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The antidiabetic effect of *Moringa oleifera* leaves extract on some biochemical paramers of diabetic rats induced alteration in cytoskeletal desmin of cardiomyocytes.

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ABSTRACTS

The purpose of this study is to evaluate the effect of aqueous leaf extract of *Moringa oleifera* on blood glucose level, insulin, urea, creatinine, cholesterol and triglycerides as well as histopathology and desmin immunoreaction of cardiac muscle in diabetic adult male albino rats. Sixty adult male albino rats of the Wistern strain weighing 99 ± 1.03 g were used. The animals were randomly assigned into 6 groups, 10 rats for each; Group I: normal control rats, Group II: control 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 alloxan intraperitoneally (served as Hyperglycemic group), Group V: Diabetic rats administered with low dose of moringa, Group VI: Diabetic rats administered with high dose of moringa. At the end of the experiment, rats were sacrificed; the cardiac muscle specimens and blood samples were collected after 14 hours of fast. Changes in the rats' blood glucose, insulin, urea, creatinine, cholesterol and triglycerides were determined in all animal groups. The histopathology of cardiac muscle by H&E and desmin immunoreaction were also demonstrated. significant increases in glucose, urea, creatinine, cholesterol and triglycerides were recorded in diabetic rats as well as a significant decrease of insulin. The treatment of diabetic rats with high dose of *Moringa oleifera* extract(400 mg/kg/d for 30 days) recorded better results as significant decrease in blood glucose level with other previous parameters and a significant increase in insulin more than low dose of moringa as compared to the control undiabetic rats. Also, histological study of cardiac muscle of diabetic rats showed disarrangement of cardiac myocytes, sarcoplasmic vacuolation, pyknotic nuclei, dilatation and congestion of blood vessels. The treatment of diabetic rats with high dose of moringa illustrated clearly recovery of normal histological appearance of cardiac myofibres with intact intercalated discs and normal nuclei. No improvement of cardiac myofibres of diabetic rats treated with low dose of moringa. By IHC, weak desmin expression in intercalated discs and Z-lines of cardiac myofibres of diabetic rats was seen, and the treatment of diabetic rats with high dose of moringa restored the desmin expression in the cardiac myofibres more than low dose. The results indicated that the moringa at a dose 400 mg/kg/d for 30 days is recommended to be used as a curative drug to reduced glucose level and improve all the previous physiological parameters as well as the disturbances in the cardiomyocytes and myofibrils assembly caused under the effect of diabetes.

Keywords: Diabetes, *Moringa oleifera*, Biochemical parameters, Cardiac muscle, IHC, Cytoskeleton, Rats

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disease characterized by alterations in carbohydrate, protein and lipid metabolism resulting from defects in insulin secretion, insulin action, or both (1). The disease also causes significant disturbances of water and electrolyte homeostasis (2). The long-term complications of *diabetes mellitus* include retinopathy, nephropathy, neuropathy and angiopathy (3) associated with oxidative stress and overwhelming free radicals. In recent years, the incidence of *diabetes mellitus* has increased drastically in both developed and underdeveloped countries.

Epidemiological studies showed higher prevalence of *diabetes mellitus* in the adult population with wide geographical distribution. Diabetic cardiomyopathy is a disorder of the heart muscle in people with diabetes. It can lead to inability of the heart to circulate blood through the body effectively, a state known as heart failure **(**4) with accumulation of fluid in the lungs (pulmonary edema) or legs (peripheral edema). Most heart failure in people with diabetes results from coronary artery disease, and diabetic cardiomyopathy is only said to exist if there is no coronary artery disease to explain the heart muscle disorder (5). Both the quantity and the type or source of carbohydrate found in food influence postprandial glucose level. Diets providing high amounts of simple carbohydrates and/or fructose are associated with insulin resistance and low plasma HDL cholesterol. In contrast, diets providing high amounts of complex carbohydrates and fibre are associated with increased insulin sensitivity (1).

Much research has been done to determine the efficacy of moringa plant. *Moringa oleifera* is a small tree that grows up to 12 feet in height. Its leaves are small and pale-green. *Moringa oliefra* is a versatile and exceptionally nutritious vegetable tree with a variety of potential uses. It is the most widely cultivated species of a *Moringaceae* family (6,7). Commonly it is known as *Moringa* or Drumstick tree or Horseradish tree in English, in Hindi as Sahjan, in Latin—*Moringa oleifera*, in Sanskrit as Surajana, in Nepali Sajiwan or Swejan etc. It is useful not only for human beings but also for animals and also in various industrial applications (8). The leaves, fruit, flowers and immature pods of this tree are used as a highly nutritive vegetable in many countries, particularly in India, Pakistan, Philippines, Hawaii and many parts of Africa. People in India have been using it as an item of their daily food for nearly 5000 years (9).

Moringa oleifera has gained usefulness in pharmacological activities—crude ethanolic extract of dried seeds, hot water infusion of flowers, leaves, roots, seeds and bark, crude methanolic extract of the roots are used as anti-inflammatory agents. Oil from dried seeds, methanol and ethanol extract of free dried leaves is also used as antioxidant agents. Defatted and shell-free seeds, fresh leaves juice, roots and bark are antimicrobial (10); aqueous extract of stem bark, ethanolic extract of leaves, ethanolic and aqueous extracts of whole pod and their parts, namely, coat, pulp and seed are used as cardiovascular agents (11). Leaves and fruits are used as antihyperlipidemic agents (12). Methanolic extract of roots are CNS depressants (7,13). Paste of leaves, ethanolic extracts of seeds are used as anticancer agents (14). Aqueous and ethanolic extract of roots and flower serves as antihepatotoxic agents (15), Ethanolic extracts and methanolic extracts of leaves and flower buds are use as antiulcer agents (16).

The aim of this work is to study the role of the leaves extract of *Moringa oleifera* on the blood levels of glucose, insulin, urea, creatinine, cholesterol and triglycerides as well as histopathology and immunostain with desmin of cardiac myocytes of diabetes induced in adult albino rats.

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 housed two weeks before study. The animals were fed with a standard diet and allowed free access of water. 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 experimental diabetes , alloxan was dissolved in acetate buffer and was injected into fasting rats at 150 mg/kg as recommended by Ajibola *et al.* (17), then after two days ,the rats were injected with 100mg/kg of alloxan (2^{nd} injection). Lastly, 3rd injection of alloxan (100 mg/kg) was applied two days after the 2^{nd} one. Note, the 2^{nd} 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. 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 for 30 days (18).

Experiment:-

All procedures were done at the Faculty of Science, Tanta University, Egypt. The animals were housed in cages, and divided into six groups (10 rats/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 14 hours and then sacrificed by decapitation, blond samples were collected and cardiac muscle tissues specimens were carefully dissected out and divided into two pieces for biochemical and immunohistochemical studies.

Blood glucose, insulin, urea, creatinine, cholesterol and triglycerides estimation:-

Blood glucose was estimated on 0, 7, 14, 21 and 30 day by using Accu-Chek Performa Apparatus according to **Brăslasu *et al. (*19)** and **Abunasef *et al .(*20).** Insulin was determined by using a rat-specific Insulin-Ak ELISA according to **Finlay and Dillard (21).**Urea was determined in laboratory by using o-Phthalaldehyde 37ºC Colorimetric methods of spinreact kits according to **Burtis et al. (22).** creatinine was determined in laboratory by using Jaffé Colorimetric – kinetic methods of spinreact kits according to **Burtis et al. (22).** Cholesterol and triglycerides was determined in the laboratory by using GPO-POD Enzymatic colorimetric methods of spinreact kits according to **Burtis et al. (22).**

Histological study:-

Haematoxylin and eosin stain (H&E) was used **(23).**

Immunohistochemical study:-

Paraffin sections of cardiac muscle and primary monoclonal antibody (RD301) against desmin were used. Clone, RD301 is a mouse monoclonal IgG2b antibody and reacts exclusively with desmin, which is expressed in smooth, cardiac, and striated muscle cells, and was obtained from Thermo Fisher Scientific Industries. Avidin Biotinylated secondary antibody was applied and followed by avidin– biotin complex (ABC). Colour reaction was developed by using 3,3' diaminobenzidine (DAB) and gave a brown colour to desmin. Sections were then counterstained with Harris haematoxylin **(24).**

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 $P < 0.001$

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RESULTS

Biochemical results:-

Effect of moringa leave extract on blood glucose value:

Diabetes caused a significant increase of blood glucose value (p=0.001) 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=0.001). 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±1.00 mg/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=0.001), (Table 1 & Fig. 1).

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 doses 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 insulin value (13.74±.38mg/l) with difference (42.67%) than the treatment with low dose of moringa (12.20±.69 mg/l) which caused a slight increase in insulin value with difference (30.34%) as compared to the corresponding value of diabetic rats at (p=0.001), (Table 2 & Fig. 2).

Effect of moringa leave extract on serum total cholesterol.

Diabetic rats showed significant increase in serum total cholesterol (156.76±7.43 , 114.19%) compared to the control normal rats (77.21±10.47). The treatment with moringa at both low and high doses to undiabetic rats caused insignificant decrease of serum total cholesterol (72.75±10.62, -5.86% & 74.53± 10.26,-3.47%, respectively) compared to control undiabetic group (77.21±10.47). The treatment with moringa at both doses to diabetic rats caused significant decrease of serum total cholesterol (113.17±20.65, -27.80% & 107.22±17.46, -31.60%) compared with untreated diabetic rats (156.76±7.43,114.19%). (Table 2 & Fig. 3).

Effect of moringa leave extract on serum triglycerides.

Diabetic rats showed significant increase in serum triglycerides (274.62±16.72, 201.26%) compared to the control normal rats (91.15±5.43). The treatment with moringa at both low and high doses to undiabetic rats caused insignificant decrease of serum triglycerides (81.65±6.58, -10.43% & 84.36±7.95, -7.46%, respectively) compared to control undiabetic group (91.15±5.43). The treatment with both moringa doses to diabetic rats caused significant decrease of serum triglycerides (158.93±28.66, -42.12% & 116.93±39.96, -57.42%) compared with untreated diabetic rats (156.76±7.43, 114.19%) (Table 2 & Fig. 4).

Effect of moringa leave extract on urea.

Diabetic rats showed significant increase in serum urea (112.90±10.44, 154.36 %) compared to the control normal rats (44.39±1.10). The treatment with moringa at both low and high dose to undiabetic rats caused significant decrease of serum urea (34.24±2.46, -22.85%&24.86±2.88,-43.97%) compared to control group (44.39±1.10). The treatment with moringa either low or high doses to diabetic rats caused significant

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decrease of serum urea (70.05±4.37, -37.95%&68.75±4.83, -39.10%) compared with untreated diabetic rats (112.90±10.44,154.36 %). (Table 2 & Fig. 5).

Effect of moringa leave extract on serum creatinine.

Diabetic rats showed significant increase in serum creatinine (2.37±0.2, 193.17%) compared to the control normal rats (0.80±0.08). The treatment with both doses of moringa to undiabetic rats caused insignificant decrease of serum creatinine (0.68±0.04, -15.88%&.62±.04, -23.31%) compared to control group (0.80±0.08). The treatment with moringa at low and high dose to diabetic rats caused significant decrease of serum creatinine (1.26±0.26, 46.83%&1.28±0.20,-45.99) compared with untreated diabetic rats (2.37±0.2, 193.17%), (Table 2 & Fig. 6).

Table (1): The effect of moringa on both low and high doses (200,400 mg/kg) three times weekly 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± SE (standard error) at p<0.001

Table(2): The effect of moringa on both low and high doses (200,400 mg/kg) three times weekly for 30 days on serum Urea, creatinine, insulin, cholesterol and Triglycerides.

GROUPS	Urea mg/dl		creatinine mg/dl		Insulin µu/L		Cholesterol mg/dl		Triglycerides mg/dl	
	X ± SE	diff%	X ± SE	diff%	X ± SE	diff%	X ± SE	diff%	X ± SE	diff%
GROUP 1	44.39±1.10	.....	0.80±0.08	.....	19.16±0.31	.....	77.21±10.47	.....	91.15±5.43	.....
GROUP 2	24.86±2.88	-43.97	0.62±0.04	-23.31	19.53±0.18	2.75	72.75±10.62	-5.86	81.65±6.58	-10.43
GROUP 3	34.24±2.46	-22.85	0.68±0.04	-15.88	19.5±0.20	0.56	74.53±10.62	-3.47	84.36±7.95	-7.46
GROUP 4	112.90±10.44*	154.36	2.37±0.21*	193.17	9.63±0.30*	-49.82	156.76±7.43*	114.19	274.62±16.72*	201.3
GROUP 5	70.05±4.37**	-37.95	1.26±0.26**	-46.83	12.20±0.69**	30.34	113.17±20.65**	-27.8	158.93±28.66**	-42.12
GROUP 6	68.75±4.83**	-39.10	1.28±0.20**	-45.99	13.74±0.38**	42.67	107.22±17.46**	-31.6	116.93±39.96**	-57.42

*Significant against group1, ** significant against group 4. All results are expressed as mean±S.E (standard error) at p ≤0.001.

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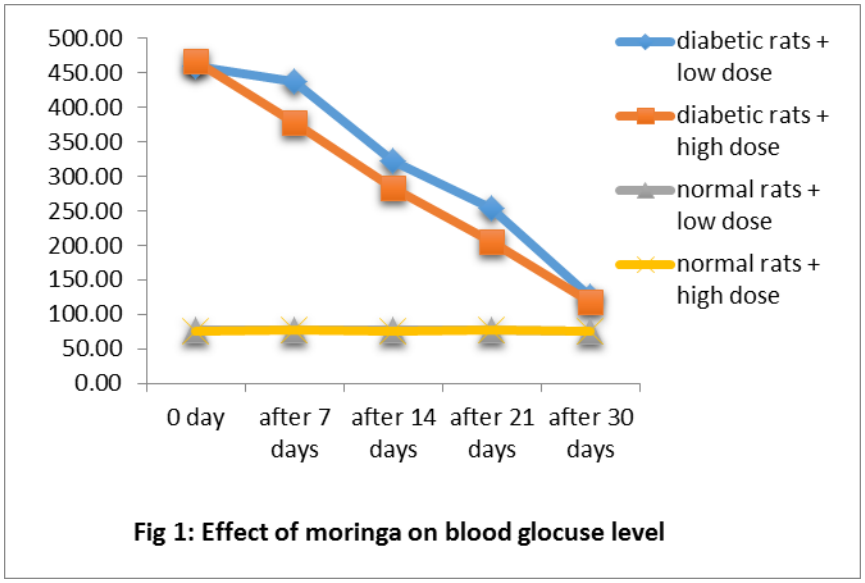

Fig 1: Effect of moringa on blood glocuse level

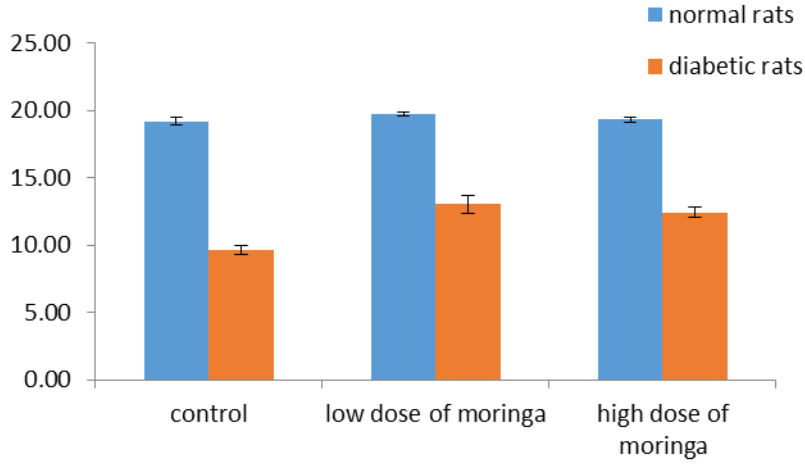

Fig 2:Effect of moringa on serum insulin

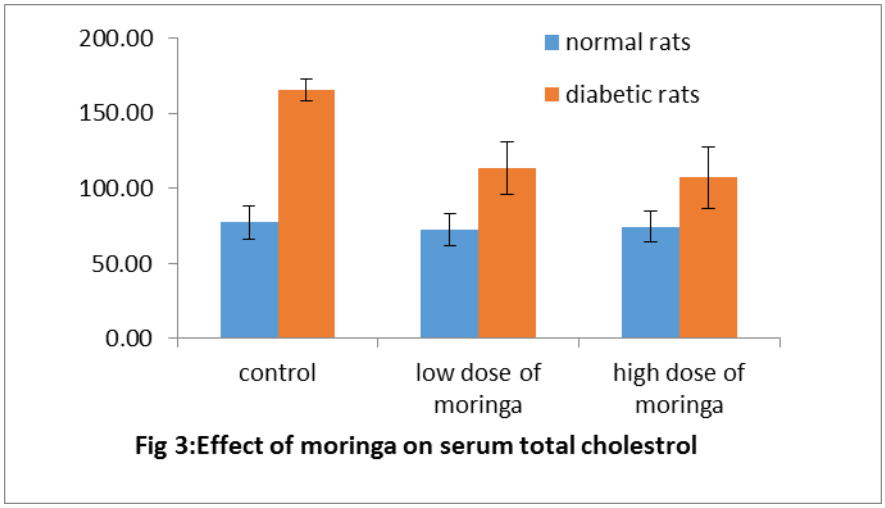

Fig 3:Effect of moringa on serum total cholestrol

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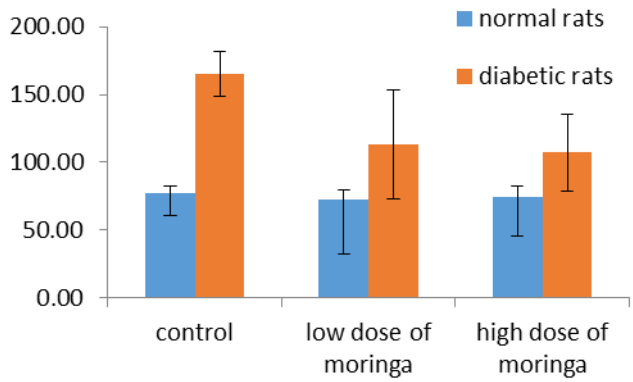

Fig 4 :Effect of moringa on serum triglycerides

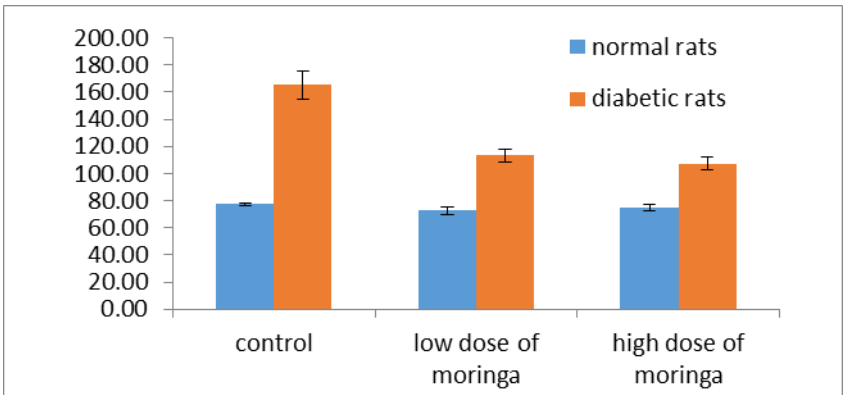

Fig 5:Effect of moringa on serum urea

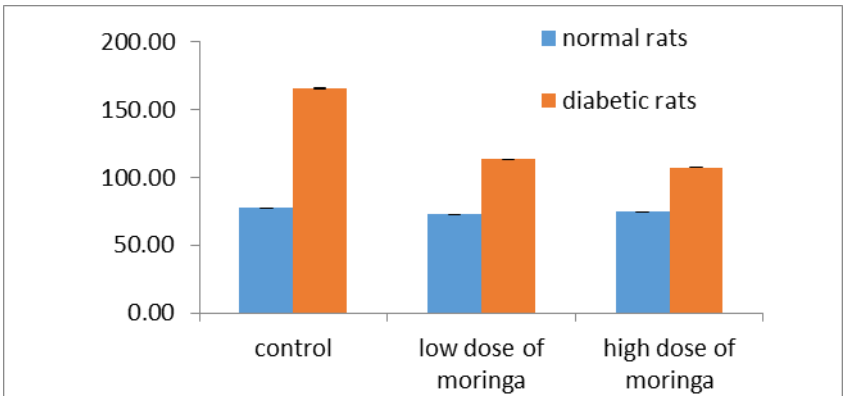

Fig 6:Effect of moringa on serum creatinine

Histological observations:-

In a normal control rat, the myocardium is striated and arranged in a linear array that branches and anatomizes in a specific pattern giving the appearance of a sheet. The cardiac muscle fibers are joined together by intercalated discs. They contain acidophilic cytoplasm with oval centrally located nuclei. The cardiac muscle fibers are separated by delicate layer of connective tissue with well evidenced myocardial blood capillaries (Fig.7). The alloxanized-diabetic rats showed disarrangement of cardiac myocytes,

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sarcoplasmic vacuolation, degeneration of muscle fibres, pyknotic nuclei, dilated blood vessels and appearance of inflammatory cells (Figs. 8 & 9).

The diabetic rats treated with low dose of moringa demonstrated no improvement in the cardiomyocytes and revealed disarrangement of cardiac myocytes, sarcoplasmic vacuolation, pyknotic nuclei, dilatation and congestion of blood vessels (Fig.10). The treatment of diabetic rats with high dose of moringa illustrated the cardiomyocytes closly similar to control form. The cardiac muscle restored their normal histological appearance with intact intercalated discs and normal nuclei (Fig.11).

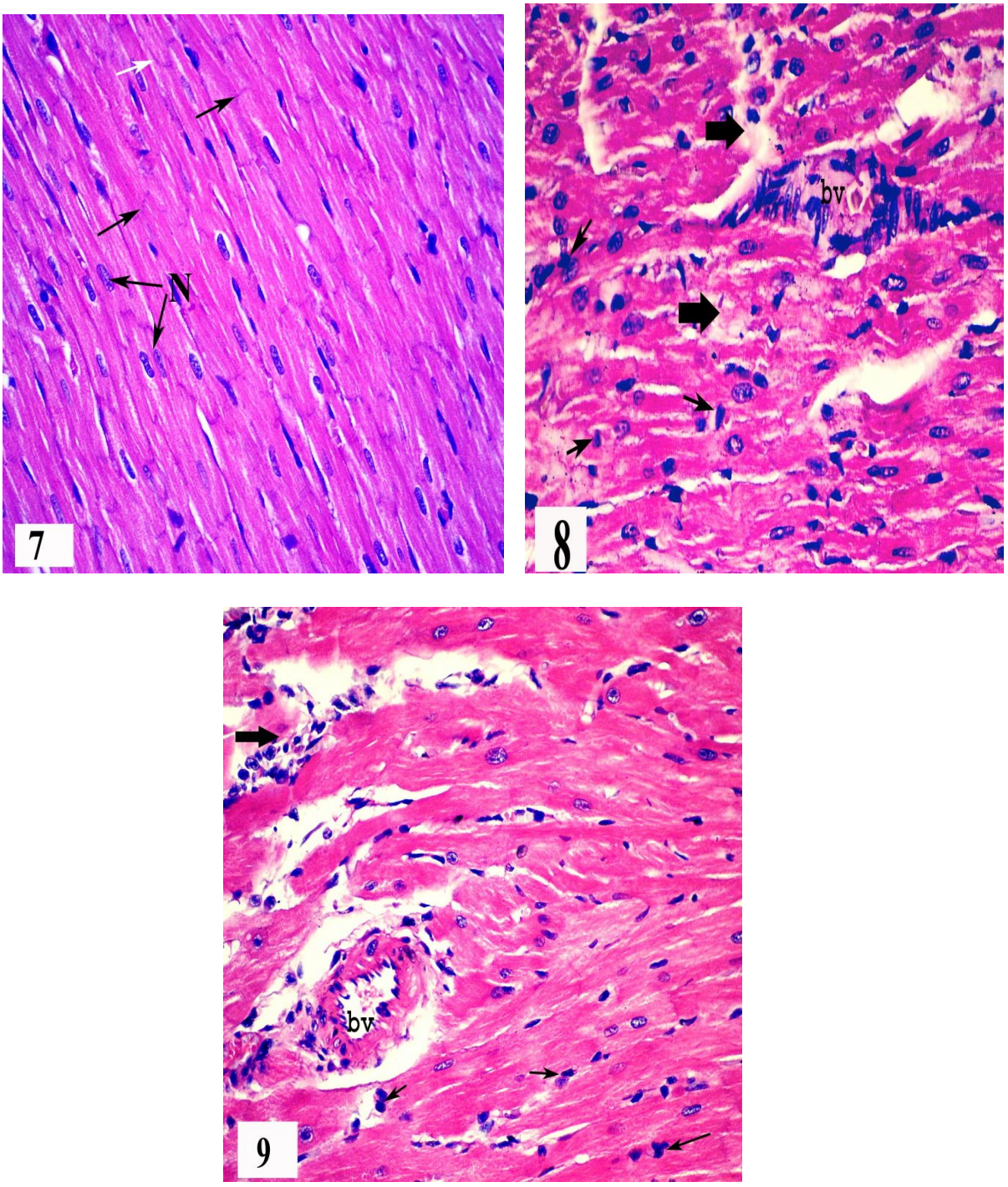

Fig.9. Longitudinal section of the cardiac muscle of an alloxanized diabetic rat showing disarrangement of cardiac myocyte, inflammatory cells (thick arrows), pyknotic nuclei (thin arrows) and dilatation of blood vessel (bv). H&E, X 400

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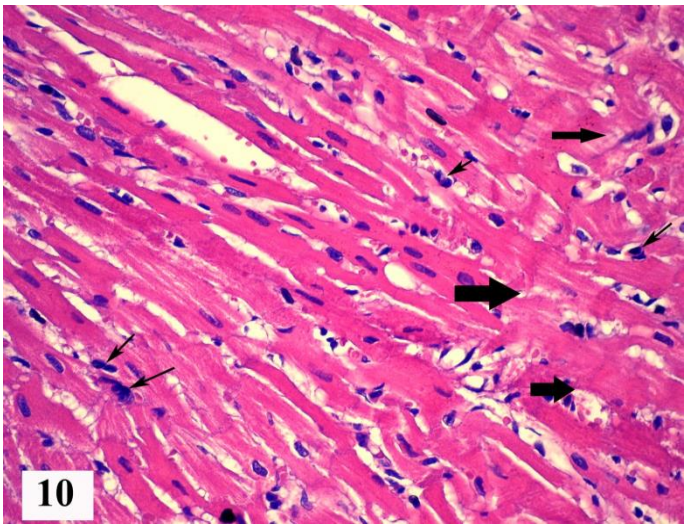

Fig.10. L.S. of the cardiac muscle of an alloxanized diabetic rat treated with low dose of moringa (200 mg/kg/d for 30 days) showing no improvement and disarrangement of cardiac myocytes , pyknotic nuclei (thin arrows) and dilatation of blood vessels(bv). H&E, X 400

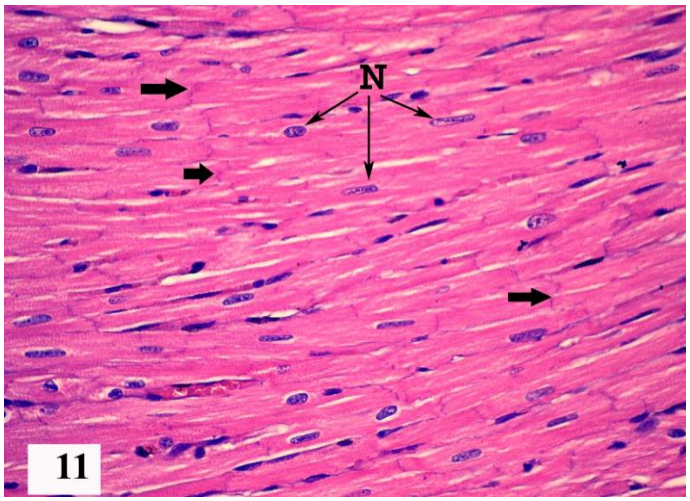

Fig.11. Longitudinal section of the cardiac muscle of an alloxanized diabetic rat treated with high dose of moringa (400 mg/kg/d for 30 days) showing clearly recovery of cardiac muscle and normal structure appearance of cardiac myofibres with intact intercalated discs(thick arrows) and normal centrally located nuclei (N). H&E, X 400

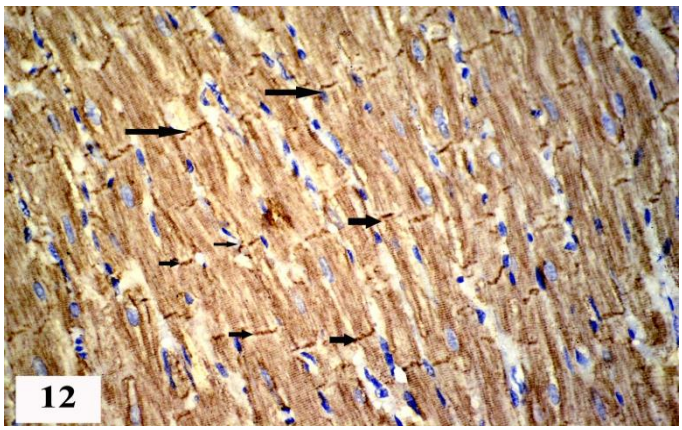

Fig 12: Longitudinal section of the cardiac muscle of control rat showing positive intense desmin expression in the intercalated discs & Z-lines (arrows). Desmin immunostain, X 400

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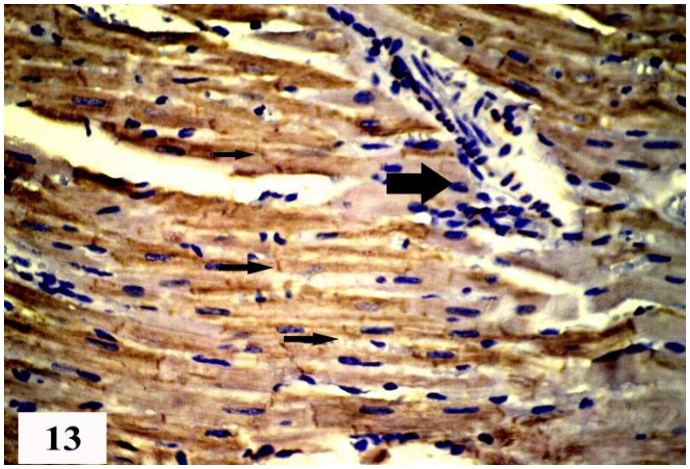

Fig 13 : L.S. of the cardiac muscle of diabetic rat revealing an obvious reduction of immunopositive expression of desmin in intercalated discs in myocytes (thin arrows). See dilated blood vessel with infiltration of inflammatory cells (thick arrows). Desmin immunostain, X 400

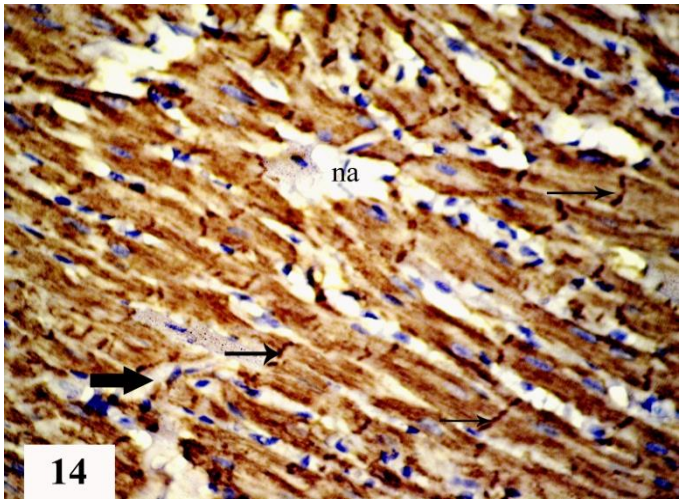

Fig 14: L.S. of the cardiac muscle of diabetic rat treated with low dose of moringa illustrating a partial recovery in immunopositive reaction to desmin in intercalated discs (thin arrows), partial arrangement of cardiac myocytes , Necrotic areas (na) and inflammatory cells (thick arrows) are still present. Desmin immunostain, X 400

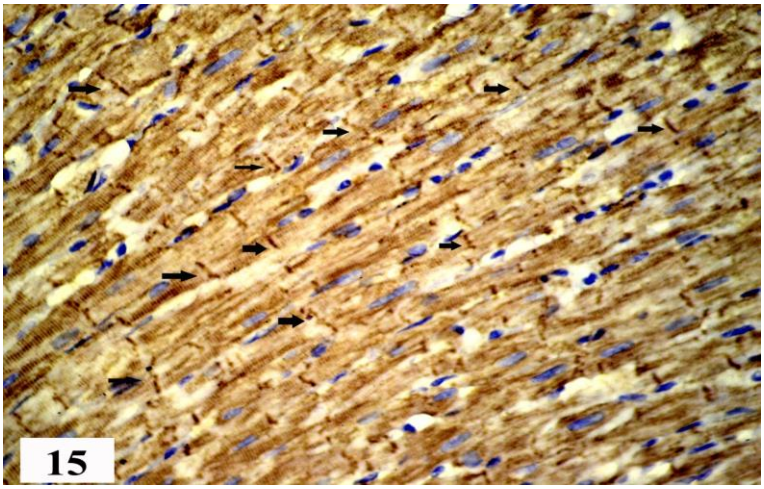

Fig 15: L.S. of the cardiac muscle of diabetic rat treated with high dose of moringa illustrating normal positive intense desmin expression in the intercalated discs (thick arrows), in Z lines (short thin arrows). Desmin immunostain, X 400

Immunohistochemical observations:-

The cardiac muscle of control rats group expressed the immunoreaction to desmin as a brown colour in the intercalated discs and Z lines of cardiac muscle fibers (Fig. 12). The alloxanized-diabetic rats group showed an obvious reduction in immunoreactivity to desmin of cardiomyocytes and expressed faint brown reaction in intercalated discs and Z lines (Fig.13). The treatment of alloxanized-diabetic rats with low dose of moringa (200 mg/kg/30 days) showed no improvement in immunoreaction to desmin in intercalated discs and Z lines , approximately similar to that of diabetic rats (Fig.14). The treatment of alloxanized-diabetic rats with high dose of moringa (400 mg/kg/30 days) expressed the strong positive intense immunostain to desmin in the cardiomyocytes in the intercalated discs and Z lines, similar to that of control ones (Fig.15).

DISCUSSION

Diabetes mellitus is metabolic disorders leading to hyperglycemia which later develops to micro- and macrovascular complications and becomes a major cause of death (25). 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 (26-28). A recent study has concluded that the increased level of plasma glucose could promote destruction in β-cells of pancreas (29,30).

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 (31).

Similarly, Oyedepo *et al.* (32) and Soliman. (33) 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 (29,30,34). 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 (35), and possibly through the mechanism of induction of free radical species (36) and oxidative stress that impaired insulin secretion in type 2 diabetes (37).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 (30). Additionally, diabetes-induced apoptosis in cardiomyocytes in diabetic rats (38).

The present results illustrated a significant increase in serum cholesterol and triglycerides in the diabetic rats. The high dose of moringa (400 mg/kg) showed a highly significant decrease in serum cholesterol and triglycerides of diabetic rats than low dose (200 mg/kg) for the same time period. These were in accordance with Oyedepo et al. (32) and Atsukwei et al. (39) who reported that the administration of moringa demonstrated a significant decrease in serum cholesterol and triglycerides, and this is due to the presence of phytochemical constituents and β-sitosterol that are the major cholesterol-reducing components of the *Moringa oleifera* leaf. β-sitosterol helps in reducing cholesterol levels by limiting the amount of cholesterol that is able to enter the body, by inhibiting cholesterol absorption in the intestines. The structure of β-sitosterol is similar to that of cholesterol. β- sitosterol takes the place of dietary and biliary cholesterol in micelles produced in the intestinal lumen and this reduces cholesterol absorption in the body. Therefore, instead of cholesterol being absorbed, the β-sitosterol in moringa oleifera will be absorbed.

Triacylglycerols are the main storage form of fatty acids. The decrease in serum triacylglycerol by the leaf extract of *M*. *oleifera* may be due to reduced lipolysis. This may deplete the store of fatty acids.

Also, the reduced serum triacylglycerol (TAG) level in the treated animals could be co-related to elevated lipoprotein lipase activity (39).

The present results showed a highly significant increase in serum urea and kreatinine in the diabetic rats, and the treatment with high dose of moringa (400 mg/kg) was more effective than low dose (200 mg/kg) that represented by a decrease of serum urea and kreatinine. In accordance, many studies reported that serum urea and kreatinine increased in diabetic rats, these are may be due to the kidney defected. Treatment of diabetic rats with 50 mg and 100 mg moringa seeds powder/kg body recorded significantly decrease (p=0.001) and ameliorated all kidney function parameters under study compared with that of the positive control group (25,40,41).

The histological and IHC observations of cardiac muscle in the current study confirmed biochemical results in which Halaby et al. (41) demonstrated that hyperlipidemia is a powerful and extremely one of the major causes of the development of cardiovascular disorders (42,43). On the othe hand, the changes in the type of diets have led to an increase frequency of lifestyle related disorders such as hyperlipidemia, diabetes mellitus and atherosclerosis (44). High fat diet is the term used to denote raised serum levels of one or more of total cholesterol, low-density lipoprotein cholesterol, triglycerides, or both total cholesterol and triglyceride (combined hyperlipidemia) (45,46).

In the current study, the diabetic rats showed disarrangement of cardiac myocytes, sarcoplasmic vacuolation, degeneration of muscle fibres, pyknotic nuclei, dilated blood vessels and inflammatory cells. The diabetic rats treated with low dose of moringa demonstrated with no improvement in the cardiomyocytes and revealed similar to that of diabetic myocytes. The diabetic rats treated with high dose of moringa illustrating the cardiomyocytes closly similar to control form. The cardiac muscle restored their normal histological appearance with intact intercalated discs and normal sarcoplasm & nuclei. These were in accordance with Halaby et al. (41).

Concerning to desmin in the present study, the cardiac muscle of control rats group expressed the immunoreaction to desmin in the intercalated discs and Z lines of cardiac muscle fibers. The diabetic rats showed an obvious reduction in immunoreactivity to desmin of cardiomyocytes and expressed faint intercalated discs and Z lines. The treatment of diabetic rats with high dose of moringa expressed strong positive intense immunostain to desmin in the intercalated discs and Z lines of the cardiomyocytes approximately similar to control ones more than with low dose of moringa. *El-Desouki et al.* (47) reported that the immobilized-stressed rats for 30 days expressed the cardiac muscle with a marked decrease of immunoreactivity to desmin in intercalated discs and Z lines and after treatment with diazepam for 30 days, the cardiac muscle illustrated an obvious recovery and increase immunopositive reaction to desmin in intercalated discs and Z lines.

In conclusion, the changes in serum glucose, insulin values, urea, kreatinine, cholesterol and triglycerides of alloxan-diabetic rats as well as the histopathological changes in cardiac muscle and the destruction of cardiac desmin immunostain were improved and recovery by treatment of moringa at a dose 400 mg/kg/d for 30 days more than by 200 mg/kg/d. of moringa for 30 days, and acts as hypoglycemic effect.

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