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Pathological and Biochemical Studies on the Effect of *Trigonella foenum* - Graecum and *Lupinus termis* in Alloxan Induced Diabetic Rats

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Abstract: This study was undertaken to investigate the histopathplogical effect of *Trigonella foenum –graecum* (*T. foenum graecum*) and *Lupinus termis* (*L- Termis*) either each alone or in combination in alloxan diabetic albino rats in addition to some biochemical parameters. Seeds of T. *foenum graecum* and *L-termis* were crushed into fine powder, suspended into double distilled water and orally administrated at a dose of 75 mg /kg B.W. for 21 days. After 7, 14 and 21 days of Treatment, five rats of each group were sacrificed., histopathological analysis of pancreas in alloxan induced diabetic rats revealed a vacuolar change with pycknosis of nuclei of cells in the islets of Langerhans along with atrophy of islets. After 21 days, there was destruction of beta cells, distortion of cells and reticular changes of islets as evidence of fibrosis. Treatment with either *T. foenum graecum* or *L.termis* each alone or in combination resulted in marked improvement in histological picture of pancreas,especially after 21 days if compared with diabetic rats in which the islets of Langerhans were somewhat returned to normal. The administration of these plants also resulted in significant reduction in blood glucose, serum cholesterol level and Triglycerides, in addition to increased intensity of glycogen storage in hepatic cells in treated diabetic rats.

Key words: Alloxan · Trigonella foenum graecum · Lupinus termis · P ancreas · Hyperglycemia · Histopathology

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and protein and increased risk of complication from various diseases [1]. Oxidative stress is currently suggested as mechanism underlying diabetes and diabetic complications. Enhanced oxidative stress and changes in antioxidant capacity, observed in both clinical and experimental *diabetes mellitus* though to be the etiology of chronic diabetic complications [2]. In search of new compounds from natural sources, the insulin like and insulin releasing effect of the isolated compounds such as 4- hydroxy isoleucine have been evaluated [3]. Sheweta *et al*. [4] isolated 4- hydroxy isoleucine (4HI) from seeds of fenugreek (*Trigonella foenum graecum L.*). Garcica *et al*. [5] concluded that quinolizisine alkaloids obtained from lupinus species increase insulin secretion from beta cells, in addition to that the blockage of beta cell K (ATP) sensitive channels is at least one of mechanism involved in enhancing secretogogue. Furthermore, Hanfy and El- Shazly [6] confirmed the anti-hyperglycemic effect of aqueous suspension of lupinus termis in alloxan diabetic rats.

The aim of the present study was to investigate the effects of *Trigonella foenum-graecum* (*T.foenum-graecum*) and *Lupinus termis* (*L.Termis*) either each alone or in combination on the histopathological changes in the pancrease in alloxan induced-diabetic rats beside some biochemical profiles.

MATERIAL AND METHODS

Plant Material: Seeds of *T. Foenum-graecum* and *L- Termis* were collected from private market at Kafr El-Sheikh city. The whole seeds were crushed into fine powder using a grinding machine. The plant powder was suspended into double distilled water (a dose of 1.5 ml of aqueous suspension /100 g B.W equivalent to 75 mg / 100 g B.W for *T. foenum graecum* [7] and also used for *L-Termis*.

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Alloxan Monohydrate (Prolabo Product) B.D.H: Alloxan monohydrate was used as 2% solution in physiological saline. The solution was injected as a single dose of 110 mg / kg B.W subcutaneously within 50-75 seconds [8].

Experimental Animals: Seventy five clinically healthy male albino rats of three month age and 180 g / rat average body weight were used in present study. Rats were purchased from the National Institute of Ophthalimologic Research, Giza, Egypt. All animals were kept for acclimatization for 2 weeks under laboratory conditions and fed with pelleted food and tap water ad libitum.

Experimental Design: Rats were divided into five equal groups of 15 rats each

Group (A): was left as normal, non diabetic group.
Groups (B), (C), (D) and (E): were served as alloxan diabetic groups
Group (B): was diabetic non treated rats.
Groups (C), (D) and (E): Were daily gastric intubated with aqueous suspension of T.*foenum-graecum, L. Termis* or combination of the two plants at a dose of equivalent to 75 mg /100 g B.W for each plant seed respectively.

After 7, 14 and 21 days of treatment, five rats of each group were sacrificed four hours after dosing and two blood samples were collected. The first was taken on sodium fluoride for estimation of plasma glucose and the second was collected in plain centrifuge tube to separate serum for measurement of some serum biochemical parameters.

Assay of Biochemical Parameters: Plasma glucose level was determined according to Trinder [9], Fructoseamine in serum was estimated according to the method described by Johnson *et al*. [10], Total cholesterol was determined in serum according to Watson [11] while Triglycerides was determined according to Fossati and Principe [12].

For determination of liver glycogen, frozen liver samples prepared according to Johann and Lentini [13] were used.

Histopathological Examination: Pancreas was subjected for routine histopathological examination and fixed in 10% formal saline. Tissues were processed and embedded in paraffin wax. Sections were cut at 5µm thickness and stained with heamatoxylin and eosin [14].

For examination of glycogen storage, specimen of liver was fixed in cold absolute alcohol at-4°C, the sections were stained with PAS technique. Light microscope examination of the sections was then carried out.

Statistical Analysis: The obtained results were statistically analyzed using student (t) test according to Tamhans and Dunlop [15] to compare between normal control and different diabetic animal groups.

$P^a > 0.05$, $P^b > 0.01$, $P^c > 0.001$ as comparison to normal control Group (A),

P*>0.05, P**>0.01, P***>0.001 as comparison to diabetic control group (B).

RESULTS

A-Clinicobiochemical Results: Biochemical parameters illustrated on Tables 1, 2 and 3 described the changes in carbohydrates, lipids profiles in alloxan diabetic rats either treated with the two tested plants or non treated. The data described the degree of diabetic control via using the two tested plants when compared with normal or diabetic control groups.

Blood Glucose: Blood glucose of alloxan diabetic rat groups (B, C, D and E) were significantly increased when compared with normal control group A while blood glucose in rat groups C, D and E treated with *Trigonella foenum –graecum* (*T. foenum graecum*) and *Lupinus termis* (*L- Termis*) and / or combination showed statistically decrease when compared with Alloxan diabetic non treated rats group B Table 1, 2 and 3.

Fructosamine: Table 1, 2 and 3 described statisticaly increase in fructosamine in all animal groups when compared with normal control group A while group C,D and E illustrated significance decrease when compared with alloxan diabetic non treated group (B).

Cholestrol: Table 1, 2 and 3 described statisticaly increase in Cholestrol in all animal groups when compared with normal control group A while group C,D and E illustrated significance decrease when compared with alloxan diabetic non treated group (B).

Table 1: Some biochemical parameters in alloxan diabetic rats 7 days from beginning of the experiment (Mean ±SE)

Parameters Animals Groups	Blood glucose mg/dl	Fructoseamine mmol/L	Total cholesterol mg/dl	Triglycerides mg/dl	Liver glycogen µg/100mg
A	91.12±6.13	0.65±.14	104±2.17	86.73±2.12	378.21±6.24
B	[c] 342.11±22.13	1.35±0.08	[c] 286.13±10.15	[c] 240.91±6.13	[c] 172.16±10.11
C	[c] 212.13±8.33**	[a]1.02±0.06*	[c] 180.74±8.23***	[c] 165.12±4.06***	[c] 205.11±12.03***
D	[c] 280.27±14.41*	[b]1.24±0.04	[c] 225.16±9.12**	[c] 182.23±6.15***	[c] 165.13±13.11*
E	[c] 201.25±16.23***	0.98±0.1*	[c] 185.14±9.07***	167.18±7.13***	[c] 235.14±10.11***

P[a]>0.05, P[b]>0.01, P[c]>0.001 as comparison to normal control Group (A), P*>0.05, P**>0.01, P***>0.001 as comparison to diabetic control group (B).

Table 2: Some biochemical parameters in alloxan diabetic rats 14 days from beginning of the experiment (Mean ±SE)

Parameters Animals Groups	Blood glucose mg/dl	Fructoseamine mmol/L	Total cholesterol mg/dl	Triglycerides mg/dl	Liver glycogen µg/100mg
A	101.15±7.06	0.76±.11	98.31±3.28	94.16±2.98	360.75±12.16
B	[c] 312.04±18.17	[c] 3.65±0.08	[c] 265.13±6.84	[c] 255.16±4.13	[c] 106.17±6.13
C	[c] 223.16±19.13**	[c] 1.78±0.09***	[c] 195.24±8.76***	[c] 172.13±6.11***	[c] 264.18±8.42***
D	[c] 255.24±14.11*	[c] 1.96±0.10***	[c] 223.16±6.14**	[c] 180.11±7.14***	[c] 173.36±7.14***
E	[c] 211.28±13.12**	[c] 1.53±0.09***	[c] 230.32±7.13**	[c] 155.13±6.11***	[b] 293.16±7.97***

P[a]>0.05, P[b]>0.01, P[c]>0.001 as comparison to normal control Group (A), P*>0.05, P**>0.01, P***>0.001 as comparison to diabetic control group (B).

Table 3: Some biochemical parameters in alloxan diabetic rats 21 days from beginning of the experiment (Mean ±SE)

Parameters Animals Groups	Blood glucose mg/dl	Fructoseamine mmol/L	Total cholesterol mg/dl	Triglycerides mg/dl	Liver glycogen µg/100mg
A	86.14±4.93	0. 56±.08	88.11±7.13	96. 05±8.41	406.33±20.11
B	[c] 280.15±16.03	[c] 3.98±0.12	[c] 242.66±9.79	[c] 263.91±7.03	[c] 143.92±8.71
C	[c] 195.14±11.03**	[c] 1.62±0.06***	[c] 155.14±8.24***	[c] 185.11±6.11***	[c] 270.16±10.06***
D	[c] 223.19±12.43*	[c] 1.77±0.08***	[c] 187.16±7.32***	[c] 213.27±9.13***	[c] 218.11±13.14***
E	[c] 203.22±9.06**	[c] 1.46±0.09***	[c] 160.13±8.14***	[c] 206.11±6.11***	[c] 258.16±14.07***

Pa>0.05, Pb>0.01, Pc>0.001 as comparison to normal control Group (A), P*>0.05, P**>0.01, P***>0.001 as comparison to diabetic control group (B).

Triglycerides: Table 1, 2 and 3 described statistical increase in Triglycerides in all animal groups when compared with normal control group A while group C,D and E illustrated significance decrease when compared with alloxan diabetic non treated group (B).

Liver Glycogen: Table 1, 2 and 3 showed statisticaly decrease in Liver glycogen in animal group B, C, D and E when compared with normal control group A while liver glycogen in group C, D and E statistically increased when compared with alloxan diabetic non treated group (B).

Histopathological Results

Pancrease

Group A: Histology of the islets of langerhans of normal control rats which sacrificed on 7th, 14th and 21th days showed no pathological changes. The exocrine pancreatic tissue composed of acini with draining ductules, the endocrine components were found as nodules within the substance of exocrine pancreas (Fig. 1).

Group B: After 7 days, pancreatic tissue of rats which treated with single dose of alloxan subcutaneously showed minimal pathological changes except some rats showed extravasated blood in exocrine pancreatic tissue (Fig.2).

After 14 days, pancreatic sections of some sacrificed rats showed vacuolar changes (Fig.3) with pycknosis of some nuclei of cells in the islets of Langerhans, some areas revealed atrophy of the islets with pycknotic nuclei (Fig.4).

After 21 days, the islets of angerhans from the pancreas of diabetic control group revealed advanced changes of diabetes as destruction of beta cells with pycknosis of nuclei. There was distortion of cells and reticular changes of islets as evidence of fibrosis (Fig.5).

Group C: Histological examination of pancreatic tissue in diabetic animals treated with *T.foenum-graecum* at dose of 75 mg/100 g B.W. did not show improvement after 7 days in which there was vacuolar changes with pycknosis of nuclei of some cells in the islets of Langerhans.

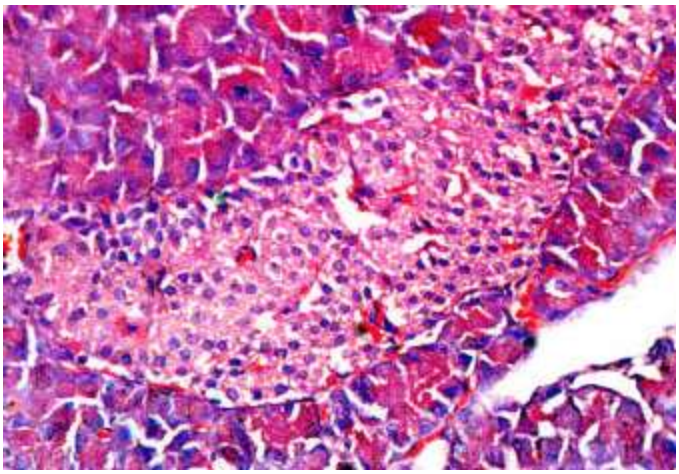

Fig. 1: Pancreas (GA), showing no pathological changes. The exocrine pancreatic tissue composed of acini with draining ductules, the endocrine component is found as a nodule within the substance of exocrine pancreas. (H&E)(10X20).

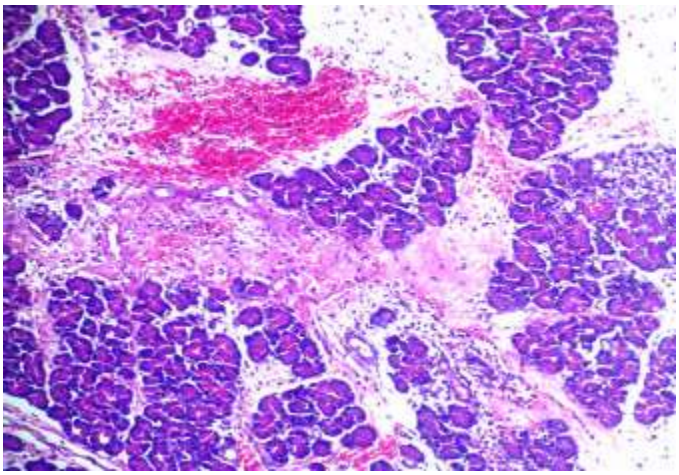

Fig. 2: Pancreas (GB), after 7 days: showing extravasated blood in exocrine pancreatic tissue (H&E) (10X10).

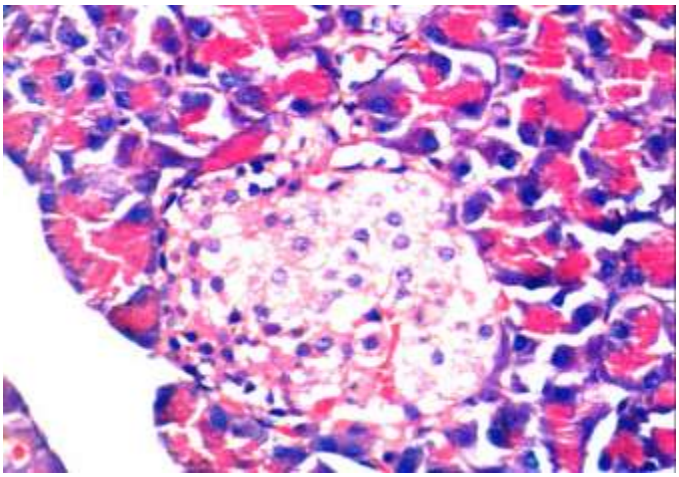

Fig. 3: Pancreas (GB), after 14 days: showing vacuolar changes with pycknosis of some nuclei of cells in the islet of Langerhans (H&E) (10X40).

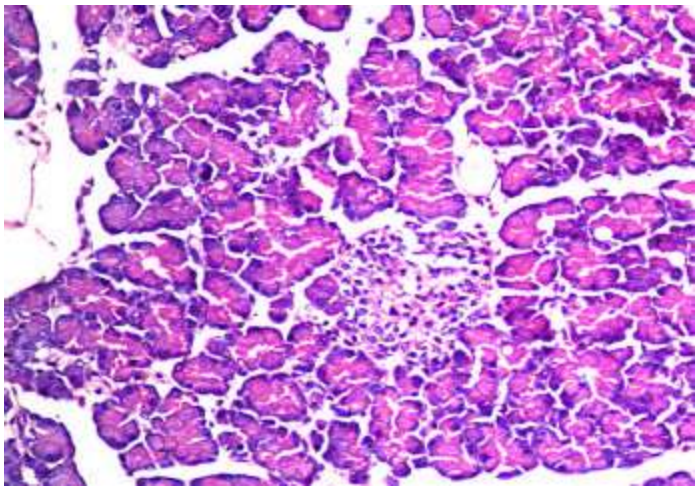

Fig. 4: Pancreas (GB), after 14 days: showing atrophoid islet with pycknotic nuclei (H&E) (10X20).

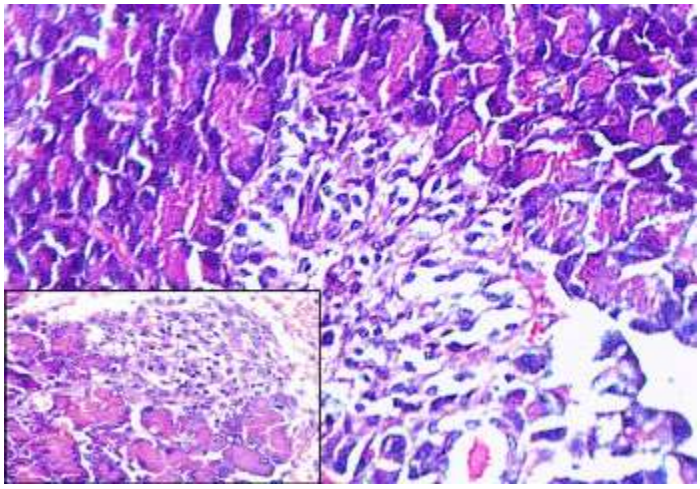

Fig. 5: Pancreas (GB), after 21 Days: showing advanced changes of diabetes as destruction of beta cells with pycknosis of nuclei. Observe distortion of cells and reticular changes of islets as evidence of fibrosis (H&E) (10X20). Higher inset, showing advanced changes of diabetes as destruction of beta cells with pycknosis of nuclei. (H&E) (10X40).

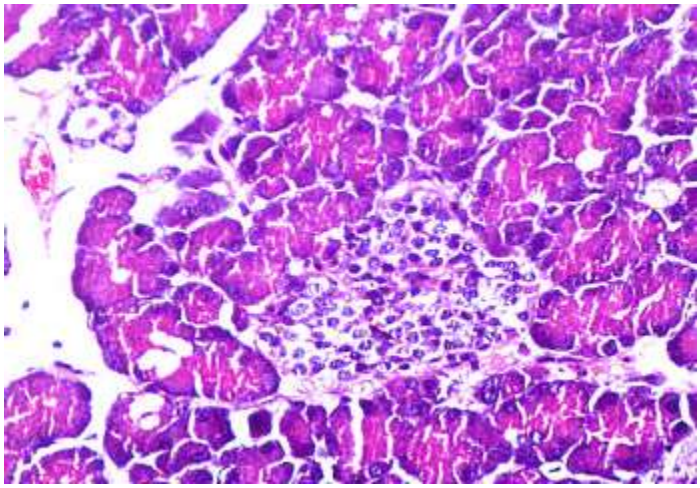

Fig. 6: Pancreas (GC), after 21 days: showing more or less improvement or restoration of normal cellular population size of islets (H&E) (10X20).

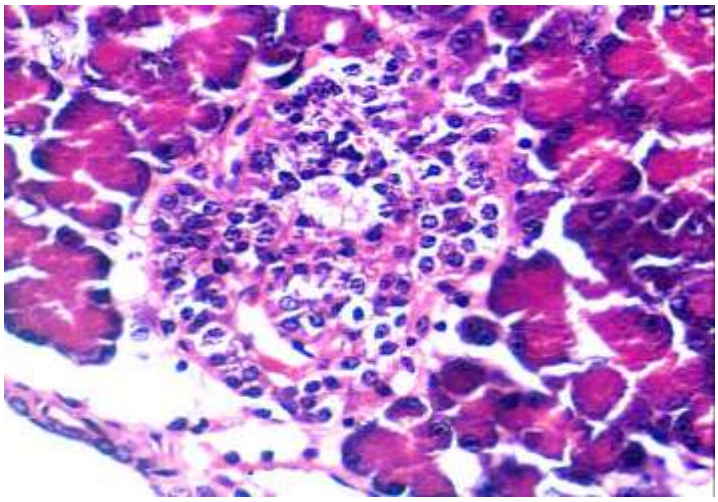

Fig. 7: Pancreas (GD), after 21 days: showing slight degenerative changes (H&E) (10X40).

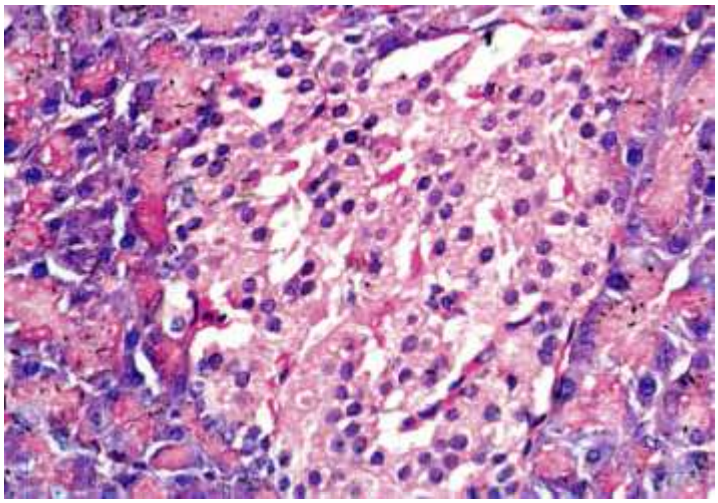

Fig. 8: Pancreas (GD), after 21 days: showing marked improvement with nearly normal islets of Langerhans (H&E) (10X40).

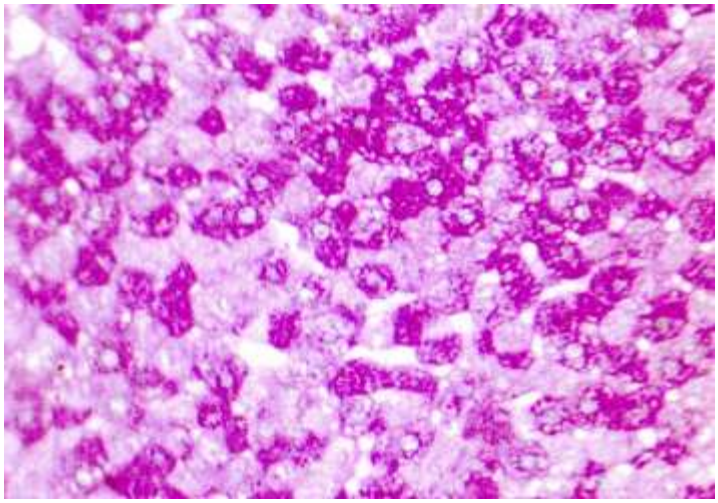

Fig. 9: Liver (GA): showing abundance of glycogen in the form of purple red granules in the cytoplasm of hepatic cells (PAS stain) (10X20).

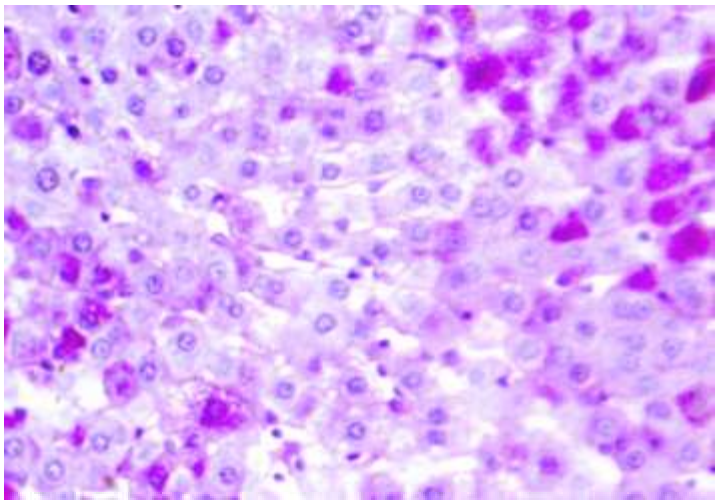

Fig. 10: Liver (GB), after 21 days: showing marked depletion of glycogen (PAS stain) (10X20).

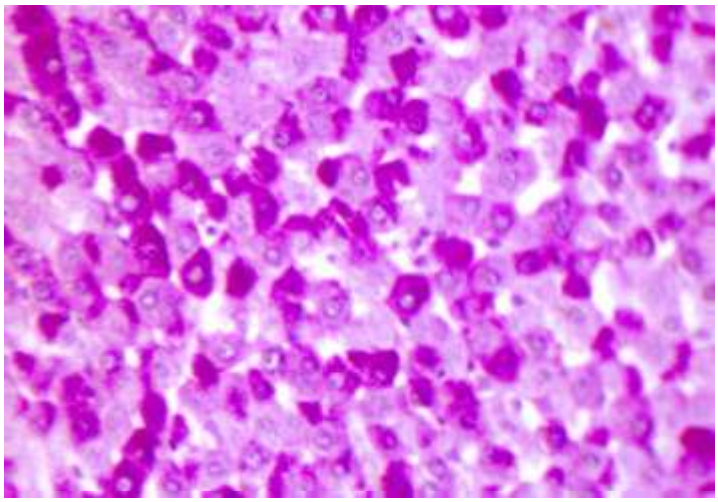

Fig. 11: Liver (GC), after 21 days: showing increased glycogen in the hepatic cells (PAS stain) (10X20).

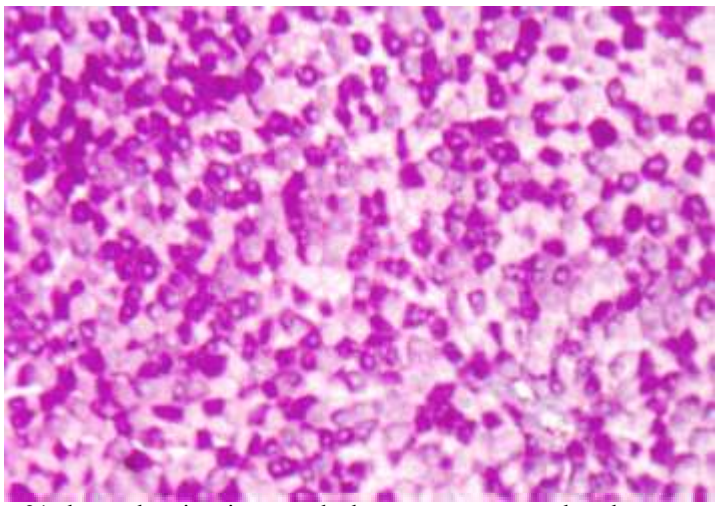

Fig. 12: Liver (GE) after 21 days: showing increased glycogen storage to be almost near to normal (PAS stain) (10X20).

After 14 and 21 days, there was more or less improvement or restoration of normal cellular population size of islets (Fig.6).

Group D: Histopathological finding of pancreas of diabetic animals treated with *L. Termis a*t a dose of 75 mg/100 g B.W revealed no improvement after 7, or 14 days. After 21 days, the islets showed slight vacuolar degeneration and in some cases, there was marked improvement in islets structure (Fig.7).

Group E: Histopathological study of pancreatic tissues in diabetic rats treated with combination of *T.foenum-graecum* and *L. Termis* was carried after 7, 14 and 21 days. The histopathological examination revealed slight improvement after 7 days.

After 14 and 21 days there was marked improvement and the islets of Langerhans was somewhat returned to normal (Fig.8).

Liver: The intensity of glycogen localization was examined in all groups of the experiment.

Group A: Examination of liver sections of control rats stained with periodic acid schiff's (PAS) Technique showed abundance of glycogen in the form of purple red granules in the cytoplasm of hepatic cells (Fig.9).

Group B: In diabetic animals, there was a slight decrease in glycogen storage in hepatic cells after 7 days. After 14 and 21 days there was a marked depletion of glycogen in hepatic cells (Fig.10).

Group C: In diabetic group of animals treated with *T.foenum-graecum*, there was a slight elevation in glycogen storage in hepatic cells. After 14, 21 days, the intensity of glycogen storage was increased as there was moderate to high content of glycogen (Fig.11).

Group D: In diabetic group of animals treated with *L-Termis*, the degree of storage of glycogen in liver was similar to group C.

Group E: The histological examination of hepatic cells for presence of glycogen in diabetic group of animals treated with combination of *T.foenum-graecum* and *L. Termis* revealed slight elevation after 7 days. After 14, 21 days the intensity of glycogen storage clearly increased to be almost near to the normal (Fig.12).

DISCUSSION

Several previous studies were undertaken to understand the mechanism underlying the effects of some plants which have action on the blood glucose. These plants may act on blood glucose through different mechanisms, some of them may have insulin like substances [16], some may increase insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes [17] and other may increase beta cells in the pancreas by activating regeneration of these cells [18].

In the present study, histopathological examination of pancreas of alloxan induced diabetic rats revealed destruction of beta cells and reticular changes of islets as evidence of fibrosis. This result was in agreement with those of many previous investigations [9- 21]. After 7 days treatment with *T.foenum-graecum* at a dose of 75 mg /100g B.W no noticeable improvement in changes induced by alloxan injection whereas there was vacuolar changes with pycknosis of nuclei of some cells in the islets of Langerhans however, after 14, 21 days of treatment, islets of Langerhans showed improvement whereas there was restoration of normal cellular population size of islets. These results are corroborative with Babuk *et al*. [22] and Elder [23] who reported that treatment of alloxan - induced diabetic rats with *T.foenum-graecum* resulted in hypoglycemia and improvement in the picture of pancreas.

Extensive work has been undertaken to work out the mechanism by which *T.foenum-graecum* could be exerting its effect. It may exert its therapeutic effect thought its alkaloids content by modulation of insulin secretion. Madar and Thorne [24] attributed it to dietary fibers present in the *T.foenum-graecum* which help in the management of metabolic abnormalities associated with diabetes such as peripheral insulin resistance and lipid abnormalities.

Petit *et al*. [25] and Yoshikawa *et al*. [26] reported the isolation of furostanol saponins called trigoneoside Ia,Ib,IIIa,IIIb; glycosides and trifoenoside A. they claimed that these saponine are the active principles owing to their hypoglycemic effects. Furthermore, Sauvaire *et al*. [27] and Borca *et al*. [28] have demonstrated evidence of insulinotropic and antidiabetic of 4-hydroxy isoleucine isolated from *T.foenum-graecum* seeds in glucose-dependant manner. They suggested that antidiabetic effect of 4-hydroxy isoleucine was at least in part, from direct pancreatic beta cell stimulation.

L-Termis are highly valued animal feed and it has hypoglycemic effect. The histopathological finding of diabetic pancreas treated with *L-Termis* showed marked improvement after 21days and minimal pathological changes. Our results are coincided with Hall *et al*. [29] who attributed the hypoglycemic effect of L.termis may have been a result of higher protein content stimulating a higher insulin response, in addition to the actions of various of phytochemicals and dietary fibere found in *L-Termis*. Another suggest mechanisms that *L-Termis* has pronounced hypoglycemic effect were reported by Newairy *et al*. [7], Mohamed and El- Shorbagi [30] and Knecht *et al*. [31] who found that *L-Termis* contain saponin,alkaloids and Tannin. Saponins have hypoglycemic activity which may be due to inhibition of liver glycogenesis or glycolysis [32].

In the present study, the marked improvement which obtained from alloxan diabetic rats which treated with *T.foenum-graecum* and *L-Termis* may be due to preventing the death of beta cells and /or helped in recovery of partially destroyed beta cells. It may be that, the activities of these plants have triggered the beta cells to increase insulin production which promotes glucose uptake and utilization by other tissues.

In the present study, there was marked depletion in glycogen storage in hepatic cells of diabetic rats. This observation is in accordance with the finding of Kader *et al*. [33] and Sharma *et al*. [34] who reported that the liver glycogen is drastically reduced in diabetic group and Jambolana seeds helped in increasing the glycogen content, but not equivalent to that observed in nondiabetic control animals. Also, Nirmala *et al*. [35] confirmed this finding, they reported that during diabetes the liver shows decrease in weight due to enhanced catabolic process such as glycogenolysis, lipolysis and proteolysis, which are the outcomes of lack of insulin and cellular glucose in liver cells. The obtained histopathological findings correlated with the biochemical determination of different animal groups treated with the tested plants and supporting the foregoing assumptions; Insulin is the main regulator of glycogenesis in the liver and muscles. The decrease of liver glycogen level observed in this study may be due to lack of insulin in diabetic rats or oxidative stress by diabetes may inactive the oxygen synthase. This result is in agreement with what recorded by Surjet *et al*. [36] who noted marked reduction in liver and muscle glycogen level (21days) in streptozotocin induced diabetic animals.

In diabetic rats which treated with either *T.foenum-graecum* or *L-Termis*, there was increasing in glycogen storage, especially after 21days if compared with diabetic non treated animals. The increased storage of glycogen in diabetic rats which treated with plant may be attributed to that the seeds of *T.foenum-graecum* decreased the activity of the key enzymes of glycolysis beside slight inhibition in the activity of gluconeogenic enzymes [37].

There were significant decrease in plasma glucose level in alloxan diabetic rats treated with *T.foenum-graecum* and *L-Termis* either each alone or in combination along the entire period of the experiment This finding was confirmed by Mohamed and El- Shorbagi [30]. They reported that the lupine seed powder produced a pronounced hypoglycemic effect in diabetic rats. Newairy *et al*. [7] and Knecht *et al*. [31] found that L.Termis contains saponin, alkaloids and tannin. Saponins have hypoglycemic activity may be due to inhibition of liver gluconeogenesis or glycolysis [32]. The hypoglycemic effect of *T.Foenum graecum* seeds powder may be due to presence of steroid saponin and this agree with that obtained by Valette *et al*. [38] who attributed the hypoglycemic activity of T.Foenum greacum seeds to the presence of saponin and proteins or may be due to the intestinal glycosidase inhibition [39].

As regard to fructoseamine in the present work, it was significantly decreased in diabetic rats treated with *T.foenum-graecum* and L.Termis. This result may be due to the decrease of glucose level as reported by Kaneko *et al*. [40] who mentioned that fructoseamine was evaluated as an index of glycemic control in normal and diabetic rats.

Lipids disorders are common in (DM) and play crucial roles on the development of diabetic cardiovascular complications. Diabetic dyslipidemia is characterized by hypertriglyceridemia, increased levels of very low density lipoproteins (VLDL) and low density lipoproteins [41].

Concerning lipid profile illustrated in tables 1-3 There were significant decrease in serum cholesterol Level and triglycerides of alloxan diabetic rats groups either treated with *T.foenum-graecum* or L.Termis and/or their combination was coincided with those obtained by Shalabi *et al*. [42] who reported that L.Termis seeds have hypocholesterolemic effect in diabetic rats. The decrease in cholesterol of *T.Foenum graecum* seeds had significantly lower level of serum cholesterol [43]. The hypocholesterolemic effect of T.Foenum greacum seeds which increase biliary cholesterol excretion could be

attributed to saponin content of the seeds or interference with cholesterol biosynthesis in the liver [25]. The obtained results come in accordance with Babuk *et al*. [22] who found that the hypolipidemic effect of fenugreek extract was more pronounced at the higher dose 400 mg/kg.

The lipids lowering effect of fenugreek might also be attributed to its estrogenic constituent, indirectly increasing thyroid hormone T4 [44].

In conclusions, Administration of *T.foenum-graecum* and L.Termis either each separate or in combination alleviated the alloxan monohydrate' lesions in all the experimental rat groups. Beside the two tested plants corrected for certain extent carbohydrate and lipid profiles resulted from diabetogenic effect of alloxan, the administration of plant combination added no synergism over using every plant singly.

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