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Histopathological Changes of Pancreatic Tissues in Hyperglycemic Male Rats Treated with Mixture of Plants Extracts

Maysaa' Adil Hadi, Haider Kamil Zaidan, Ali Hmood Al-Saadi

Department of Biology, College of Science, University of Babylon/ Hilla-Iraq

Abstract : The present study aims to evaluate the hypoglycemic, and protective effects of mixture of methanol-watery extracts of five selective medicinal plants: *Trigonella faenum-graecum* seeds, *Nigella sativa* seeds, *Zingiber officinale* rhizomes, *Olea europeae* leaves and *Fraxinus ssp.* seeds, and determine the efficacy of this mixture in the treatment of diabetes mellitus type 2.

Animals were randomly divided into six groups: group I: normal negative control, group II: diabetic control, group III: normal rats treated with mixture of plants extracts for 60 days, group IV, V, VI: diabetic rats treated with mixture of plants extracts for 45, 60, 75 days respectively.

The results revealed significant decrease ($P<0.05$) in body weight of the diabetic rats, diabetic rats treated with mixture of plants extracts for 45, 60 days as compared with normal control, and significant increase ($P<0.05$) in the diabetic rats treated for 75 days as compared with the diabetic control, while normal rats treated with mixture of plants extracts for 60 days which showed significant decrease ($P<0.05$) as compared with normal control, but it was considered significant increase as compared with diabetic rats. The result of fasting blood glucose levels showed significant decrease ($P<0.05$) in all treated groups as compared with diabetic control. On the other hand, significant decrease ($P<0.05$) was shown in serum insulin levels and pancreas/body weight ratios in diabetic group as compare with negative control while treatment with mixture of plants extracts for different periods caused non significant differences in all treatment periods as compared with negative control and returned pancreas/body weight ratio near the normal value. Treatment of normal rats with mixture of plants extracts for 60 days caused non significant differences in fasting blood glucose, insulin and pancreas / body weight ratio.

Histological sections of diabetic pancreas revealed degeneration, vacuolization of the islets of Langerhans and the exocrine pancreas manifested inflammatory cells infiltration, and vascular congestion, while treated groups exhibited normal appearance of islets of Langerhans especially β-cells and pancreatic acini and some pancreatic sections showed with inflammatory cells infiltration.

In conclusion, type II DM caused histopathological changes in pancreas, many of these changes could be prevented or reduced by using mixture of plants extracts used in this study. The effect of this mixture had more positive effects when given orally for longer period (75 days).

Keywords : Histopathology, Pancreas, Hyperglycemic Rats, Plants Extracts.

Introduction

Diabetes mellitus (DM) is a common heterogeneous metabolic disorder of multiple causes characterized by chronic hyperglycemia and disorders of carbohydrate, fat and protein metabolism associated with defect in insulin secretion (type 1) or resistance to insulin action (type 2)[1] with micro and macro vascular complications that results in significant morbidity and mortality in the world[2]. DM is a multifactorial disease involving interaction of genetic and environmental factors[3-6]. It is caused by inherited and/or acquired deficiency in the production of insulin by the β-cells of pancreas, or by ineffectiveness of insulin produced (an absolute or relative insulin deficiency)[7]. Hyperglycemia and hyperlipidemia thereafter exert additional damaging or toxic effect on the β-cell[3].

Several pathogenic processes are involved in the development of DM. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action and becomes insufficient to control systemic glucose levels[8].

Diabetic patients are at risk of the micro and microvascular complications through the body[9,10]. The microvascular complications, include nephropathy, retinopathy, neuropathy, and macrovascular complications including heart disease and stroke[9]. Diabetes has been associated with reproductive impairment in both men and women[10]. The mild thyroid dysfunction in DMT2 was linked to significantly changes in body weight ,lipid profile and likely represents risk factor for healthy and obesity[11].

Although insulin treatment and other chemical therapies can control many aspects of diabetes, numerous complications are common in the disease[12,13]. As continuous oral administration of insulin can culminate in many side effects and toxicity[14]. Also, the use of biguanides, sulphonylurea and other drugs are valuable in the treatment of diabetes mellitus; their use, however, is restricted by their limited action, pharmacokinetic properties, secondary failure rates and side effects[15].

Recently, attention has been paid to the search of effective drugs in the field of traditional medicine[10]. Medicinal plants are frequently considered to be less toxic and free from side effects than the synthetic ones[16]. This study was designed to use the mixture of plant extracts of five medicinal plants to treatment of DM and aimed to evaluate the possible hypoglycemic, protective effect and determine the efficiency of this mixture in the treatment of type 2 DM which may occur against harmful effect and damage in pancreatic tissues of alloxan-induced diabetes in rats.

2. Materials and methods

2.1: Preparation of solutions[17].

2.1.1: Formalin fixative(10%): Each 1ml of formalin 40% added to 9 ml of tap water.

2.1.2: Haematoxylin-Harris stain: The solution was prepared by dissolving 1g of haematoxylin in 10 ml ethyl alcohol. 20g of potassium alum dissolved in 200 ml of DW and then boiled. Haematoxylin then added and the solution boiled for ½ minutes. 0.5g of mercuric oxide added. The solution cooled rapidly and a few drops of acetic acid were added ; it is optional but its inclusion gives more precise and selective staining of nuclei.

2.1.3: Eosin stain: Prepared by mixing 1 gm of eosin Y in 99 ml ethanol.

2.1.4: Acid alcohol 1%: Prepared by mixing 1ml of HCl in 100 ml of 70% ethanol.

2.2: Plants collection and identification

The plants used in this study were purchased from a local herbal markets except olive leaves were collected from gardens of Babylon university. The plants were identified by Plant Harbarium /College of Science/ university of Babylon. The plants parts used in this study were *Trigonella faenum-graecum* seeds, *Nigella sativa* seeds, *Zingiber officinale* rhizomes, *Olea europeae* leaves, and *Fraxinus ssp.*seeds. Oilve leaves were rinsed with water to remove dust, insecticides, and contaminated materials then dried in dark. All plants materials were grained into fine powder.

2.3: Preparation of plant extracts: The plants extracts were prepared according to Sato *et al*. (1990). The plant powder was extracted with mixture of methanol and distilled water in a ratio of 20 % methanol: 80% distilled water (V/V) in average of 1 gm of plant powder :3 gm of mixture using blender for 30 min at room temperature. The suspension were filtered by guase and the filtrate concentrated in oven at 45 ^{o}C. The crude extracts were stored at 4 ^{o}C until use.

2.4: Animals of Experiments: Adult albino male rats aged 2-3 months were used in this study. The overall number of animals used was 36. The animals were provided with food and water *ad libitum*. After adaptation animals were used for experimental studies. After the induction of T2 DM, the animals were divided into different groups that included 6 animals in each group. Some of diabetic rats died without treatment.

2.5: Detection of plants extracts doses:

Treatment by mixture started by giving different oral doses of plants extracts (100-1000 mg/kg body weight) to normal and diabetic rats. The treatment performed by mixing 0.5 ml of each plant extract immediately and administrated orally by orogastric tube.

2.6: Induction of type 2 DM:

Diabetes was induced by injecting the animals with 3 doses of alloxan 120 mg/kg dissolved in 0.5 ml normal saline immediately for the induction of T2DM. Fastig blood glucose level (FBG) of fasting rats were measured weekly by using the glucometer and rats with FBG > 200 mg /dl were considered diabetic and used in this study[14].

2.7: Experimental design of the study: Rats were randomly divided into six groups:

1- Normal Control group(Negative control): Included healthy intact animals which were given normal saline intraperitonialy (i.p.) and distilled water orally by orogastric tube.

2- Diabetic control group: Included animals which had been given alloxan i.p. and distilled water orally but did not treated with mixture of plants extracts (35 days).

3- Normal group treated with mixture of plants extracts: Included healthy intact animals treated orally with mixture of plant extracts only for 60 days.

4- Treatment groups: Included 18 rats. They were treated with alloxan i.p. and then, after the induction of DM, they treated orally with mixture of plants extracts. These diabetic treated animals were subdivided into three subgroups (n=6) treated for 45, 60 and 75 days.

Through the study period glucose and body weight of animals were measured. The percentage of weight changes was calculated according to the formula:

$$\frac{\text{Initial body weight}}{\text{Final body weight} - \text{Initial body weight}} \times 100$$

2.8: Blood sampling:

Through the course of the study, blood was obtained by puncturing the caudal vein by a sterile needle, then a blood drop was put in contact with the strip of glucometer to measure the blood glucose level. After one week from the end of experiment, the animals were sacrificed and blood was collected directly by heart puncture. Also, pancreas were removed and weighted to calculate pancreas/ body weight ratio by using the formula:

$$\text{Organ /body weight ratio (\%)} = \frac{\text{Organ weight (gm)} \times 100}{\text{Body weight after experiment (gm)}}$$

2.9: Serum insulin measurement:

Insulin level (ng/dl) was measured according to Rat Insulin Elisa Kit (Cosabio Biotech Co.)

2.10: Histological processing and staining:

Ordinary histological processing are prepared for pancreas in order to study the histopathological changes. The pancreas was harvested and trimmed of fat. Its macroscopic appearance was recorded and the gland was fixed in 10% formaldehyde. Dehydration, clearing, embedding in paraffin, sectioning at 5 µ and stained with hematoxylin and eosin according to Bancroft and Steven (1982) was carried out.

2.11: Statistical analysis: Analysis of data was performed by using Statistical Package for Social Science (SPSS) system/ version 17. Results expressed as mean ± S.E .The analysis of variance (ANOVA) and the paired sample T- test were used for this purpose.

3. Results

3.1: Body Weight: Results of this study showed significant decrease ($P<0.05$) in the body weight of diabetic group from (212.67 ± 16.65) gm to (187.67± 14.03) gm compared to negative control which increased from (216 ± 8.103) gm to (255 ± 7.302) gm. In normal group administrated with mixture of plants extracts for 60 days, there was a little increase in body weight which statistically considered as significant decrease ($P<0.05$) in the body weight from (305 ± 5.477) gm to(312.33 ± 7.60) gm compared to negative control. Diabetic groups treated with mixture of plants extracts registered a body weight gain with increasing period of treatment. Diabetic group treated with mixture of plants extracts for 45 days still had significant decrease in their body weight from (299 ± 35.704) gm to (280 ±29.828) gm when compared to negative control and non significant difference as compared to diabetic control, but there was significant increase ($P<0.05$) in the weight gain from (329.33 ± 4.77) gm to (333. 66 ± 6.296) gm and from (323 ± 2.19) gm to (354 ± 7.669) gm in diabetic groups treated with mixture of plants extracts for 60 and 75 days respectively when compared to diabetic control. Diabetic group treated for 75 days could revert to normal body weight gain as in negative control as shown in Table 1.

Table (1): Changes of body weight in experimental rats

Weight change	Body weight (mean ± S.E)		Group
	Final Body weight (gm)	Initial Body weight (gm)	
39 a	255 ± 7.302	216 ± 8.103	Negative control
-25 b	187.67± 14.030	212.67 ± 16.650	Diabetic control
7 c	312.33±7.600	305 ± 5.477	Plant extracts mixture (60 days)
-19 bcd	280 ± 29.828	299 ± 35.700	DM + Plants extracts mixture (45 days)
4 ce	333. 66 ± 6.296	329.33 ± 4.770	DM + Plants extracts mixture(60 days)
31 a	354 ± 7.669	323 ± 2.191	DM + Plants extracts mixture (75 days)

Different letters refer to significant difference between groups
Similar letters refer to non significant difference.
n= 6 for each group S.E :Standard error

3.2: **Fasting blood glucose (FBG) & fasting serum insulin**

The FBG levels in negative control and all experimental groups were analyzed (Table 2) and variation of FBG was observed (Figure 2). Alloxan injection caused significant increase ($p<0.05$) in mean of FBG levels (471 ± 37.98) mg/dl as compared to negative control (124 ± 4.219) mg/dl. Furthermore, there was decrease in

mean of FBG levels but was non significant in normal rats treated with mixture of plants extracts for 60 days as compared to negative control while this group had significant reduction in mean of FBG level (79.66± 4.168) mg/dl as compared to diabetic control. Diabetic groups which were treated with mixture of plants extracts for 45, 60, 75 days showed significant reduction ($p<0.05$) in mean of FBG levels as compared to diabetic control and could revert FBG to normal value in this groups compared to negative control.

Also, there was no significant differences in means of insulin level (0.193 ± 0.012) ng/dl in normal rats treated with mixture of plants extracts for 60 days as compared to negative control while the same group caused non significant increase in insulin level as compared to diabetic control (Table 2).

Serum level of insulin was significantly reduced (0.162 ± 0.003) ng/dl in alloxan –induced diabetic rats compared to negative control (0.303 ± 0.072) ng/dl. This alteration was ameliorated by administration of mixture of plants extracts which caused significant increase ($p<0.05$) in insulin level (0.4± 0.073) ng/ dl and (0.43 ± 0.11) ng/ dl in diabetic rats treated with mixture of plants extracts for 45 and 60 days, respectively. The 75 days treatment caused preservation of insulin level near normal value as compared to negative control (Table 2).

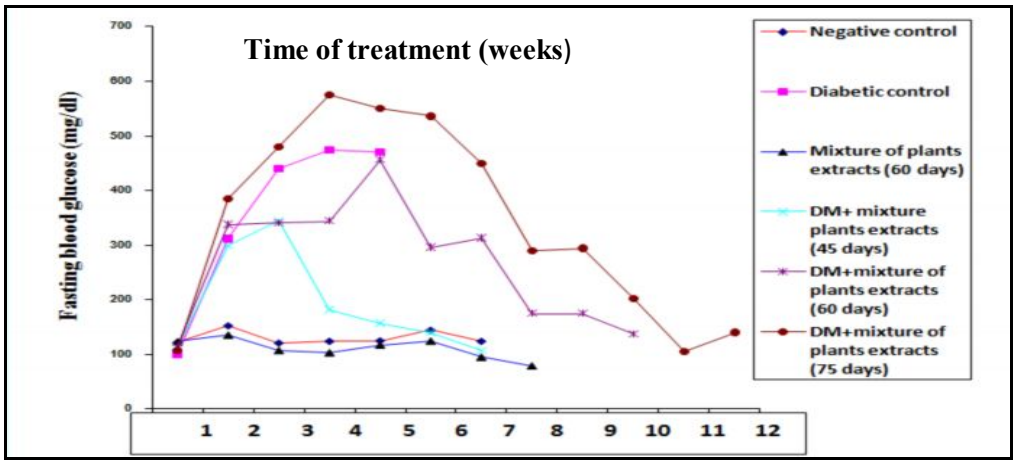

Figure (2): Variation in fasting blood glucose (mg/dL) of experimental rats.

Table (2): Changes of FBG (mg/dL)& Fasting serum insulin (mg/dL) in experimental rats (mean ±S.E).

Fasting serum insulin (ng/dL)	FBG (mg/dL)	Group
0.303 ± 0.07 a	124 ± 4.22 a	Negative control
0.162 ±0 .003 b	471 ± 37.9 b	Diabetic control
0.193 ± 0.012 abc	79.66 ± 4.17 ac	Mixture of plants extracts (60 days)
0.4 ± 0.07 a	107 ± 2.56 acd	DM + Mixture of plants extracts (45 days)
0.43 ± 0.11 a	137.66 ± 12.08 ad	DM + Mixture of plants extracts (60 days)
0.31 ± 0.01 abc	140 ± 5.84 ad	DM + Mixture of plants extracts (75 days)

Different letters refer to significant difference between groups
Similar letters refer to non significant difference
n=6 for each group S.E: Standard error

3.3: Pancreas / body weight ratio

As shown in Table 3, injection of alloxan markedly decreased tissue weight of pancreas and caused significant decrease ($p<0.05$) in pancreas / body weight ratio (0.330 ± 0.01) % as compared to negative control (0.905 ± 0.158) %. Although mixture of plants extracts caused non- significant difference in pancreas / body

weight ratio in normal rats as compared to normal control, yet it could return pancreas / body weight ratio near normal value in treated groups and caused significant increasing ($p<0.05$) in pancreas / body weight ratio (0.778 ± 0.034) % and (0.856 ± 0.097) % in diabetic rats treated with mixture of plants extracts for 45 and 60 days, respectively and non- significant increasing in diabetic rats treated for 75 day as compared to diabetic control.

Table (3): Changes of pancreas / body weight ratios in experimental rats (mean ± S.E).

pancreas / body weight ratio	Group
0.905 ± 0.16 a	Negative control
0.330 ± 0.01 b	Diabetic control
0.775 ± 0.07 ac	Mixture of plants extracts (60 days)
0.778 ± 0.03 ac	DM + Mixture of plants extracts (45 days)
0.856 ± 0.09 ac	DM + Mixture of plants extracts (60 days)
0.728 ± 0.07 ac	DM + Mixture of plants extracts (75 days)

Different letters refer to significant difference between groups
Similar letters refer to non significant difference
n=6 each group , S.E: Standard error

3.4:Histopathological study of Pancreas

Histological section of the negative control group showed that the pancreas composed of the hormone –producing cells of the pancreas which is islet of Langerhans containing several secretory cell types and exocrine part composed of acini as seen in Figure (3 A). The pancreata of the normal group treated with mixture of plants extracts for 60 days had normal islets and normal acini (Figure 3 B).

Histologically, some changes of the pancreata of experimental rats have been observed in sections stained hematoxylin /eosin stain. In diabetic control, there was degenerative changes in both exocrine and endocrine pancreas. The most characteristic changes of the endocrine pancreas was a decrease in the size and number of pancreatic islets, abnormal appearance of many islets which had less number of cells compared to islets of negative control group. Islets consisted of many islet cells with vacuolated or shrunken cytoplasm indicating the degranulation of the islet cells (Figures 4 A&B). Also, another islets of Langerhans had severe damage with presence of inflammatory cells and fibroblast in islets (Figure 4C). Exocrine pancreas had moderate vascular congestion and degenerative acini. The presence of a few lymphocyte between acini and connective tissue in the pancreata of the diabetic rats was observed (Figures 4 D&E). Diabetic groups which were treated with mixture of plants extracts for different periods (45, 60, 75 days respectively) showed regeneration of both islets and acini and had less number of inflammatory cells. These groups histologically had normal architecture of many islets and acini (Figures 5 A). Few islet still had few vascular congestion (Figures 5 B) and degeneration (Figure 5 C&D) while other islets showed a bigining of regeneration (Figures 5 E). Also, some islets showed presence of a few inflammatory cells at boundaries of islets (Figure 5F).

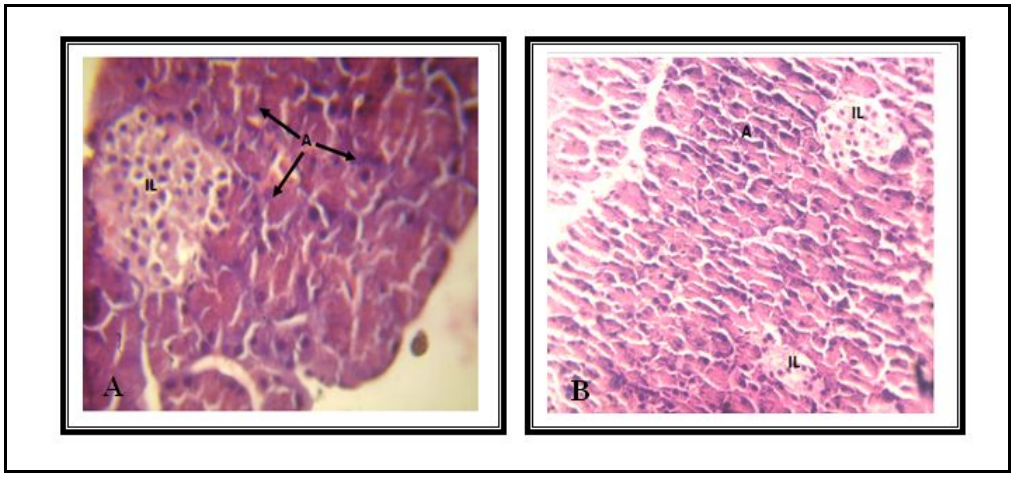

Figure (3): Sections of pancreatic tissues show: A- Negative control group shows normal islets of Langerhans (IL) and normal acini (A) H&E (100X). B- Normal group treated with plants extracts mixture for 60 days shows normal islets of Langerhans (IL) and acini (A) H&E(40X).

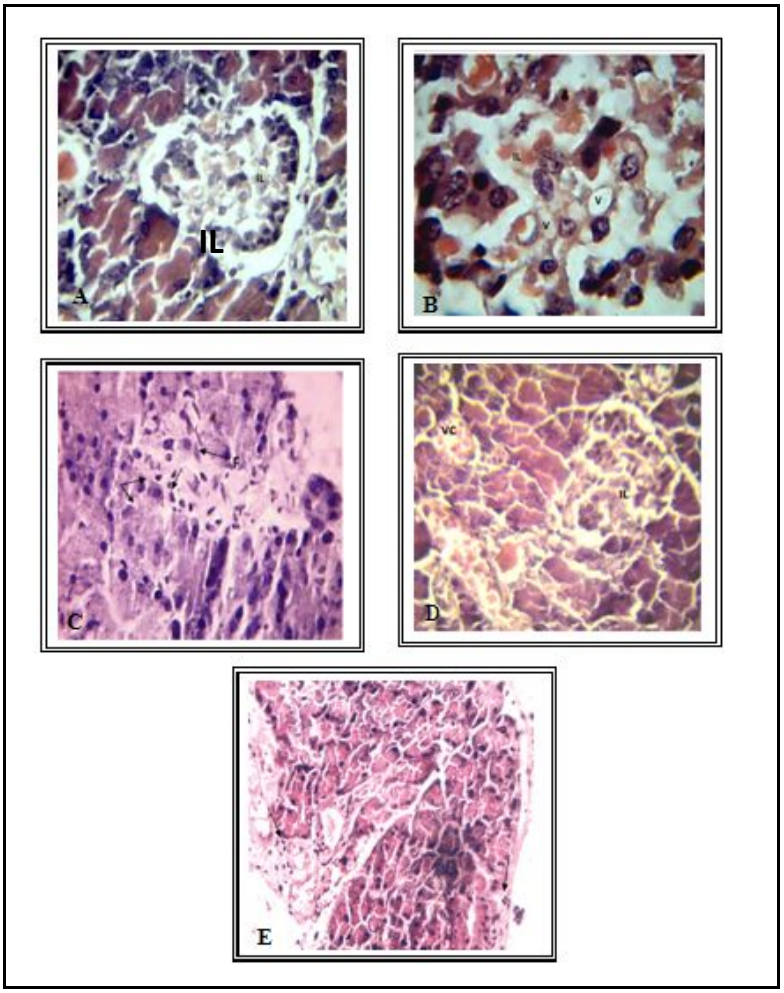

Figure 4: Sections in pancreatic tissues of diabetic group show :A & B: Degenerative changes of islets of Langerhans (IL) and vacuolation of cytoplasm (V). H&E (400X&1000X). C- Islet of Langerhans (IL) with sever damage and presence of inflammatory cells (→) and fibroblast (F) in islets. H&E (400X). D-Degenerated islet of Langerhans (IL) with presence of vascular congestion (VC). H & E (400X). E-Degenerated acini with presence of inflammatory cells between acini and connective tissue. H&E (100X).

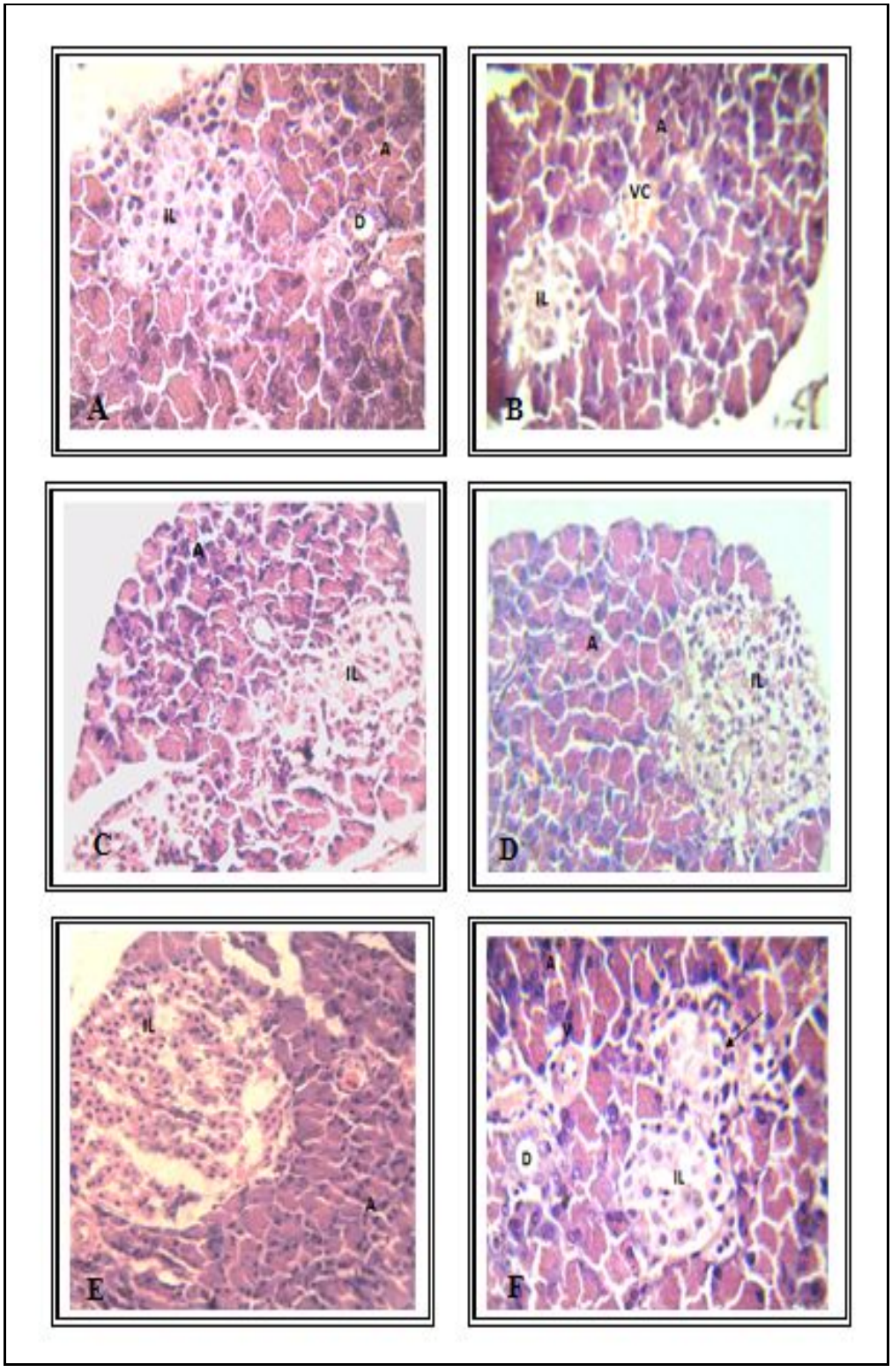

Figure 5: Sections in pancreatic tissues of diabetic group treated with mixture of plants extracts: A: Treatment for 45 days showed normal islets of Langerhans (IL) and acini (A), Duct (D). B: Few vascular congestion (VC). C&D: Some degenerated islets of Langerhans (IL) and normal acini (A). E- Treatment for 60 days shows bigining of regeneration of islets of Langerhans (IL) and normal acini (A). F- Treatment for 45 days shows inflammatory cell at the boundaries of normal islets (IL) and normal acini (A),Vessels(V), Duct(D). H&E(400X).

4. Discussion

Results of the present study showed that the alloxan-induced group exhibited a significant decrease in the whole body weight compare with negative control group. This result was expected because it is well known that diabetes causes decrease in the whole body weight which is considered one of the most important diagnostic symptoms of diabetes[8] (ADA, 2014). In addition, in diabetes there is inability to store fat and protein along with breakdown of existing fat and protein stores (Ravi *et al.,* 2005) reflected in decreasing body weight. The decreased mean body weight in diabetic rats is due to excessive break down of tissue protein (Ganesh *et al*., 2010). This result is consistent with other studies that showed a decrease in body weight in diabetic rats[12]

The data showed in Table (2) and Table (3) demonstrated significant increase ($P<0.05$) of FBG and significant decrease ($P<0.05$) of fasting serum insulin levels and pancreas/body weight ratio in diabetic group and persistent hyperglycemia in diabetic animals may be due to the partial destruction of pancreatic β-cells by the direct effect of alloxan, because alloxan is well known to cause selective hydropic degeneration, degranulation, necrosis of the pancreatic β-cells, and fibrosis of the islets in a dose depending pattern. Weight reduction may be due to the effects of alloxan which causes degeneration and necrotic effects (Singh and Gupta, 2007).

Decreasing both β-cell function and number can contribute to insulin deficiency in type 2 diabetes ,the average of β-cell mass is about 39% lower in type 2 diabetes subjects compared to matched control (Rahier *et al*.,2008). Electron microscopic observation demonstrated the presence of more dead β -cells in islets of diabetics than from non-diabetic controls. The type of cell death observed seems to be apoptosis or autophagy-associated cell death, altered levels or impaired function of autophagy, possibly defects in the process of lysosome fusion and/or proteolytic enzyme activation, may contribute to the reduced β-cell mass by accelerating β -cell death (Masini *et al*. 2009). Also, alloxan-induced diabetes cause degenerative changes in endocrine and exocrine pancreas which may reflected in its weight. Various tissues in the diabetic state are more prone to oxidative damage resulting in various complications of DM[15].The reduction of pancreas area and size may assist finding of this study about pancreas reduced weight.

Histopathological observation in diabetic control showed degenerative changes in both endocrine and exocrine pancreas such as atrophy of islets, vacuolization of islets cells, vascular congestion, and presence of inflammatory cells. A probable explanation may be related to oxidative stress resulting from hyperglycemia decreases the antioxidants levels and increases ROS (Al-Kufaishi, 2012; Shen and Pierce, 2015) and the activities of antioxidant enzyme (three primary scavenger enzymes: super oxide dismutase, catalase, and glutathione peroxidase) were altered in diabetic rats (Arulselvan and Subramanian, 2006) and these effects further exacerbate the development and progression of diabetes complications through the damage of protein, lipids, and DNA, and then the cells (Johansen *et al*., 2005), and these may represent the causes of degeneration revealed in histological sections.

The presence of chronic inflammatory cells may refer to the presence of inflammation because hyperglycemia, which may have increased inflammation by increasing inflammatory markers such as migration inhibitory factor (MIF) and C-reactive protein (CRP), and decreases the antioxidant endothelia nitric oxide (Sojoholm and Nystrom, 2006). The vascular congestion may be due to specific defect in the blood capillaries that nourish the pancreas, or it may be a result from increased capillary blood pressure which may be due to increased systemic blood pressure because diabetes are usually associated with hypertension as one of diabetic complications, as it was referred previously that people with type 2 diabetes also have high rates of high blood pressure[8] (ADA, 2014). This results is in agreement with Shaffie *et al* (2010) who noticed that the islets of Langerhans showed severe necrotic changes, congestion and increase in connective tissue leading to relative reduction in size of islets.

The results of this study found out that the group of normal animals treated with mixture for 60 days showed a little increase in body weight that is statistically considered as significant decrease in the body weight, and this mixture of plants extracts may affect on metabolism when given to normal cell in normal group primarily. This action is not correlated to the toxicity of the mixture of plants extracts, since no sign of toxicity detected in this study.

The treatment for 60 and 75 days caused significant increase in the body weight which may be due to the enhancement of the blood glucose reflecting on enhancement of carbohydrates, protein, and fat metabolism due to the longer period of treatment. Many mechanisms explain enhancement of body weight as the mixture increase appetite. Besides, this may be because it contains high protein content. The increasing body weight may be due to increase in pancreas weight as we have seen in this study and increasing of other organs such as spleen (Bajallan, 2006), testis and epididymus (Al-Salammi,2004) after fenugreek extracts administration and increasing testis and epididymus weight when *Z. officinale* extract used (Bahar, 2011).Or this may be because olea leaves and fenugreek seeds increased glycogen content in liver and liver /body weight ratio which due to induction significant insulin release (Al-Hamadani, 2002). Also, Petit *et al* (1995) reported that saponin isolated from fenugreek seeds increased food intake and body weight of rats and suggested that fenugreek caused hypoglycemic effect and activation of glycogenesis in the liver which may be reflected in increasing body weight. This steroid saponin converted in the body to corticosteroids which caused hypertrophy of pancreas and stimulate insulin secretion. In addition *in vivo* administration of dry olive leaf extract significantly reduced clinical signs of T1DM (hyperglycemia and body weight loss) and led to complete suppression of histological changes in pancreatic islets (Cvjeticanin *et al*.,2009).This property of antidiabetic attributed to oleorupein and oleonolic acid (Sato *et al*., 2007).

There was non significant and significant decrease in FBG level in the normal and three diabetic groups treated for different periods respectively. This reflects the efficiency of this mixture in the treatment of hyperglycemia and it can revert FBG to normal value in diabetic group treated for 75 days. The significant effect of this mixture on hyperglycemia may be due to its effect in the enhancement of insulin sensitivity and increase cellular uptake then decreasing it´s level in plasma or decreasing hepatic glucose protection. This hypoglycemic and normoinsulinimic activity of mixture may be due to the ability of hypoglycemic compound in the mixture to act synergistically to improve diabetic status. The mixture may enhance and increase the release of endogenous insulin from pancreatic β-cells and induce regeneration of pancreas. They also promote and facilitate peripheral tissue uptake and utilization of glucose. Alternatively, its action can be related to possessing insulin-like action or glucagon inhibition, which increases in DM and stimulates gluconeogenesis in liver, leading to enhancement of glucose metabolism which is impaired in DM.

Also, the mixture contain compound that have antidiabetic/insulin mimetic effects as fenugreek seeds. Also, Broca *et al*. (2000) reported that 4-hydroxyisoleucine (4-OH-Ile), an amino acid extracted and purified from fenugreek seeds, displays an *in vitro* insulinotropic activity and cause improvement of the diabetic state. For instance, flavonoids are reported to regenerate the damaged pancreatic β-cells in diabetic animals and have been reported to activate PPARs (Saravanan and Leelavinothan, 2006).

These results are consistent with previous studies who reported that the administration of *Trigonella foenum-graecum* seeds powder to diabetic animals has been shown to lower blood glucose levels and partially restore the activities of key enzymes of carbohydrates and lipid metabolism to near normal levels in various animal models (Vats *et al*. 2003).

However, earlier studies have shown the presence of saponin compounds diasgenin, alkaloids and trigonelline – inhibit intestinal glucose uptake in vitro (Al-Habori *et al*. 2001). The treatment with *Trigonella* seeds powder (TSP) and vanadate could increase glucose utilization and reduce glycosylation of proteins, ROS formation and lipid peroxidation by controlling hyperglycaemia (Baqure *et al*., 2011). In addition, inhibition in renal glucose reabsorption by *Fraxinus excelsior* was reported (Donga *et al*., 2011).

Different mixture phytoconstituents had proven to reduce FBG as oleuropein extracted from olive leaves (Omar, 2010) and [6]-gingerol of the ginger rhizome (Ali *et al*.,2008), flavanoids, , saponins, polyphenolic compounds, tannins, glycosides, alkaloids and terpenoids (Tiwari and Rao, 2002; Baeshen *et al*., 2010) had proven effective against diabetes. Preliminary study showed the presence of different phytoconstituents which may be beneficial for the anti-hyperglycemic activity of this mixture (Al-Joubori *et al*., 2013).

In treatment groups, insulin levels returned near normal value, this result reflects the hypoglycemic effect of the mixture suggesting that the hypoglycaemic effect may be mediated through potentiation of pancreatic secretion of insulin from β-cell of islets. Another possible hypoglycaemic mechanism of the mixture of plants extracts may increase the sensitivity of tissue to available insulin. This effect of mixture may be

because containing natural antioxidant compound which reduce ROS formation in β-cell induced by alloxan and enhance the defense antioxidant mechanism against ROS production in diabetes type 2.

In all treatment study groups, pancreas / body weight ratio significantly increased and the mixture can reverted the pancreas / body weight ratio near normal value .This may be due to ability of the mixture to enhancement the pancreas function as induction of β-cells repair or decreasing apoptosis. Also, it acted to reduce degenerative changes in histological structure of pancreas and induce regeneration of islets of Langerhans as shown in histopathological study. Thus, in addition to FBG lowering effect, histopathological observation also supports the idea that this mixture of plants extracts produces significant antihyperglycenic activity by protecting pancreas and β-cell against alloxan action and enhance defence mechanisms. Hence the possible mechanism by which mixture of plants extracts brings about its antihyperglycemic action may be by the stimulation of surviving *β*-cells to release more insulin. This was clearly evidenced by the increased level of insulin in diabetic rats treated with the mixture.

This ability of this mixture in regeneration pancreatic tissue may be due to the precense of *N.sativa* which had a positive effect on the regenerative of Langerhans islets and attenuates the damage to β-cell of the pancreas initially distorted by STZ, was observed at the end of the experiment period (17 days) of treatment with thymoquinone (25mg/kg bw/day) or neutral lipid fraction (100mg/kg bw/day) (Widad *et al*., 2011). Also, there was increased number of β cells in the islets of Langerhans when treatment with crude *Nigella* for 21 days in adult male rats (Khanam and Dewan, 2008). In addition, fenugreek oil significantly improved blood glucose levels and insulin, less pancreatic islet and β-cells damage were observed after the administration of fenugreek oil to diabetic rats and reveals the efficacy of fenugreek oil in the amelioration of diabetes, hematological status, and renal toxicity which may be attributed to its immunomodulatory activity and insulin stimulation action along with its antioxidant potential (Hamden *et al*.,2010).

Reducing inflammatory cells may be because the mixture contains many plants acts as anti-inflammatory as *Z. officinale* (Raji *et al*.,2002). *Zingiber officinale* dried rhizomes ethanol extract and gingerols, the most biologically active components of ginger, produces its antiinflammatory effect by inhibiting the release, synthesis and/or production of inflammatory mediators, including polypeptide kinins, prostaglandins and so forth, like diclofenac (Jiang *et al*., 2006).

These enhancement in both glucose and insulin levels which reverted near normal levels are also seen in histopathological study of pancreas which indicates protective effect of the mixture of plants extracts used in this study on pancreatic tissues. This results in consistent with previous studies which reported that DM has negative effect in male testis and epididymis of alloxan-induced rats and may contribute in reduction of fertility while the same mixture of plants extracts used in this study can reduce most degerenative changes occurred in testes and epididymis in diabetic groups[(10)]. Also, cytogenetic study showed significant increasing ($P<0.05$) in both mitotic index and chromosomes aberrations in diabetic group while treatment with the same mixture of plants extracts significantly reduced mitotic index and chromosomes aberrations in all treatment groups and for different period of study (45, 60 and 75 days)(Al-Joubori *et al*.,2014)..

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