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Evaluation of anti- hyperglycemic effect of *Moringa oleifera* leaves Extract on some physiological parameters of diabetic rats induced apoptosis in the pancreas

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ABSTRACT

Aims: The purpose of this study is to evaluate the effect of *Moringa oleifera* leaves extract on blood glucose level, insulin, growth rate, ALT, AST and total protein as well as apoptosis in diabetic adult male albino rats. Sixty adult male albino rats of the Wistar strain weighing 99 ± 1.03 g were used for this study. These 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 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, insulin, growth rate, total protein, ALT and AST were determined in all animal groups. The immunostain with caspase-3 for apoptosis was also examined. **Results:** a significant increase in blood glucose and a significant decrease in insulin of diabetic rats were recorded when compared with the normal control group. Low dose of moringa recorded slight decrease in plasma glucose and a slight increase in insulin. The treated diabetic rats with high dose of *Moringa oleifera* extract recorded a significant decrease in blood glucose level and a significant increase in insulin. Also, a significant decrease in growth rate of diabetic rats was recorded as compared to the control undiabetic rats. Moringa treatment to diabetic rats with high dose (400 mg/kg/d) for 30 days was better than low dose of it, and a recovery of the body weight by a significant increase in body weight gain was recorded compared with untreated diabetic rats. The mean values of ALT&AST were not significantly affected by diabetes. Treating diabetic rats with the two doses of moringa under study did not affect the studied liver enzymes. The alloxanized-diabetic rats showed insignificant decrease in serum total protein and significant decrease in both liver and pancreatic tissues homogenate. Diabetic rats treated with both low & high doses of moringa have insignificant increase of total protein contents in serum and a significant increase of total protein content in both liver and pancreatic tissues homogenate. Immunostain with caspase-3 of the pancreas of alloxanized- diabetic rats that treated with high dose of moringa revealed a reduction in the apoptotic cells of acinar and islet cells. **Conclusion:** oral administration of aqueous leaf extract of *Moringa oleifera* reduced the glucose level associated with diabetes with the improvement of insulin, growth rate, total protein, ALT, AST and a reduction of the apoptotic pancreatic cells were recorded.

Keywords: Diabetes, *Moringa oleifera*, Physiological parameters, Apoptosis IHC, Rats

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder that results from a reduction of insulin available for normal function of many cells in the body. DM is characterized by disturbance of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both **(1)**. The diseases can also be recognized during less overt stages, most usually by the presence of glucose intolerance. The effect of DM includes long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, livers, hearts and blood vessels **(2).**

Type 2 diabetes is caused by beta-cell dysfunction and declining beta-cell mass in insulin resistant subjects. Apoptosis or "programmed cell death", characterized by DNA fragmentation and cellular shrinkage, is increased in pancreatic beta cells in type 2 diabetes leading to loss of beta-cell mass (**3).** Examination of pancreases obtained from healthy and type 2 diabetic human donors showed that beta-cell mass is decreased and apoptosis is increased in type 2 diabetes **(4).** Staining of human pancreatic sections with TUNEL and Ki67 revealed that this decrease in beta-cell mass that was caused by increased apoptosis, and not by decreased beta-cell replication. Apoptosis is difficult to detect in islets because of rapid-turnover and clearance of apoptotic cells by neighboring macrophages. Because of this difficulty, most of the studies of apoptosis in type 2 diabetes have been carried out on animal models. Several animal models have shown loss of beta-cell mass and increased number of TUNEL positive apoptotic beta cells in type 2 diabetes **(5).**

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 **(6).** Oxidative damage due to free radicals was associated with vascular disease in people with types 1 and 2 diabetes mellitus **(7).** There are several potential of 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 type 1 or 2 DM was lower than that of age-matched controls, and this might be attributed to lower levels of vitamin C, vitamin E, or other factors including in blood **(8)**. The increased susceptibility of tissues such as the liver and kidney of diabetic animals to diabetic complications may be due to increased lipid peroxidation **(9).** In addition, increased lipid peroxidation under diabetic conditions resulted due 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 **(9).**

Moringa oleifera Lamarack is the cultivated species of the genus Moringa of the family Moringaceae **(10)**. Several health benefits were reported as a result of supplementation with moringa leaves or seeds or their extract **(11).** *M. oleifera* is described as the miracle tree, tree of life and God's Gift to man **(12).** Moringa root wood reduced the elevated urinary oxalate and lowered the deposition of stone forming constituents in the kidneys of calculogenic rats as a result of ethylene glycol treatment **(13).** Moringa improved nutrition, boost food security, foster rural development support sustainable land care, forage for livestock **(14).** Moringa ameliorated liver fibrosis in rats and reduces liver damage and symptoms of liver fibrosis, decreased the CCl4-induced elevation of serum aminotransferase activities and globulin level, reduced the elevated hepatic hydroxyproline content and myeloperoxidase activity (**11).**

Moringa crude extract is a good scavenger for nitric oxide radicals and has a potential source of natural antioxidant **(15).** Moringa has also nutraceutical uses and is used in treatment of hypercholesterolemia and hyperglycemia, and also, as a nutritional supplementation, it can be prescribed as food appendage for coronary artery disease patients along with their regular medicines **(16).** Moringa also increases wound healing of normal and dexamethasone suppressed wound in rats **(17).** The antioxidant and antidiabetic activity of aqueous extract of moringa leaves indicated potential benefits as a potent antidiabetic on streptozotocin-induced diabetic albino rats **(18).**

The present work was designed to study the role of the leaf extract of *Moringa oleifera* on the blood glucose and insulin levels, growth rate, total protein, ALT, AST and immunostain with caspase-3 for pancreatic tissues apoptosis of the diabetes induced in adult albino rats.

Materials and methods

1. 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**.**

2. 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.* (19),** then after two days ,the rats were injected

with 100mg/kg of alloxan (2nd injection). Lastly, 3rd injection of alloxan (100 mg/kg) was applied two days after the 2nd one. Note, the 2nd and 3rd 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 **(8 & 20)**.

3. Experiment:-

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/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 and pancreatic tissue samples were carefully dissected out and divided into two pieces for biochemical and immunohistochemical studies.

4. Blood glucose, insulin, total protein, ALT and AST estimations:-

Blood glucose was estimated on 0, 7, 14, 21 and 30 day by using Accu-Chek Performa apparatus according to **Brăslasu *et al.* (21)** and **El-Desouki *et al.* (8).** Insulin was determined in the Laboratory by using a rat-specific Insulin-Ak ELISA according to **Finlay and Dillard (22).** The serum and tissue homogenate ALT& AST activities were determined using biomaxima kits colorimetric method according to **Reitman and Frankel (23).** Total protein was determined using chrom biomed kits colorimetric method according to **Yatzidis (24).**

5. Immunohistochemical staining:-

Caspase -3 of pancreatic tissues with immunolocalization technique was performed as previously described **(25).** The immunoreactivity 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 1h to prevent non-specific binding followed by incubation with the primary monoclonal antibody for 1h 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 ℃. Staining was visualized using diaminobenzidine (DAB, Sigma), then slides were washed and counterstained with haematoxylin, cleared, mounted and examined by light microscopy. Finally, the cytoplasmic sites of reaction were stained brown and nuclei stained blue.

6. 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).

Results

A) Biochemical results:-

(1) Effect of moringa leaves extract on blood glucose value:

Inducing of diabetes in rats 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 at a day zero (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).

The diabetic rats have 503.7±3.4 mg/L glucose value with a difference 534.68 % at the end of experiment 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=0.001), (Table 1 & Fig. 1).

(2) Effect of moringa leaves 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 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).

(3)Effect of diabetes and moringa leaves extract on growth rate :-

The chemical induction of DM reduced the weight gain (0.73) of diabetic rats compared to the control undiabetic rats (1.75). The treatment of moringa to the undiabetic rats with both low & high doses (200&400 mg/kg/d , respectively) for 30 days caused decreasing in body weight gain (0.25 & 0.29 ,respectively) compared to control undiabetic rats (Table 2) . Moringa treatment to diabetic rats with high dose (400 mg/kg/d) for 30 days caused increase in body weight gain (0.91) compared with untreated diabetic rats (0.73), (Table 3 & Fig. 3).

(4) Effect of moriga leaves extract on serum and liver tissue homogenate enzymes activity:

(a)Aspartate aminotransferase (AST):-

Diabetic rats showed insignificant increase in serum AST activity (14.17 ±0.27,-5.05%) compared to the control normal rats (7.50 ± 1.01) and insignificant increase in liver tissues homogenate (6.87±0.21, -13.09%) at p=0.001 compared to the corresponding value of control normal rats (7.24±2.03). The treatment with moringa at low dose to undiabetic rats caused insignificant decrease of AST activity of serum and insignificant decrease of liver tissues homogenate (7.31±1.19, -1.63% & 5.79±2.03, -0.55%, respectively) compared to control group. High dose of moringa leaves extract caused insignificant decrease of serum AST activity and insignificant decrease of liver tissues homogenate AST activity (7.38 ± 1.10, -0.36 % & 5.75±1.29, -1.06 %, p=0.001, respectively). The treatment with moringa at low dose to diabetic rats caused insignificant decrease of AST activity of serum and insignificant decrease of liver tissues homogenate (12.18±2.53, -14.04% & 6.81±0.65, -0.87%, p=0.001, respectively) compared with untreated diabetic rats. High dose of moringa leave extract powder caused insignificant increase of serum AST activity and insignificant increase of liver tissues homogenate ALT activity (16.66±2.79, 17.57 % & 8.02±1.73 , 16.73 %, p=0.001, respectively), (Table 4 & Figs. 4&5).

(b) Alanine aminotransferase (ALT):-

Diabetic rats showed insignificant increase in serum ALT activity (9.96 ±1.11, 25.03%) compared to the control normal rats (7.97 ± 1.01) and insignificant decrease in liver tissue homogenate (5.166±0.12, -13.09%) at p=0.001 compared to the corresponding value of control normal rats (5.82±1.05). The treatment with moringa leave extract at low dose to un diabetic rats caused insignificant increase of ALT activity of serum and insignificant increase of liver tissues homogenate (7.43± 0.87 , - 6.82% & 7.12±1.12, -0.55 %, p=0.001 , respectively) compared to control group . High dose of moringa leave extract caused insignificant decrease of serum ALT activity and insignificant increase of liver tissues

homogenate ALT activity (7.91±0.76, - 0.68 % & 7.21±1.15, -1.06 %, p=0.001, respectively). The treatment with moringa leave extract at low dose to diabetic rats caused insignificant increase of ALT activity of serum and insignificant increase of liver tissues homogenate (14.30± 2.89, 43..57 % & 10.38±1.4 , 101.1 %, p=0.001, respectively) compared with untreated diabetic rats . High dose of moringa leave extract powder caused insignificant increase of serum ALT activity and insignificant increase of liver tissues homogenate ALT activity (11.57±1.83 , 16.16 % & 11.42±2.30 , 121.3 %, p=0.001, respectively), (Table 4 & Figs. 6&7).

(5) Effect of moriga leaves extract on serum and tissue homogenate of Total protein:-

It is clear from the present data that the alloxanized-diabetic rats showed insignificant decrease in serum total protein and significant decrease in both liver and pancreatic tissues homogenate (6.21±0.25 , -9.46 % & 1.65±0.08 , -77.76% &1.56±0.09 , -76.97 %) compared to the control normal rats (6.9±0.09 & 6.93±0.20 & 6.89±0.23) at p=0.001. Moreover, there is insignificant decrease in serum and liver tissue homogenate after administration of moringa leave extract at both low and high doses to undiabetic rats (6.27±0.28 , -10.34% & 5.37±0.17 , -22.54 % & 6.46±0.42, -7.57% & 5.55±0.13, -19.96 %) and insignificant decrease in pancreatic total protein (5.32±0.35 , -21.95% & 5.72±0.21 , -16.88%). Diabetic rats treated with both low & high doses of moringa have insignificant increase of total protein contents in serum (6.45±0.34, 3.86% & 6.64±0.16, 6.92%) and significant increase of total protein content in both liver and pancreatic tissues homogenate (6.12±0.78, 341.81 % & 7.29±0.23, 270.9% & 6.04±0.09, 287.17% & 6.50±0.21 , 316.66%), (Table 5 & Figs. 8,9 & 10).

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± SE (standard error) at p<0.001

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< 0.001.

Table(3): The effect of moringa on both low and high doses (200,400 mg/kg) for 30 days on growth rate.

Groups	Body weight				Growth rate
	Initial weight(W0)		Final weight (WF)		
	X ± SE	diff%	X ± SE	diff%	
GROUP 1	92.5±1.03	.....	145.1±5.33	.....	1.75
GROUP 2	71.89±2.42	-22.22	79.39±1.21	-45.28	0.25
GROUP 3	75.49±2.34	-18.34	84.20±0.86	-41.97	0.29
GROUP 4	91.03±2.00	-1.51	113.1±1.78*	-22.05	0.73
GROUP 5	89.33±1.04	-3.38	105.87±0.88	-6.39	0.615
GROUP 6	87.42±1.93	-5.92	116.6±4.74	3.09	0.91

*Significant against group1, ** significant against group 4. All results are expressed at mean± SE (standard error) at p<0.001.

Table (4): The effect of moringa on both low and high doses (200,400 mg/kg) for 30 days on liver functions enzymes of the normal and diabetic rats.

groups	Alanine aminotransferase (ALT)				Aspartate aminotransferase (AST)			
	serum U/L		liver tissue Homogenate U/L		serum U/L		liver tissue Homogenate U/L	
	X ± SE	diff%	X ± SE	diff%	X ± SE	diff%	X ± SE	diff%
Group 1	7.97 ± 1.01	.....	5.82±1.05	.....	7.50 ± 1.01	.....	7.24±2.03	.....
Group 2	7.43± 0.87	-6.82	7.12±1.12	-0.55	7.31±1.19	-1.63	5.79±2.03	-0.55
Group 3	7.91±0.76	-0.68	7.21±1.15	-1.06	7.38 ± 1.10	-0.36	5.75±1.29	-1.06
Group 4	9.96 ±1.11	25.03	5.166±0.12	-13.09	14.17 ±0.27*	-5.05	6.87±0.21	-13.09
Group 5	14.30± 2.89	43.57	10.38±1.40	101.1	12.18±2.53	-14.04	6.81±0.65	-0.87
Group 6	11.57±1.83	16.16	11.42±2.30	121.3	16.66±2.79	17.57	8.02±1.73	16.73

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

Table (5): The effect of moringa on low and high doses (200,400 mg/kg) for 30 days on total proteins.

groups	Total proteins					
	Serum U/L		**Liver tissue U/L**		**Pancrease tissue U/L**	
	X ± SE	diff%	X ± SE	diff%	X ± SE	diff%
Group 1	6.9±0.09	.....	6.93±0.20	.....	6.89±0.23	.....
Group 2	6.27±0.28	-10.34	5.37±0.17	-22.54	5.32±0.35	-21.95
Group 3	6.46±0.42	-7.57	5.55±0.13	-19.96	5.72±0.21	-16.88
Group 4	6.21±0.25	-9.46	1.65±0.08*	-77.76	1.56±0.09*	-76.97
Group 5	6.45±0.34	3.86	6.12±0.78	270.9	6.04±0.09	287.17
Group 6	6.64±0.16	6.92	7.29±0.23**	341.81	6.50±0.21**	316.66

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

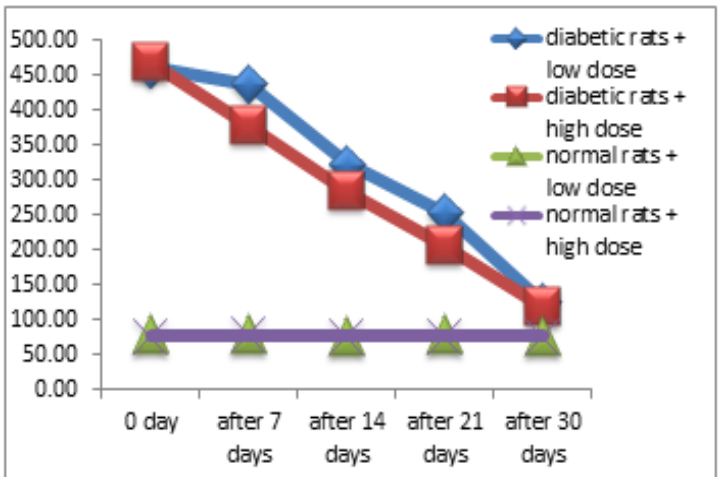

Fig 1: Effect of moringa on blood glocuse level (table 1)

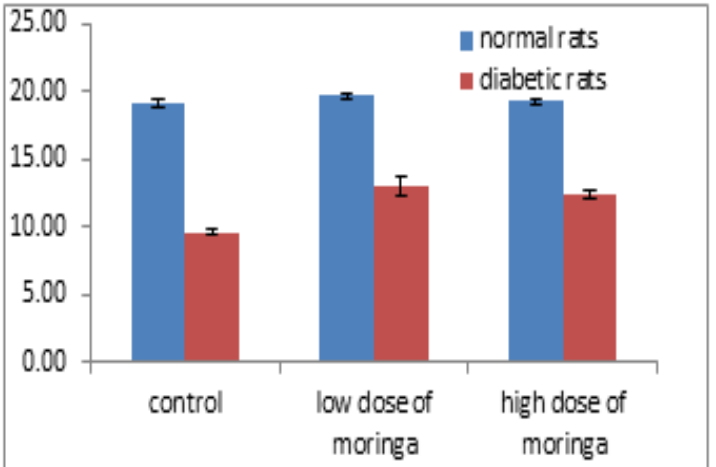

Fig 2: Effect of moringa on serum insulin(table 2)

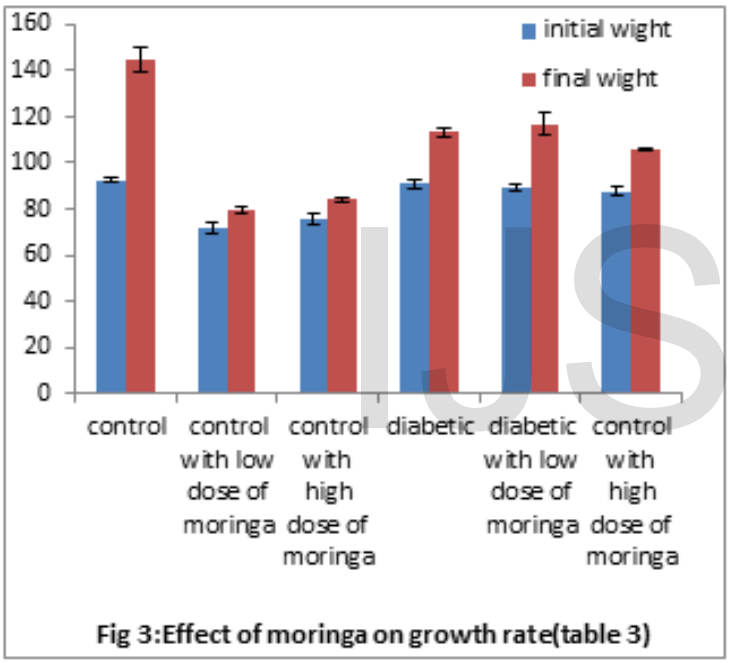

Fig 3:Effect of moringa on growth rate(table 3)

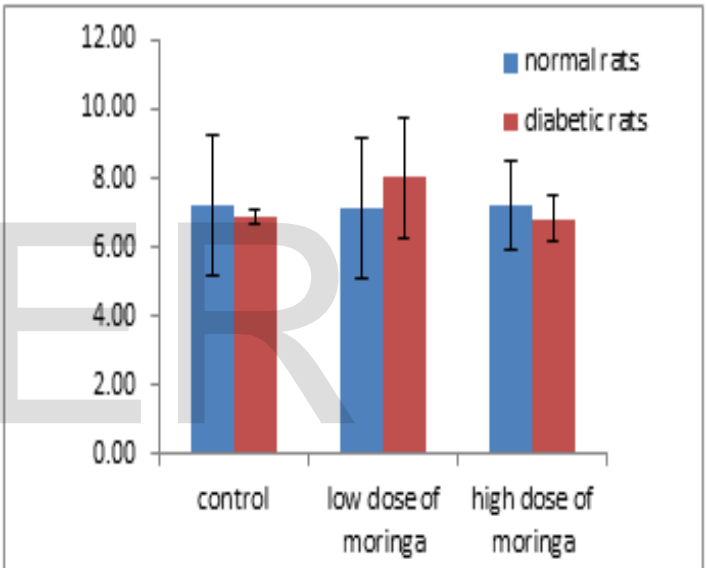

Fig 4:Effct of moringa on serum AST activity(table 4)

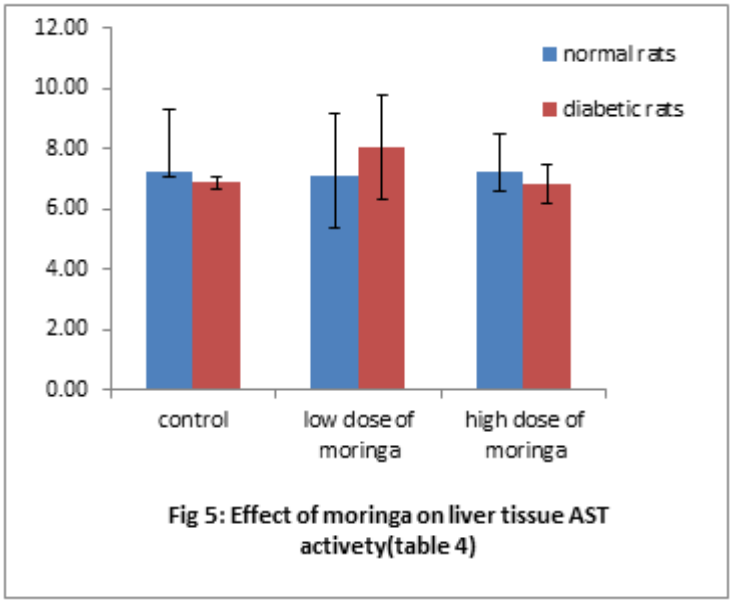

Fig 5: Effect of moringa on liver tissue AST activety(table 4)

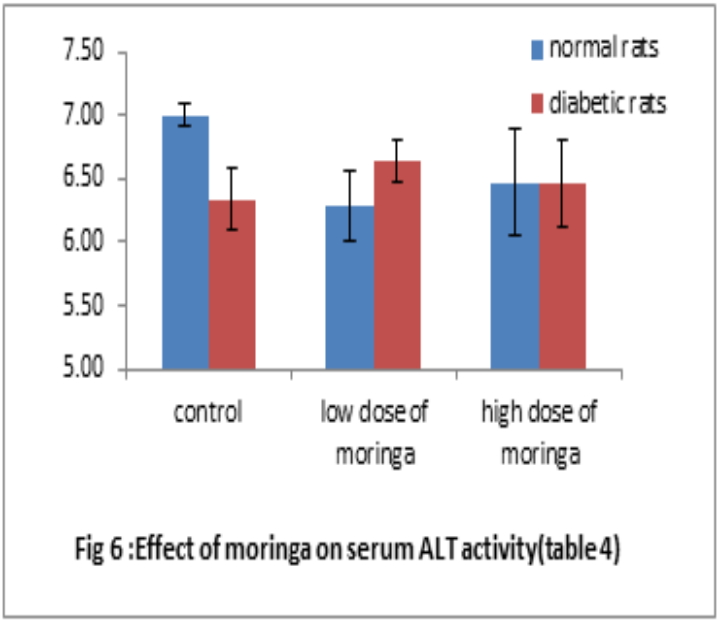

Fig 6 :Effect of moringa on serum ALT activity(table 4)

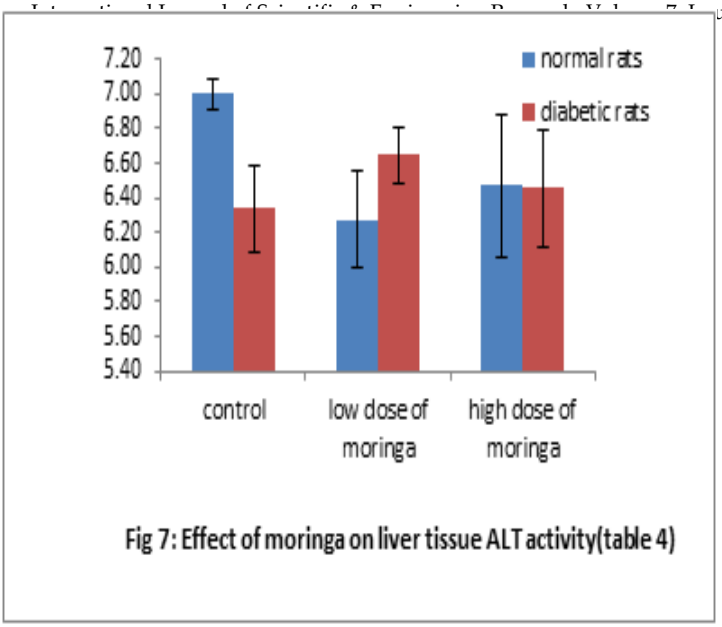

Fig 7: Effect of moringa on liver tissue ALT activity(table 4)

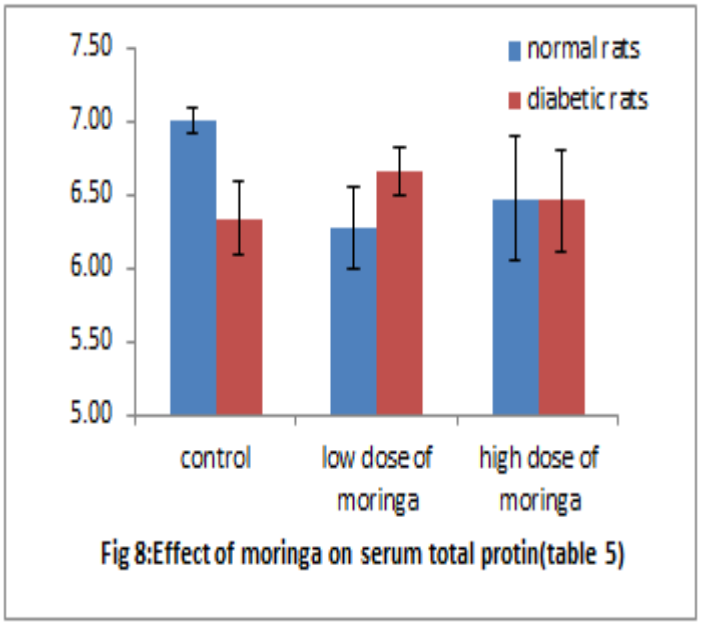

Fig 8:Effect of moringa on serum total protin(table 5)

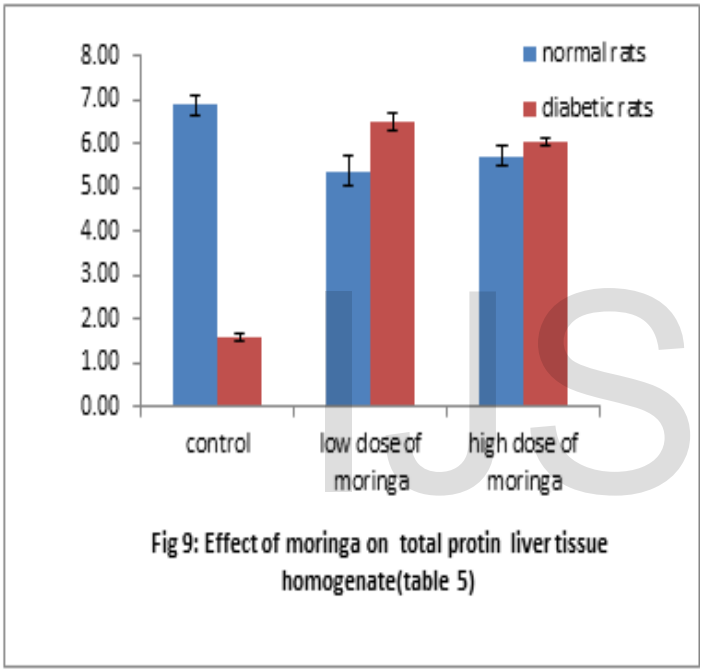

Fig 9: Effect of moringa on total protin liver tissue homogenate(table 5)

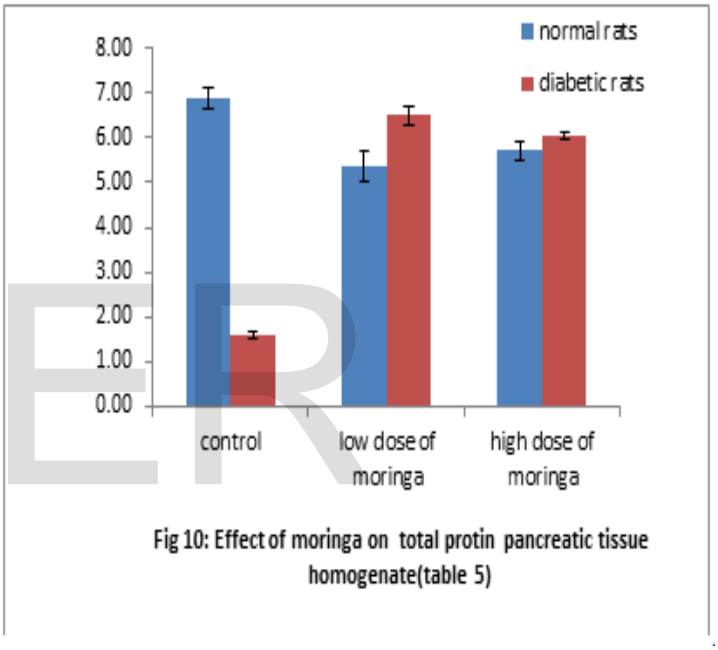

Fig 10: Effect of moringa on total protin pancreatic tissue homogenate(table 5)

B) Caspese- 3 immunostain observations:

Caspese- 3 is a main effector caspase of the apoptotic cascade. The control pancreas of normal rats group revealed normal few caspase-3 positive cells in acinar and a little bit cells in Langerhans islets cells (Fig. 11).

The alloxanized-diabetic rats group showed the increament of apoptosis in immunopositive cells of caspase-3 in islet cells in which the apoptosis was seen in the form of brown granules present in acinar and islet cells (Figs. 12&13).

The alloxanized-diabetic rats treated with low dose of moringa showed caspase-3 expression in the islet and acinar cells similar to diabetic rats group with high immunopositive reaction (Fig. 14), that means the low dose of moringa did not affect as an anti – apoptosis.

The treatment of diabetic rats with high dose of moringa demonstrated obviously the reduction of the expression of caspase-3 in pancreatic cells (Fig. 15) and appeared approximetaly similar to normal ones. It means that the high dose of moringa exerted an anti – apoptotic effects.

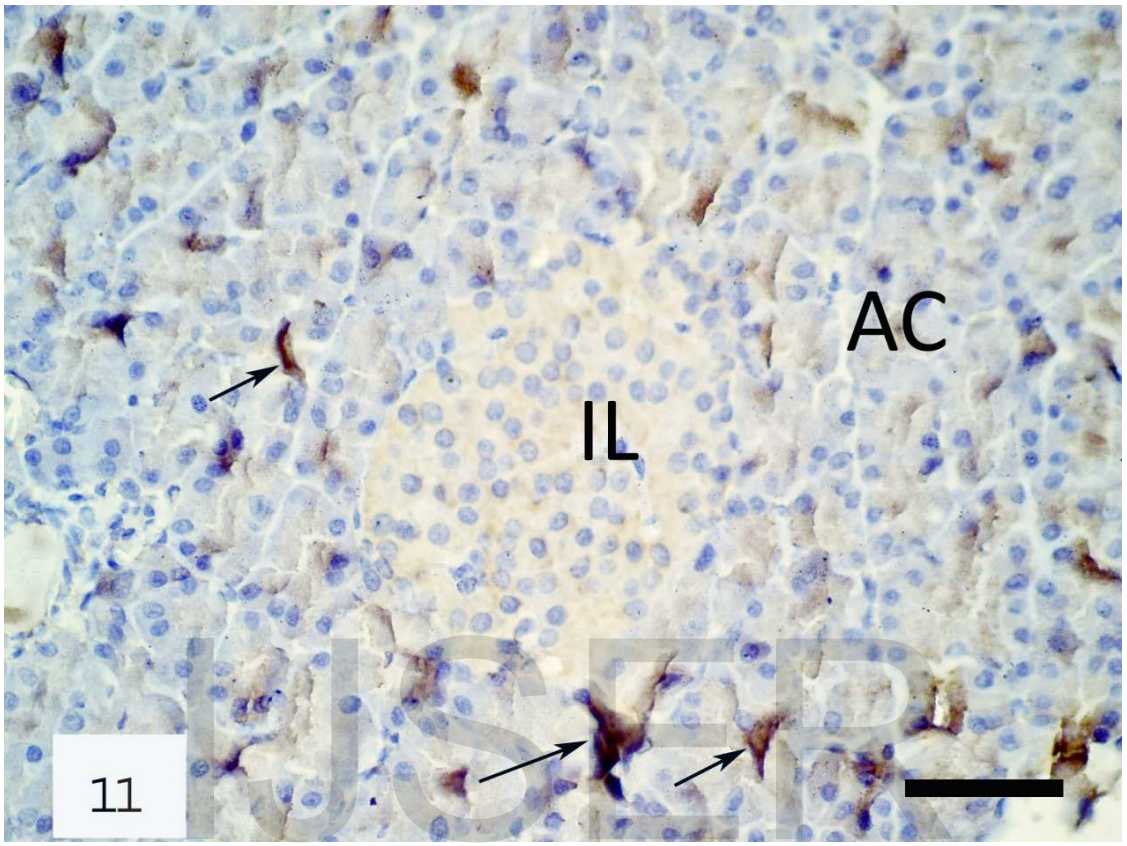

Fig 11: A section in a pancreas of a control rat showing normal appearance of Langerhans islet (IL) with a little bit of apoptic cells and a few scattered apoptic cells in the acinar cells (AC) (arrows) . Caspase -3 immunostain, Scale bar = 6.25 µm.

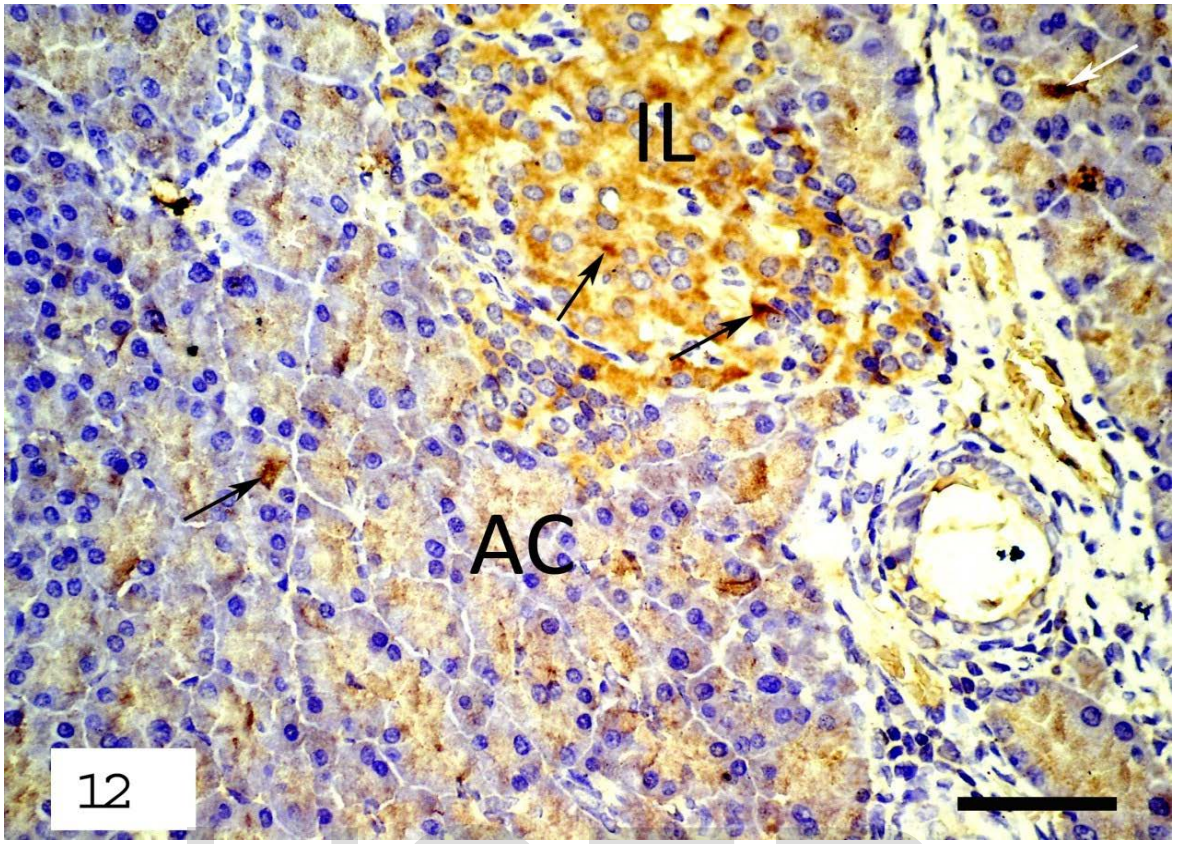

Fig. 12: A section in a pancreas of an alloxanized-diabetic rat showing the increment of apoptosis in Langerhans islet (IL) and acinar cells (AC) (arrows). Caspase -3 immunostain, Scale bar = 6.25 μm.

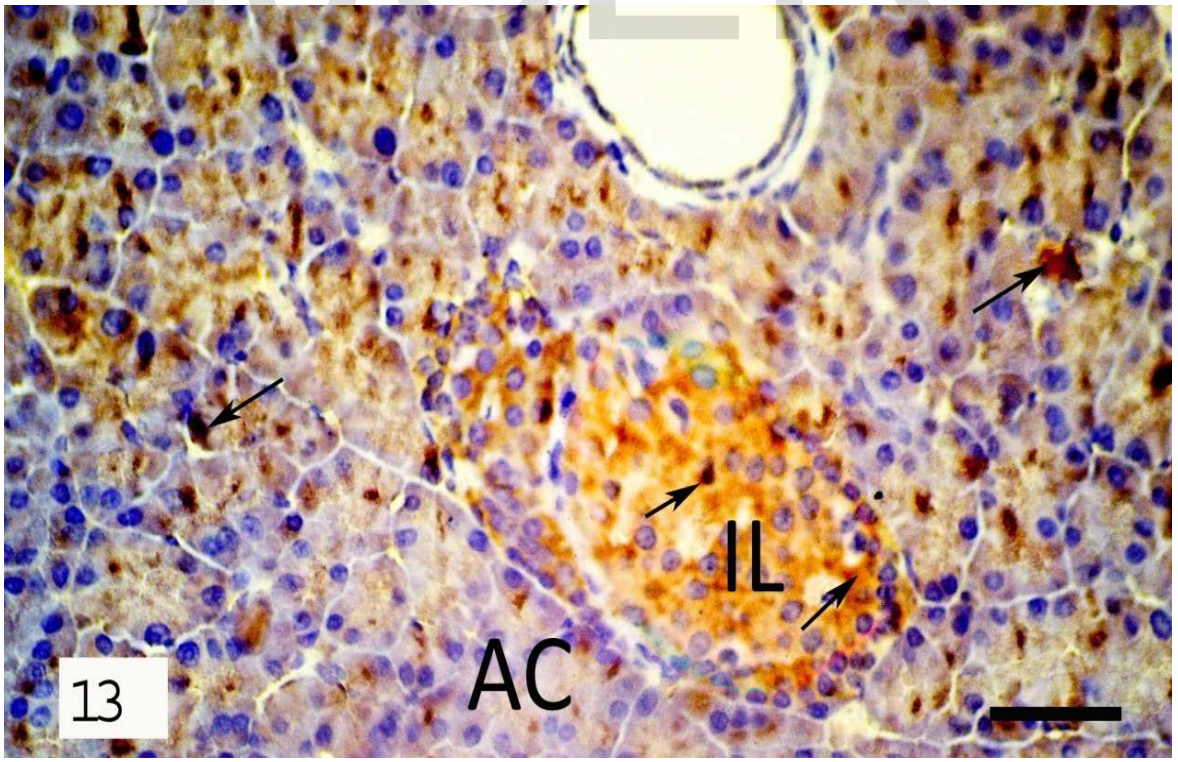

Fig. 13: A section in a pancreas of an alloxanized-diabetic rat detecting clearly apoptosis in Langerhans islet (IL) and in acinar cells(AC) (arrows). Caspase -3 immunostain, Scale bar = 6.25 μm

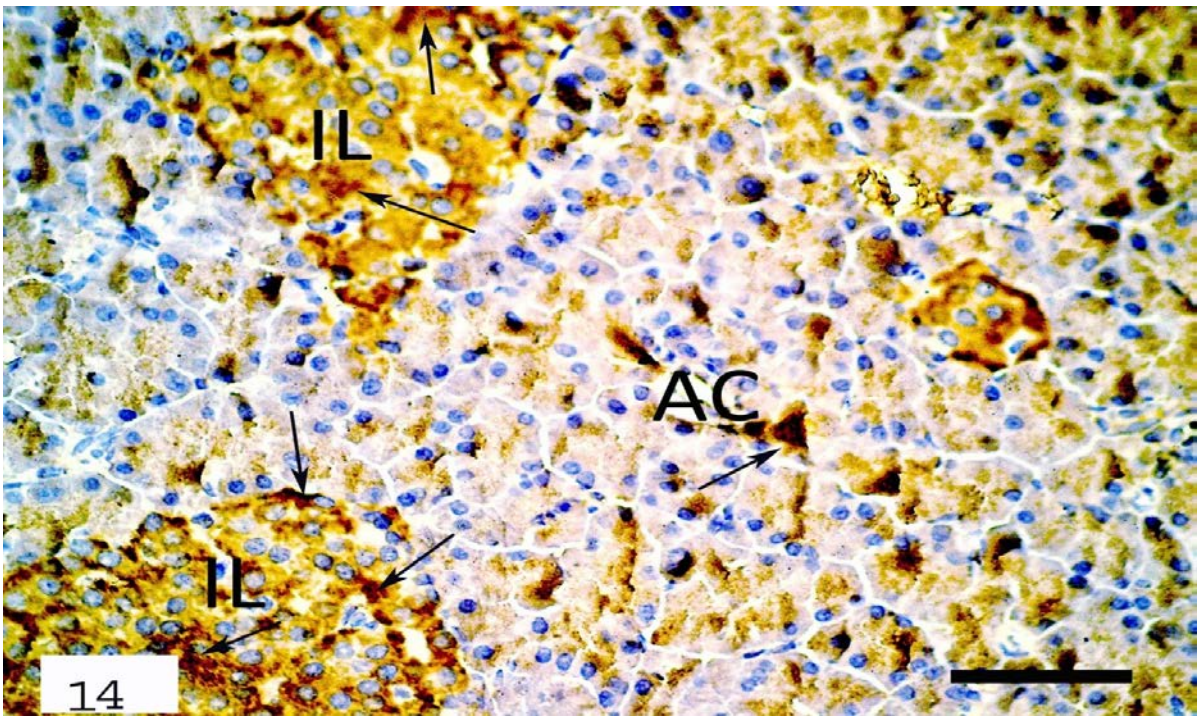

Fig. 14: A section in pancreas of an alloxanized - diabetic rat treated with low dose of moringa revealing high immunoreactivity of apoptosis in Langerhans islet (IL) and acinar cells (AC) (arrows). Caspase -3 immunostain, Scale bar = 6.25 µm.

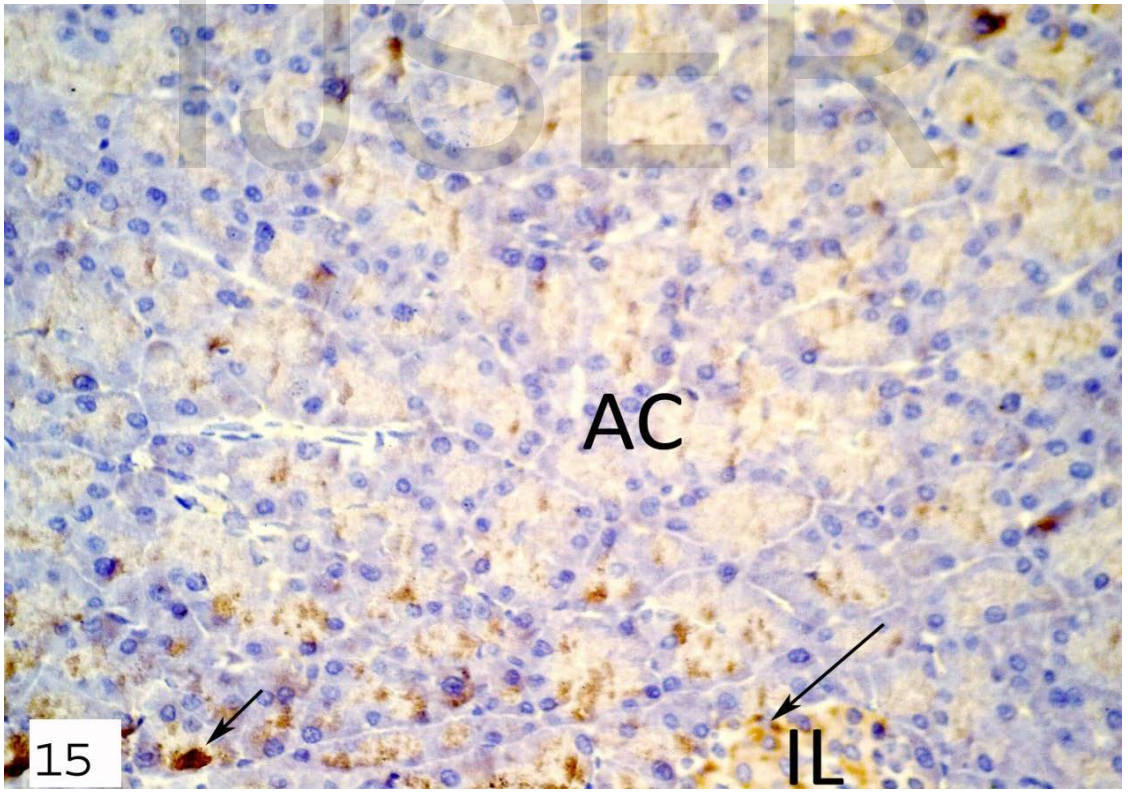

Fig. 15: A section in a pancreas of an alloxanized - diabetic rat treated with high dose of moringa revealing the reduction in the expression of apoptotic cells in both Langerhans islet (IL) and acinar cells (AC) (arrows). Caspase -3 immunostain, Scale bar = 6.25 µm.

Discussion

Diabetes mellitus is metabolic disorders leading to hyperglycemia which later develops to micro- and macrovascular complications and becomes a major cause of death **(26)**. 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 **(27-30).**

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 30 days) treated 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 **(31).** 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 **(32)**. Similarly, the treatment of diabetic rats with moringa extract (400 mg/ kg) for 28 days was significantly decrease blood glucose level **(8, 33&34)**.

Moreover, many researchers recorded a decrease in insulin level in alloxan- diabetic rats **(8, 32&35)**. From this study, it is suggested that *Moringa oleifera* seeds 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 **(36)**, and possibly through the mechanism of induction of free radical species **(37)** and oxidative stress that impaired insulin secretion in type 2 diabetes **(8 & 38)**.

Additionally the treatment of diabetic rats with a natural extract of the marine algae (spirulina) (2g/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 in the levels of the glucose and NO levels; and a significant increase in the antioxidant SOD and CAT values **(31).**

It is an established fact that growth in all life forms results from a surplus in energy balance between intake and expenditure. This usually is compromised in pathological conditions where more often food is barely tolerated and the entire metabolism of the individual is adversely affected **(39).** Changes in weight are usually a fundamental index of physiological or pathological state of an experimental animal. From this study, the sharp decrease in body weight of diabetic control compared to normal control and experimental treated animal during experimental period is an indication in tissue wasting as result of poor glycemic control in diabetes mellitus and this usually foster protein and fat mobilization **(40).** However, weight

gain in the other hand was observed in the diabetic groups treated with the moringa extract. This increase in body weight was compromise in groups that were co-administered with high dose of moringa (400 mg/kg) (3.09%) with growth rate (0.91) than low dose (200mg/kg) (-6.39%) with growth rate (0.615). This could be due to a better control of hyperglycemic state in the diabetic rats and decreased fasting blood glucose level could improve body weight in alloxan-induced diabetic rats **(41).** More so, a metabolic impairment leading to imbalance in energy can also result from impressed factors including drastic changes in environmental conditions, exposure to drugs, toxicants, pollutants and the likes **(42)**. Also, **Zafar *et al.* (43)** recorded that there is an association between hyperglycemia and decrease in body weight of experimental animals**.**

The present results illustrated the reducion in the level of total protein in liver and pancreatic tissue homogenate due to alloxan injection, this is may be due to attributed to hepatocellular damage, oxidative stress which increase the amount of (ROS radicals), impaired liver function and also may due to the decrease capacity of the hepatocytes to synthesize protein **(44),** and the decrease of acid and alkaline phosphatases histochemically by using azo dye method in the pancreatic tissue of diabetic rats **(45) .** Much research has been carried out to demonstrate the relation between diabetes and the pathology of liver and kidney .Diabetes mellitus is one of the diseases that emerges hepatic complications and causes intoxication and liver damage. The insulin – dependent diabetic cases are associated with inhibition of insulin biosynthesis and secretion **(31).** The diabetic rats treated with high dose of moringa detecting highly significant increase in total protein of liver and pancreatic tissue than low dose**.**

The present result showed that there was insignificant decrease in serum total protein of alloxanized - diabetic rats. This were in accordance with **Yassin *et al.* (46)** and **Ibrahim and Abdelatif (47)** who reported that total protein of alloxanized diabetic rabbits have no significant changes. Also, **El-Desouki *et al.* (45)** demonstrated no change in the total protein contents histochemically by using bromophenol blue dye in the pancreatic tissue either acinar or islets cells of alloxanized-diabetic rats. Conversely, other researchers **(48)** reported that total protein decreased significantly in alloxan- diabetic rats **(32).** The present results showed insignificant increase in serum and liver tissue homogenate of ALT & AST of alloxanized diabetic rats or groups treating with moringa at (p= 0.001). This might be due to the highly antioxidant activity of liver this were in accordance with **Al-Malki and El Rabey (26).** Conversely, other researchers reported that ALT and AST decreased in diabetic rats **(49).**

In the current study the control pancreas of normal rats group revealed few

caspase-3 positive cells in acinar cells, and very few in islets cells, while the alloxanized-diabetic rats group showed high apoptosis in immunopositive cells of caspase-3 in islet cells higher than in acinar cells. These results were in accordance with many authors **(29, 50-52)** who reported that alloxan and streptozotocin are toxic materials caused apoptosis. The alloxanized-diabetic rats treated with low dose of moringa group showed caspase-3 expression in the acinar and islet cells similar to diabetic group while the treatment of diabetic rats with high dose of moringa reduce the expression of caspase-3, and the cells appeared approximately like normal ones. In accordance, **Rifaai *et al.* (51)** reported that Querectin, which is one of the most widely distributed flavonoids present in foods, reduces the expression of caspase-3 immuostain in pancreatic tissue and ilucidated faint in the islet cells.

In conclusion, the changes in plasma glucose , insulin values, body weight , ALT & AST, total protein and the IHC observation of apoptosis in alloxanized-diabetic rats in the present study 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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