).

Experiment

All animals were kept under the same laboratory conditions of temperature and natural photo period. All animals received a standard food diet and drinking tap water *ad libitum.* All procedures were approved by the Animal Care and Bioethics of the Egyptian committee, and all procedure were done at the Faculty of Science, Tanta University, Egypt. The animals were housed in cages, and divided into six groups (10 rats for each) as follows:

Group I: control rats daily injected with 0.1 ml diluent solution.
Group II: undiabetic rats administered with low dose of moringa (200mg/kg/d) for 30 days.
Group III: undiabetic rats administered with high dose of moringa (400 mg/kg/d) for 30 days.
Group IV: alloxan- diabetic rats (served as Hyperglycemic group)
Group V: diabetic rats administered with low dose of moringa (200mg/kg/d) for 30 days.
Group VI: diabetic rats administered with high dose of moringa (400mg/kg/d) for 30 days.

At the end of 30 days of experiment, rats were fasted for 16 hours and then sacrificed by decapitation and pancreatic tissue samples were carefully dissected out and divided into three pieces for biochemical, histological and immunohistochemical studies.

Blood glucose and insulin estimation

Blood glucose was estimated on 0, 7, 14, 21 and 30 day by using Accu-Chek Performa Apparatus according to Brăslasu *et al.* (2007) and (Abunasef *et al.,* 2014). Insulin was determined by using a rat-specific Insulin-Ak ELISA according to Finlay and Dillard (2007).

Histological study

The pancreatic tissues were harvested from the sacrificed rats after dissection and washed with saline, cut into small pieces and then specimens were fixed in 10% neutral formalin. The fixed specimens were sliced, processed, and embedded into paraffin blocks. The blocks were cut into 5 µm thick paraffin sections by a rotary microtome. The sections were stained with Hematoxylin and Eosin H&E (Bancroft and Gamble, 2002) for histological observation.

Immunohistochemical staining (IHC) study

IHC detection of pancreatic tissues with immunolocalization technique for anti-insulin monoclonal antibody was performed as previously described (Hsu *et al.,* 1981). IHC reaction was carried out by using avidin biotin peroxidase method by Nova Castra Laboratories Ltd, UK. Endogenous peroxidase activity was inhibited by incubation with 0.3% H2O2 for 30 min. The sections were blocked with normal goat serum for 1 h to prevent non-specific binding followed by incubation with the primary insulin monoclonal antibody for 1 h at room temperature. The sections were incubated with the secondary antibody (biotinylated anti-mouse IgM) for 30 min. The sections were then incubated with ExtrAvidin (Sigma) for 45 min at 37 °C. Staining was visualized using diaminobenzidine (DAB, Sigma), then slides were washed and counterstained with haematoxylin, cleared, mounted and examined by light microscopy. Finally, the insulin secreting β-cells cytoplasmic sites of reaction were stained brown and nuclei stained blue.

Statistical Analyses

All results were expressed as mean ± SEM. Statistical analysis was carried out by one way analysis of variance (ANOVA). Differences in means were considered significant at $P < 0.05$.

RESULTS

Biochemical results

Effect of moringa leave extract on blood glucose value

Inducing of diabetes caused a significant increase of blood glucose value (p=0.05) to 513.2±4.62 mg/l with a difference 551.27 % compared with normal control rats before beginning of experiment (78.8±1.77 mg/l). After seven days of moriga leave extract treatment with both low and high dose to undiabetic rats, it caused a difference (-2.48 & -2.58, respectively) compared with the control normal rats. The moringa treatment to diabetic rats with high dose caused a significant decrease of blood glucose value (377.5±53.5 mg/l) with difference (-23.98 %) than the treatment with low dose of moringa (437.3±29.72 mg/l) with difference (-11.9%) as compared to the corresponding value of diabetic rats at (p=0.05).

At the end of experiment, the diabetic rats have 503.7±3.4 mg/l glucose value with a difference 534.68 % compared to the control normal rats. The moringa treatment to diabetic rats with high dose caused highly significant decrease of blood glucose value (118±1.00mg/l) with difference (-76.57%) than the treatment with low dose of moringa (124.8±2.48 mg/l) with difference (-75.22 %) as compared to the corresponding value of diabetic rats at (p=0.05), (Table1).

Effect of moringa leave extract on serum insulin:

Diabetic rats showed significant decrease in serum insulin (9.63±0.30, -49.82%) compared to the control normal rats (19.16±0.31). The treatment with moringa at both low and high dose to undiabetic rats caused insignificant increase of serum insulin (19.5±0.20, 0.56%&19.53±.18, 2.75%) compared to control group (19.16±0.31).

The moringa treatment to diabetic rats with high dose caused highly significant increase of insuline value (13.74±.38mg/l) with difference (42.67%) than the treatment with low dose of moringa (12.20±.69 mg/l) whigh caused a slight increase in insulin value with difference (30.34%) as compared to the corresponding value of diabetic rats at (p=0.05), (Table2).

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Table (1): Effect of moringa on both low and high doses (200, 400 mg / kg/d) for 30 days on blood glucose value at times intervals.

GROUPS	0 days		After 7		After 14 days		After 21 days		After 30 days	
	X ± SE	diff%	X ± SE	diff%	X ± SE	diff%	X ± SE	diff%	X ± SE	diff%
GROUP 1	78.8±1.77	……	79.1±1.59	……	78.6±1.98	……	80.1±1.85	……	79.3±2.41	……
GROUP 2	77.4±2.73	-1.78	77.2±2.65	-2.48	76.8±2.48	-2.24	77.1±2.71	-3.77	75.9±1.01	-4.28
GROUP 3	76.1±1.32	-3.30	77.1±3.24	-2.58	76.3±2.94	-2.88	76.4±1.94	-4.67	75.1±2.02	-5.12
GROUP 4	513.2±4.62*	551.27	496.6±32.96*	527.4	500.6±15.1*	569.04	503±23.32*	527.65	503.7±3.47*	534.68
GROUP 5	460.1±22.2*	484.01	437.3±29.72	-11.9	324.2±48.8**	-35.23	252.8±19.3**	-49.74	124.8±2.48**	-75.22
GROUP 6	468.2±16.46 *	494.16	377.5±53.5**	-23.98	283.8±33.6**	-43.30	205.2±2.71**	-59.20	118±1.00**	-76.57

*Significant against group1, ** significant against group 4. All results are expressed as mean ± S.E (standard error) at $p < 0.05$.

Table (2): Effect of moringa on both low and high doses (200,400 mg/kg) for 30 days on serum insulin

GROUPS	G1		G2		G3		G4		G5		G6	
	X±SE	diff%	X ± SE	diff%	X ± SE	diff%	X±SE	diff%	X±SE	diff%	X±SE	diff%
INSULIN	19.16±.31	…….	19.53±.18	2.75	19.5±0.20	0.56	9.63±0.30*	-49.82	12.20±.69	30.34	13.74±.38**	42.67

*Significant against group1, ** significant against group 4. All results are expressed as mean ± S.E (standard error) at $p < 0.05$.

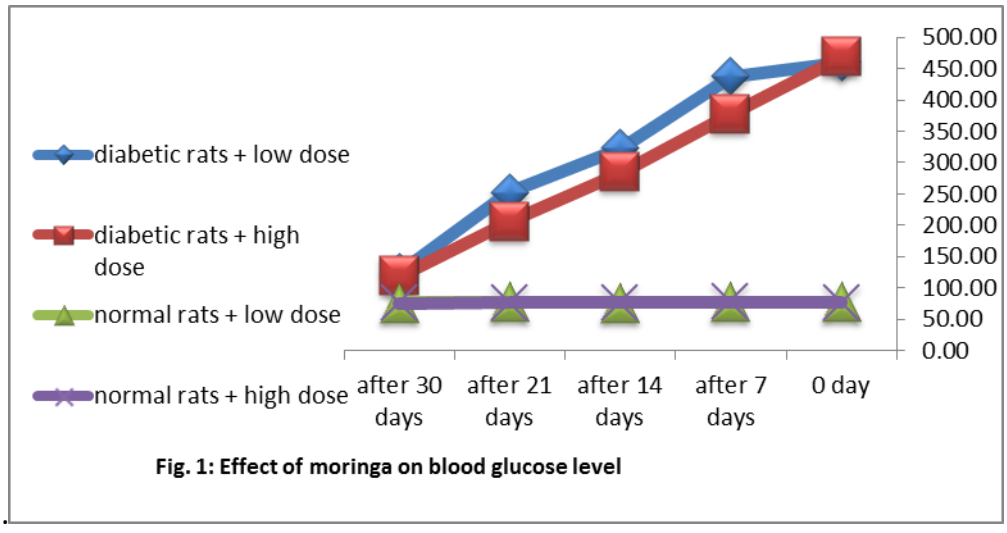

Fig. 1: Effect of moringa on blood glucose level

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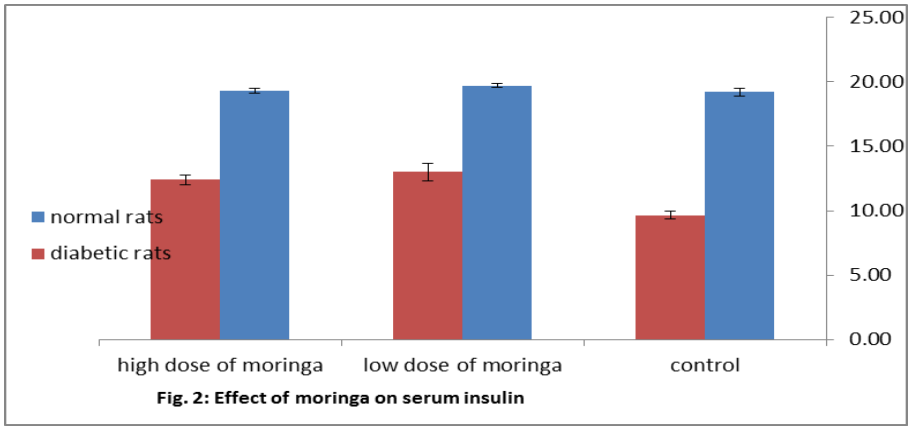

Fig. 2: Effect of moringa on serum insulin

Histological observations

Control rats (Group I)

Pancreas is a mixed gland which formed of exocrine and endocrine parts. The exocrine part includes the pancreatic acini which secrete pancreatic enzymes, and the endocrine part includes the islets of Langerhans which secretes pancreatic hormones (Fig. 3). Pancreatic acini consist of pyramidal cells contain basal rounded nuclei. The apical region of each pyramidal cell contains acidophilic granules (zymogenic granules), and the basal part is a basophils. The islets of Langerhans are present in clusters and scattered between the acini. They are rounded or oval in configuration and appeared faintly stained with H&E. The islets received their rich blood supply from the connective tissue elements of the exocrine pancreas (Fig.3).

Undiabetic rats treated with low and high doses of moringa (Groups II & III)

The pancreatic acini and islets of undiabetic rats administered with either low (200 mg/kg/d) or high dose (400 mg/kg/d) of moringa for 30 days showed a normal architecture which was almost similar to the control ones (Figs. 4 & 5) .

Alloxanized – diabetic rats (Group IV)

The diabetic rats illustrated degeneration and vacuolation in the pancreatic islet cells, appearance of many necrotic areas and pyknotic nuclei. The acinar cells were seen with a reduction in the zymogenic cells, widen of their lumens and dilation of the intercalated duct. The formation of fibers periphery to dilated blood vessels and ducts was too seen (Fig. 6).

Diabetic rats treated with low dose of moringa (Group V)

Minimized improvement in the pancreatic tissues of diabetic rats treated with low dose of moringa was observed.The acinar and islet cells appeared with abnormal shape with irregular architecture and almost similar to diabetic form. The dilated blood sinusoid and increment of fibers around the intercalated ducts were still seen (Fig. 7).

Diabetic rats treated with high dose of moringa (Group VI)

Many obvious improvements were demonstrated in the pancreatic cells of diabetic rats treated with high dose of moringa. The acinar cells restored their normal architecture. The islet cells demonstrated with no degeneration with disappearance of the vacuolated cells and reduction of necrotic areas. The noticeable reduction of dilated blood sinusoids and ducts were also illustrated (Fig. 8 a,b).

IHC observations

In the normal control rats group, the insulin secreting cells or β-cells represent the major cell population of the islets, occupying mainly the central zone. Positive expression of normal strong

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immunoreactivity to the insulin secreting β-cells was seen in the form of dark brown granules present in the cytoplasm of beta-cells (Fig. 9).

The alloxanized-diabetic rats group showed the pancreas with a marked reduction in the expression of insulin secreting β-cells in the islets of Langerhans (Fig.10).

The diabetic rats treated with low dose of moringa showed a minimized recovery of few insulin secreting cells expression (Fig.11). The diabetic rats treated with high dose of moringa demonstrated an increment and recovery in the expression of insulin secreting cells (β-cells) similar to that of the control ones. Also, there was an extension & proliferation of insulin secreting β- cells in the islet of Langerhans (Fig.12).

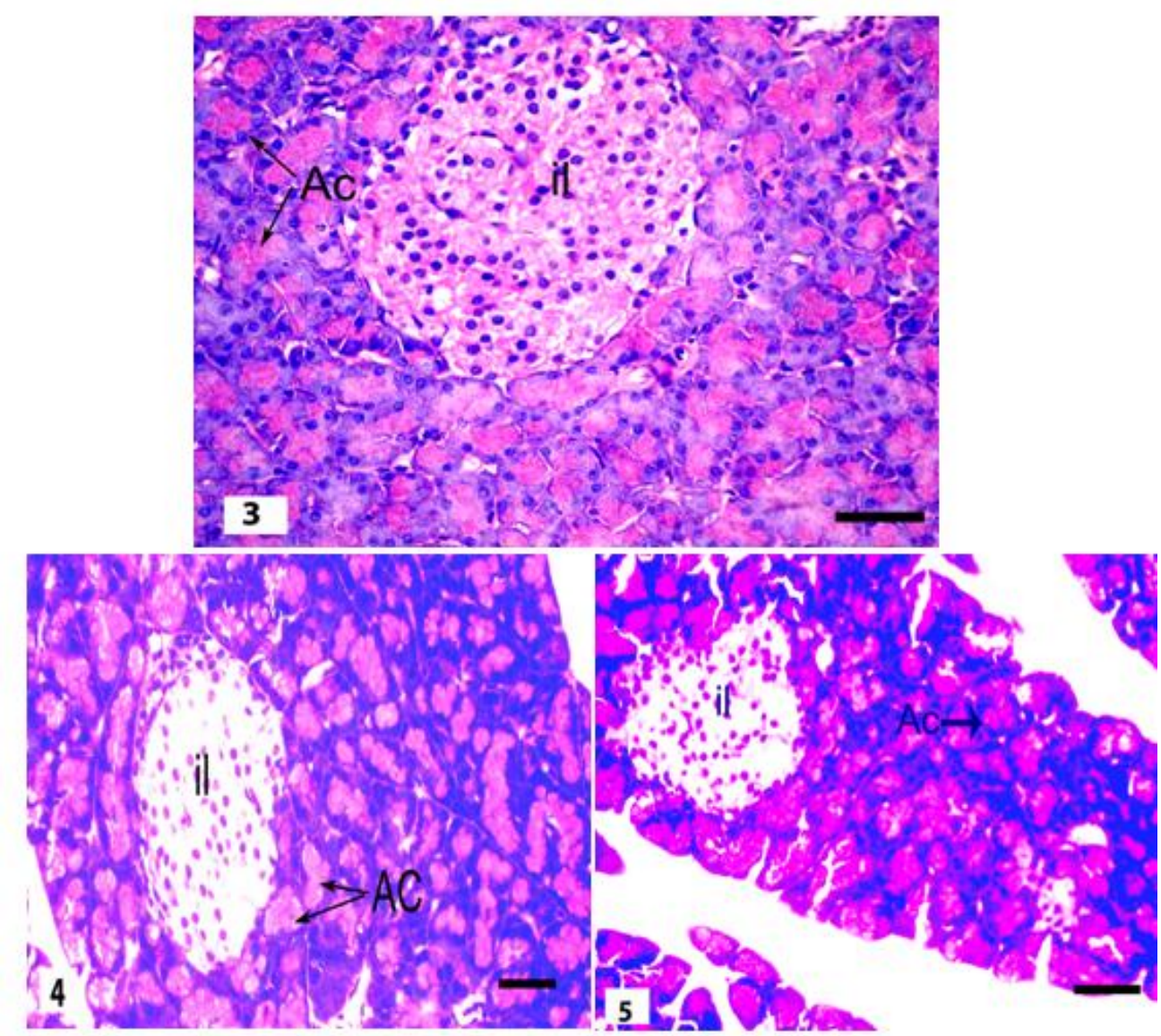

Fig. 3: A section in pancreas of normal control rat showing the normal appearance of acini (Ac) and islet of langerhans (il). H&E, scale bar=6.25 µm

Fig. 4: A section in pancreas of control rat treated with low dose of moringa illustrating the normal appearance of acini (Ac) and islet of Langerhans (il). H&E, scale bar=6.25 µm

Fig. 5: A section in pancreas of control rat treated with high dose of moringa detecting the normal appearance of acini (Ac) and islet of Langerhans (il). H&E, scale bar=6.25 µm

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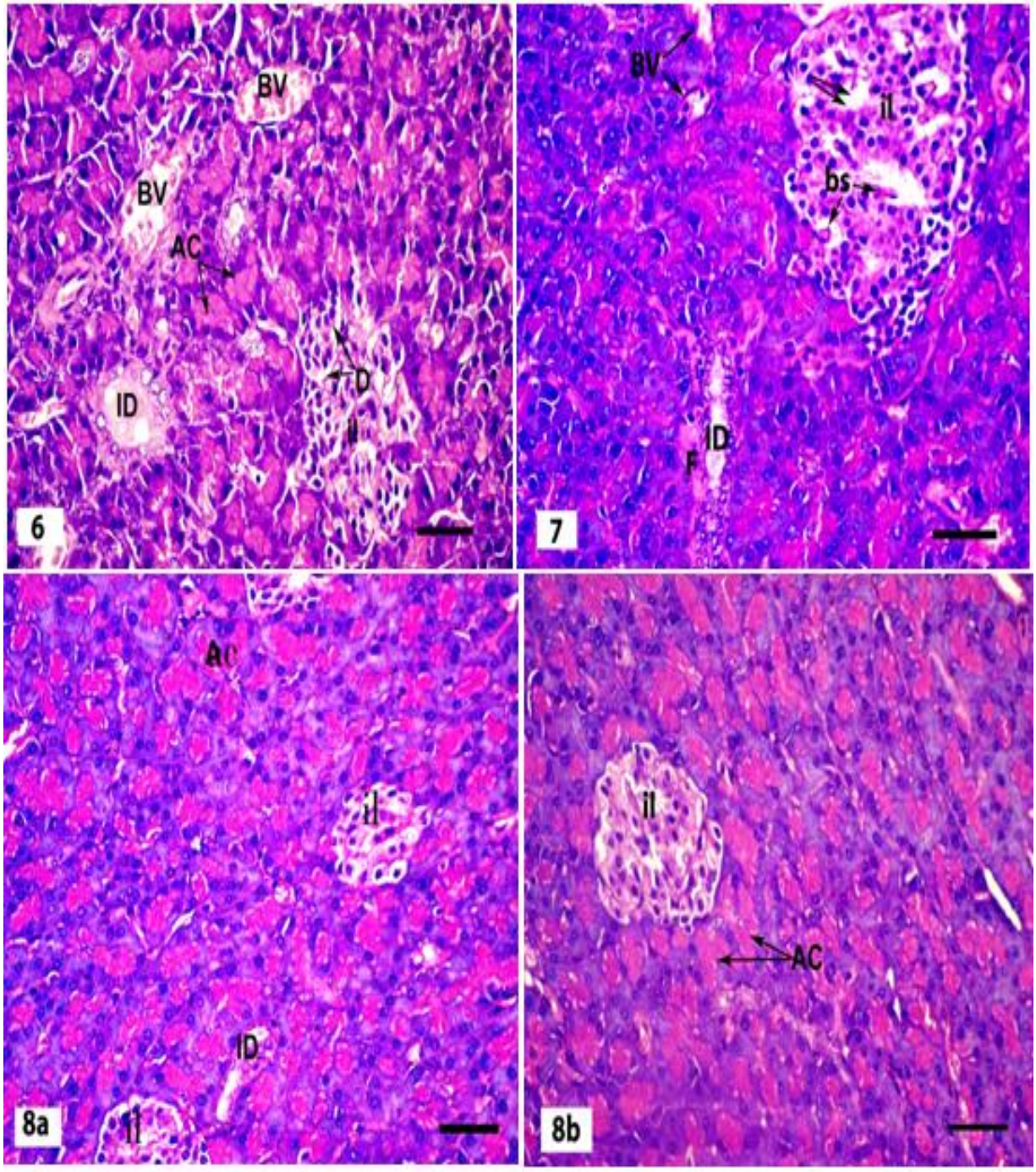

Fig. 6: A pancreatic section of an alloxanized -diabetic rat showing disturbance of islet (il) shape with degenerated many cells (D), abnormal architecture of acinar cells (Ac), dilated blood vessel (BV) and widen intercalated duct (ID). H&E, scale bar = 6.25 µm

Fig. 7: A section in the pancreas of an alloxanized -diabetic rat treated with low dose of moringa showing degeneration of islet cells (il) with necrotic area (double arrow) and with dilated blood sinusoid (bs). Abnormal architecture of many acinar cells (Ac), fibrous (F) perphery to intercalated duct (ID) and normal blood vessel (BV) are seen. H&E, scale bar = 6.25 µm

Fig. 8a & b: A section of pancreas of an alloxanized -diabetic rat treated with high dose of moringa showing a reduction of necrotic areas with a restoration of many islet cells (il) and appearance of normal form of acinar cells (Ac) with no fibers around intercalated duct (ID). H&E, scale bar = 6.25 µm

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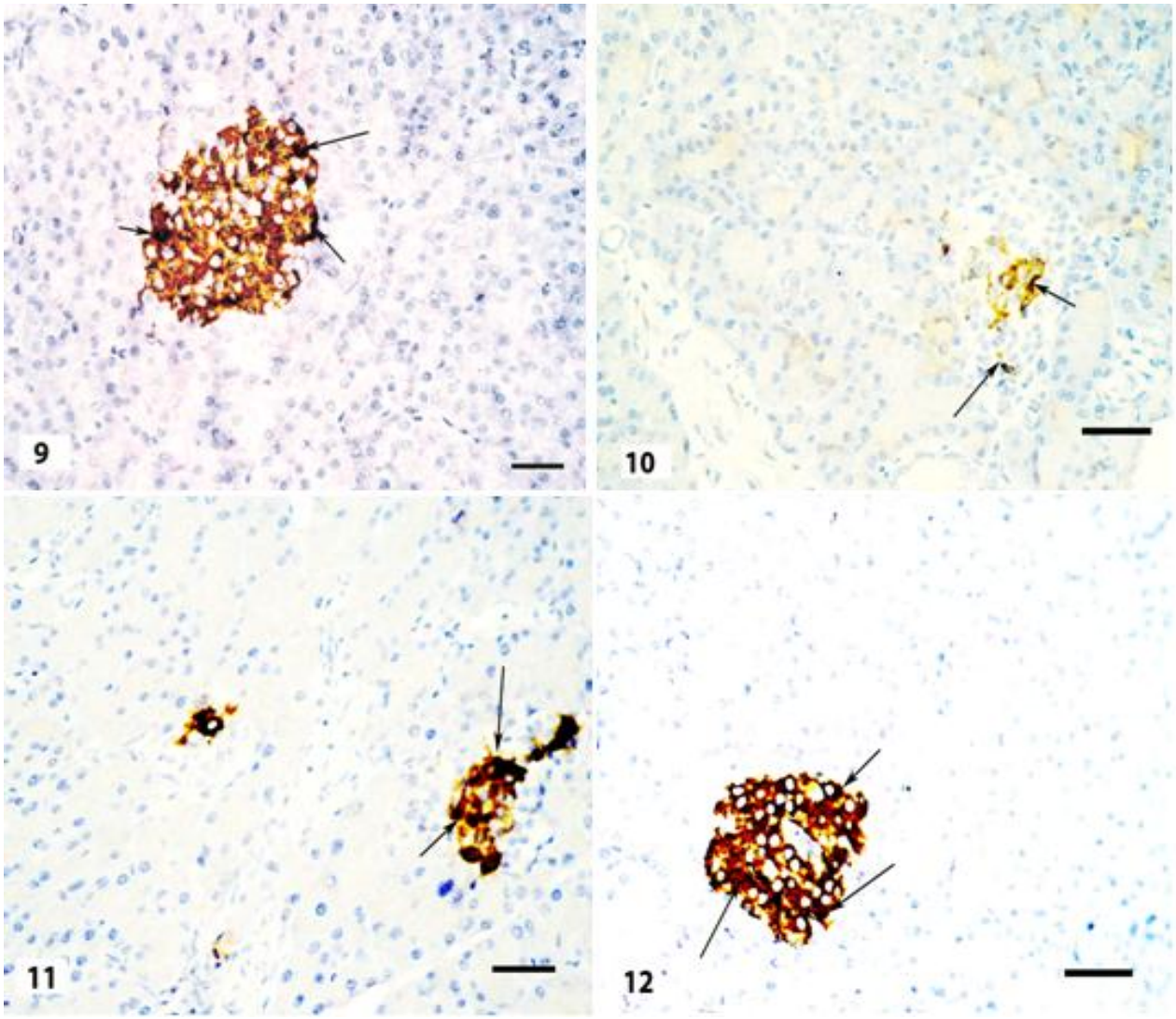

Fig. 9: Pancreatic section of a control rat illustrating the normal expression of insulin secreting β-cells in Langerhans islet (arrows). Anti-insulin immunostain, Scale bar = 6.25 µm

Fig. 10: A section of pancreas of an alloxanized -diabetic rat showing a reduction in the expression of insulin secreting β-cells in Langerhans islet (arrows). Anti-insulin immunostain, Scale bar = 6.25 µm

Fig 11: A section of a pancreas of an alloxanized -diabetic rat treated with low dose of moringa showing partially improvement & a recovery in few cellular distribution of insulin receptor in islet β- cells (arrows). Anti-insulin immunostain, Scale bar = 6.25 µm

Fig. 12: A pancreatic section of an alloxanized -diabetic rat treated with high dose of moringa showing an obvious increment and recovery of insulin secreting β- cells (arrows) in islet of Langerhans. Anti-insulin immunostain, Scale bar = 6.25 µm

DISCUSSION

Diabetes is a complex disease and causes numerous cellular damages in different organs (Haligur *et al.,* 2012). Alloxan-induced hyperglycaemia has been described as a useful experimental model to study the activities of hypoglycemic agents because it selectively destroys the pancreatic ß-cells of rats (*Junod et al., 1969;* El-Desouki, 2004*;* Tuorkey, *et al., 2015).* A recent study has concluded that the increased level of plasma glucose could promote destruction in β-cells of pancreas (El-desouki *et al.,* 2015).

The present results showed a significant increase in the blood glucose value and a highly significant decrease in insulin. The high dose of moringa leave extract (400mg/kg/d for 30days) to the diabetic rats

ameliorated the diabetic complications by declined the glucose levels and enhanced again insulin levels reflecting a restoration of the pancreatic β-cells activity. In accordance, the hyperglycemic response of streptozotocin (STZ) was found to be significantly reduced in animals pretreated with moringa pods extract (150&300mg/kg) for 21 days (Gupta et al., 2012). Similarly, Oyedepo *et al.* (2013) and Soliman (2013*)* postulated that the treatment of diabetic rats with moringa extract (400 mg/ kg) for 28 days was significantly decrease blood glucose level. Moreover, many researchers recorded a decrease in insulin level in alloxan-diabetic rats *(*Adewole *and* Martins*,* 2006; Gupta *et al., 2012;* El-Desouki *et al.,* 2015). From this study, it is suggested that *Moringa oleifera* seed extract was able to reverse the inhibition of insulin secretion from the pancreatic beta cells and reduced the blood glucose level.

These changes are a result of inhibition of insulin secretion from the pancreatic beta cells that is attributed to the induction of beta cell toxicity (Lenzen, 2008), and possibly through the mechanism of induction of free radical species (Szkudelski, 2001) and oxidative stress that impaired insulin secretion in type 2 diabetes (Robertson, 2006). However, the treatment of diabetic rats with a natural extract of the marine algae (Spirulina) (2gm/kg) for three weeks successfully ameliorated the diabetic complications by declined the glucose levels and enhanced again insulin levels reflecting a restoration of the immunostain pancreatic β-cells activity and caused a significant decrease of the NO levels and increased in the antioxidant SOD and CAT values (El-Desouki et al., 2015).

The histological and IHC observations confirmed the biochemical data in the current study. The pancreatic tissue of diabetic rat demonstrated degeneration and vacuolations in the Langerhan's islet cells, dilation blood sinusoid, widen of the intercalated duct and the formation of fibers periphery to dilated blood vessels and ducts. Moreover, the expression of insulin secreting cells (β-cells) by using monoclonal anti insulin markedly demonstrated the destruction and evident reduction of β-cells immunoreaction in diabetic rats. Treatment with high moringa dose (400 mg/kg/d.) for 30 days restored the pancreas to normal architecture, and enhanced the expression of β- cells in the islets of Langerhans, and exerted stronger anti-hyperglycemic effects than in moringa low dose of moringa (200 mg/kg/d.) for 30 days) treatment. Similar results have been reported by many authors; Marchetti *et al*. (2010) demonstrated the reduction of islet number and/or diminished beta-cell mass/volume in the pancreas of type 2 diabetes and morphological changes in several beta-cell organelles. Moreover, Patel *et al*. (2014) showed a decrease counting analysis in the number of pancreatic β-cells immunostain of obese-hyperglycemic mice that indicated the islets were losing the ability to secrete insulin efficiently.

Ajibola *et al.* (2014) recorded a significant decrease in the blood glucose level after six hours and also after fourteen days of both oral and intraperitoneal treatment of the mild hyperglycemia with 400mg/kg body weight of aqueous *Moringa oleifera*seed extract. Also there was a 48.6% and 42.8% decrease in the blood glucose level of the mildly hyperglycemic rats on treatment with both oral and intraperitoneal *M. oleifera* seed extracts and a 69.7 % and 89.6% decrease in the blood glucose level of the severely hyperglycemic rats on treatment with both oral and intraperitoneal moringa seed extracts respectively. Thus *M. oleifera* seed extract exhibited a hypoglycemic effect on both the mild and severe alloxan induced hyperglycemic rats.

Similar results were recorded by Bolkent *et al.* (2005) who reported a decrease in the number of immunoreactivity of β-cells in the streptozotocin diabetic rats compared to the control group. They demonstrated the immunoreactivity of β-cells of diabetic group was not different from any of the treated groups with Aloe vera leaf gel and pulp extracts. Moreover, Abunasef *et al.* (2014) reported that the control rats showed strong immunoreactivity of insulin in beta-cells, and the pancreas of streptozotocin diabetic rats saw a marked reduction in immunostain expression of insulin in beta-cells. They illustrated the treatment of diabetic rats with high dose of caffeine (100 mg /kg) caused an evident increase in insulin expressing beta-cells with normal density more than in 10 & 50 mg /kg of caffeine comparable to diabetic rats.

The ability of the seed extract of *Moringa oleifera* to significantly reduce hyperglycemia induced by alloxan may be as a result of its phytochemical and micronutrient constituents. A major phytochemical constituent of the extract that have been reported is flavonoids, which has been further characterized by structure and functional relationships as; flavans, flavanones, flavones, flavanols, flavanonols, cetechins, anthocyanidins and isoflavones. Bioflavonoids are well known for their multi-directional biological activities including hypoglycemic effects (Szkudelski, 2001). Also the *Moringa oleifera* contains many powerful antioxidant phytochemicals, especially quercetin and kaempferol. Kaempferol has been shown to have

hypoglycemic activities (Fuglie 1999; Luangpiom, 2013). Also, the mechanisms of actions could be either by increasing the tissue utilization of glucose (Gray *et al.,* 2000), by *Moringa oleifera* inhibiting hepatic gluconeogenesis or absorption of glucose into the muscles and adipose tissues (Mbikay, 2012; Soliman, 2013; Ajibola *et al.,* 2014).

In conclusion, the changes in plasma glucose & insulin values, histopathological and the destruction of pancreatic β-cells immunostain of alloxan-diabetic rats were improved and recovery by 400 mg/kg/d more than by 200 mg/kg/d. for 30 days, and acts as hypoglycemic effect.

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