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**Original Article**

# MODULATING EFFECT OF THE AERIAL PARTS OF PUMPKIN METHANOL EXTRACT IN LIVER AND PANCREATIC ISLETS ON EXPERIMENTAL DIABETIC RAT: HISTOLOGICAL, BIOCHEMICAL AND IMMUNOCHEMICAL STUDIES

**AHMED, F. E.,[1] SELIM, A. O.,[1] SELIM, S. A.[1]? AND MOHAMED, A. M.[2]**

[1]Department of Histology & Cell Biology and [2]Department of Clinical Pharmacology, Faculty of Medicine, Zagazig University, Zagazig, Egypt. E. mail: sa.selim73@gmail.com

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***Abstract: Nowadays, the herbal is considered a novel therapeutic approaches in the treatment of diabetes. Pumpkin extract is the most recommended one is seen to help in regulation of blood sugar. It is prescribed widely even when its biologically active compounds are still unknown. This work is designed to evaluate the protective role of methanol extract of aerial part of Cucurbita maxima (MECM) on experimental streptozotocin (STZ) induced pancreatic islets and hepatic injury. Forty male rats were divided into 4 groups: I: control; II: STZ induced diabetes; III: diabetic rats received daily 5 mg/kg of Gilbenclamide (GLB) and IV: diabetic rats received daily 200 mg/kg of MECM. At the end of experiment (30 days), the rats were sacrificed and blood samples were collected for different biochemical analysis. Livers and pancreases were removed and prepared for histopathological examination. It was found that the liver of diabetic rats revealed histological and chemical changes versus the control. The administration of MECM decreased glucose level as well as the lipid profile to/towards near the normal. On the other hand, the normal histological features were nearly resumed in the liver and pancreas which further evidenced the hepatoprotective activity of MECM. The present study proved a lessening effect of MECM on the diabetic liver. It is advisable to widen the scale of its use, for patients at high risk of diabetes mellitus in a trial to alleviate the hepatic hazards.***

**Key words:** Streptozotocin, Pumpkin, Liver and pancreas, Rat, Histology.

## INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder of endocrine system. This dreadful disease is found in all parts of the world and is becoming a serious threat to mankind health. According to International Diabetic Federation the estimated diabetes prevalence for 2010 has risen to 285 million, with a prediction that by 2030 this number will have risen to 438 million. It has become the fourth leading cause of death in developed countries [1].

Type 1 DM is an autoimmune disease characterized by inflammation and destruction of insulin-producing β-cells in the pancreatic islets [2]. Its chronic hyperglycemia is associated with long-term damage, dysfunction and failure of various organs [3]. The liver is the organ that is severely dam-aged in diabetes. These damages include inflammation, necrosis, fibrosis, hepatocellular carcinoma and acute liver failure. The mortality rate from the hepatic affection is greater than that from the cardiovascular complications [4].

Also, the abnormalities in lipid profile are one of the most common complications in DM, which is found

in about 40% of diabetes [5]. Although, insulin and hypoglycemic drugs such as glibenclamide (GLB) constitute the main treatment in diabetes, but in these days great attention is being given to its management with medicinal plants along with dietary restriction in some countries [6].

Wide variety of herbs and plant parts, especially which are commonly consumed as vegetables had also evoked interest of researchers involved in discovery of plant based remedies. Pumpkin or Curcubita maxima (C. maxima) belong to family Cucurbitaceae which are widely distributed in the warmer regions of the world. Both of its fruits and aerial parts (stem and trifoliate leaves) are commonly consumed. However, the plant has been used traditionally in many countries such as China, India and America as anti-diabetic [7]. Traditionally, pumpkin juice and pulp preparation are used to treat diabetes in rural area of India but there is no scientific evidence for its hypoglycemic potential effect [8].

A daily supplement of pumpkin fruit (Fig. 23) powder was found to reduce blood glucose levels. Its hypoglycemic chemicals include polysaccharides from the fruit pulp, oil and protein from germinated seeds. Also, several polysaccharides, phenolic glycosides and octadecatrienoic acid from their leaves have been reported. In addition, a phytochemical study showed the presence of flavonoid in its aerial part. Number of studies showed potent antidiabetic activity of flavonoids isolated from various plants [9]. The present study was carried out to evaluate the possible protective activity of aerial parts of *C. maxima* methanol extract on liver and pancreas in experimentally STZ induced diabetes in adult albino rat.

## MATERIALS AND METHODS

**Experimental animals:** Forty male albino rats (10-12- weeks-old, 160-180 gm) were obtained from Faculty of Veterinary Medicine, Zagazig University. On arrival, rats were housed at constant environmental conditions (room temperature 24±2 ºC with a 12-h light/dark cycle and 50% humidity). Before starting the experiment, the rats were acclimatized to the laboratory conditions for a period of one week. They were fed with standard laboratory diet and water ad-libitum. All the experimental procedure and protocols were reviewed and approved by the Institutional Animal Ethical Committee and all the experiments were carried out by following the guidelines of CPCSEA [10].

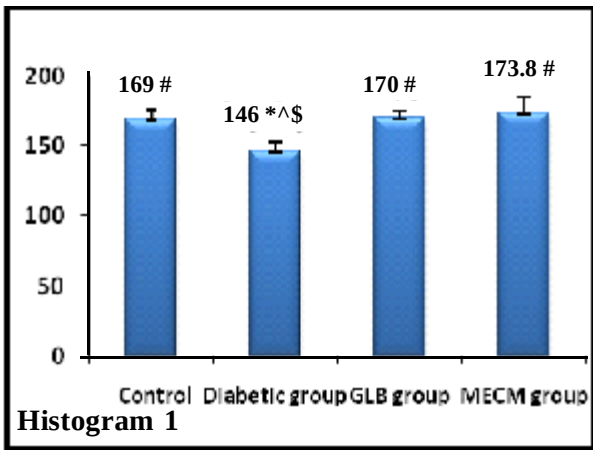
**Drug used - Glibenclamide:** was supplied by (Sedico Company) for treatment of diabetic rats. Tablets were dissolved in distilled water. It was administered by oral gavage in a dose of (5mg/kg b.wt.) orally for 30 days[10].

**Induction of the diabetes:** Animals were fasted for 8 hours before the injection of STZ, but had free approach to water. STZ was obtained in powder form, Sigma Chemical Company (St. Louis, USA). STZ was dissolved in sodium citrate buffer (0.09 M and pH 4.8). Diabetes induced by a single intraperitoneal injection of freshly dose of STZ (45 mg/kg b.wt.). One week after injection, animals with blood glucose level above 200 mg/dl were taken as diabetic for this study. Other rats were excluded [11].

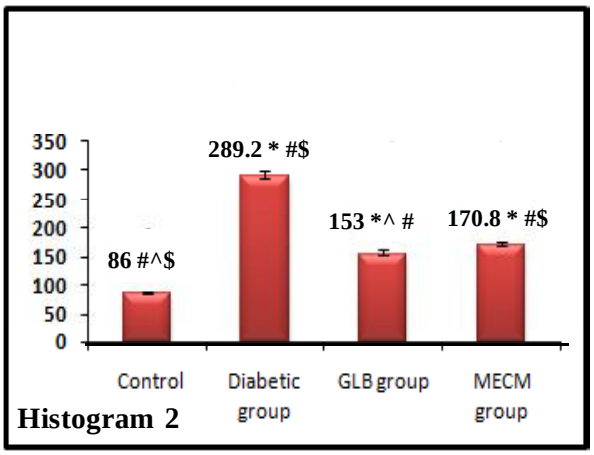
**Preparation of plant extract:** The aerial parts of C. maxima were dried and powdered in a mechanical grinder. The powdered material was extracted with

**Explanation of figures**

**Histogram 1:** Comparison between mean values of body weight (gm) in different studied groups. Showed a significant decrease (*) observed in the diabetic rats compared with the control, the body weight gain showed a significant increase (#) with MECM and GLB compared with diabetic group. **Histogram 2:** Comparison between mean values of blood glucose (mg/dl) in different studied groups. Showed a significant increase in blood glucose level (*) observed in the diabetic rats compared with the control. Treatment with oral GLB & MECM showed significant decrease of blood glucose level (#)versus to diabetic group. **Histogram 3a,b:** Comparison between mean values of serum ALT & AST levels (IU/L) in different studied group. Showed a significant increase of ALT and AST (*) level in diabetic rats when compared with the control groups. There was a significant decrease effect of GLP and MECM showed on ALT and AST levels (#) versus diabetic group. **Histogram 4a,b:** Comparison between mean values of serum TC & VLDL levels (mg/dl) in different studied group. Showed a significant increase of TC&VLDL in diabetic rats versus control groups (*). In rats treated with GLB and MECM, there was a significant decrease of serum TC & VLDL (#) levels versus diabetic group. **Histogram 5a,b:** Comparison between mean values of size and number of islets in different studied group. Showed a highly significant decreased of the size (micron) and numbers of Langerhans islets in diabetic rats compared with control groups (*). In rats treated with GLP and MECM, there was a significant increase in size and numbers of Langerhans islets (#) versus control group. ( *= significant with control group, # = significant with diabetic group. $ = significant with GLB. ^ = significant with MECM)




Histogram 1

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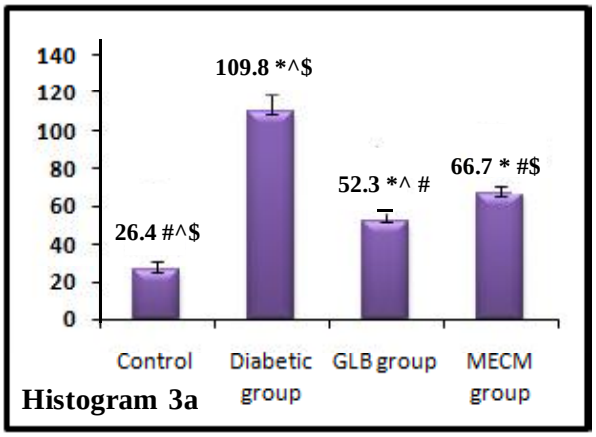
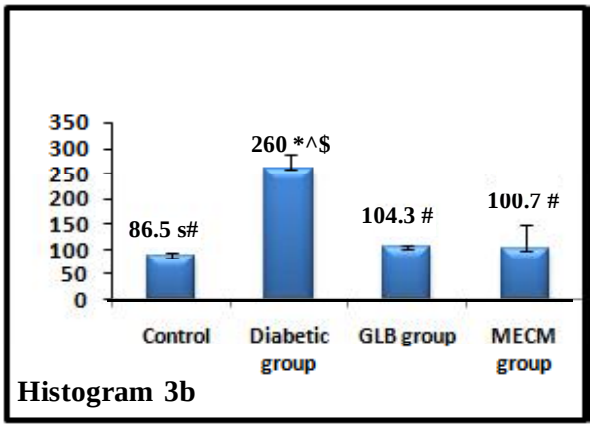
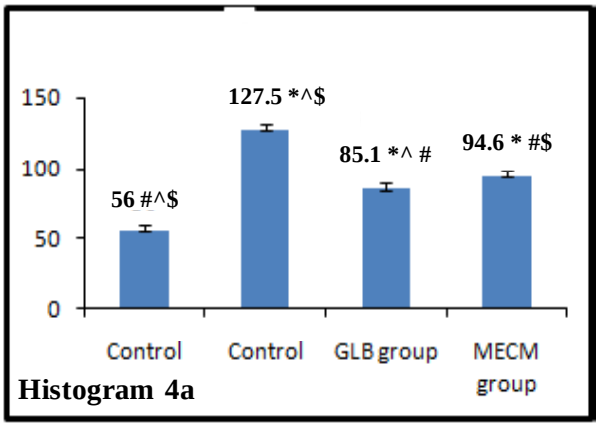


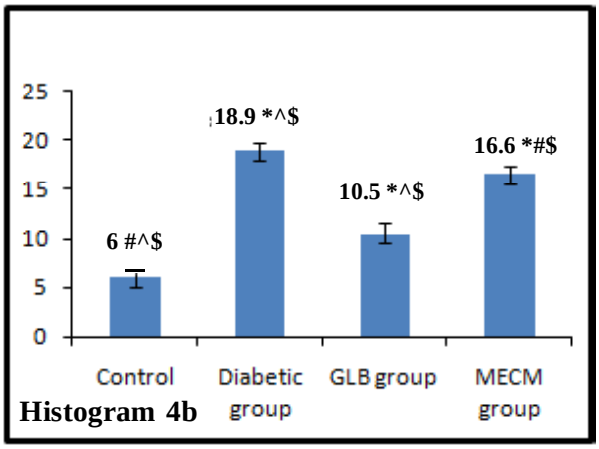

Histogram 3a



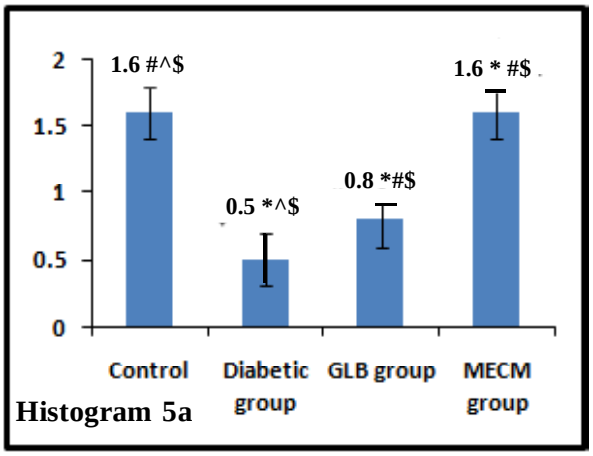

Histogram 3b



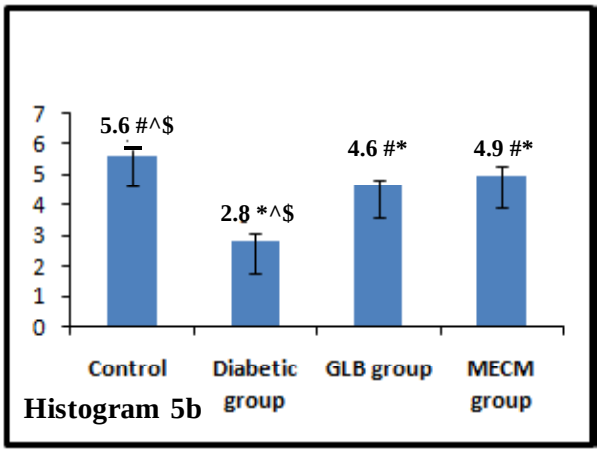

Histogram 4a




Histogram 4b




Histogram 5a




Histogram 5b

methanol. This extract was filtered, concentrated in vacuo and kept in a vacuum dessicator for complete removal of solvent. Aqueous suspension of methanol extract of C. maxima (MECM) was prepared using 2% Tween-80 that used for oral administration [7].

**Experimental design:** Animals were classified into four main groups:

**Group I (control group):** This group include 10 animals, were subdivided into two subgroups.

**(a) Subgroup Ia**: This was the negative, the included rats were received only vehicle (sodium citrate buffer pH 4.8 in a dose of 2 ml/kg b.wt. intraperitoneally) for 30 days.

**(b) Subgroup Ib:** This was the positive, the included rats that received daily MECM 200mg/kg b.wt. orally for 30 days.

**(2) Group II (untreated diabetic group):** Included the rats that had blood glucose level after STZ injection > 200mg/ dL. [11].

**(3) Group III (treated diabetic group with glibenclamide (GLB):** Diabetic rats were treated with daily glibenclamide (5mg/kg b.wt.) orally for 30 days. GLB was used as standard antidiabetic drug [8,10].

**(4) Group IV (treated diabetic group with MECM):** Diabetic rats were received a daily MECM 200mg/kg b.wt. orally for 30 days [7,12].

**Recording of body weight:** The weighs of all animals was recorded at the end of experiment.

**Seriological study:** At the time of sacrifice, animals were anesthetized with ether inhalation. Blood samples were drawn by means of a capillary tube from eyeballs for estimation of the following parameters

**Blood glucose level:** Blood glucose level was measured in four groups. Blood sugar estimation was done by using a glucometer (Horizon, from Lifescan, Johnson Company) [13].

**Liver enzyme activities:** Serum Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) activities were assayed enzymatically. All the analysis was determined by the colorimetric method using a diagnostic kit supplied by Plasmatex (Germany) [14].

**Lipid profile:** Total cholesterol (TC) and very low density lipoprotein (VLDL) were estimated by using Star 21 bio auto analyzer (E114947) at 505 nm by standard kits (Span diagnostics Ltd. India) [14].

## Explanation of figures

**Figures 1 to 4 are H & E stained sections (x 400): Fig. 1:** A photomicrograph of an islet of Langerhans **(**IL) from adult male albino rat in group I (control rat) showing a regular, well-circumscribed an islet of Langerhans. It is formed of many cells with basophilic nuclei (thick arrow) and multiple blood capillaries (thin arrow) scattered in between these cells. **Fig. 2:** A photomicrographs of an islet of Langerhans (IL) from adult male albino rat in group II (diabetic rat) showing islets of Langerhans with small darkly stained nuclei (arrow) and also many vacuoles (V) between their cells. **Fig. 3:** A photomicrographs of an islet of Langerhans (IL) from adult male albino rat in group III (diabetic rat + GLB) showing an improvement in the islet of Langerhans and their cells regain their normal appearance (thick arrow). Blood capillaries (thin arrow) are scattered in between these cells. **Fig. 4:** A photomicrographs of an islet of Langerhans (IL) from adult male albino rat in group IV (diabetic rat + MECM) showing an apparently normal islet of Langerhans. It is formed of many cells with rounded basophilic nuclei (thick arrow) and also multiple blood capillaries (thin arrow) scattered in between these cells.

**Figures 5 to 8 represent sections revealing Insulin immunoreactivity (x 400). Fig. 5:** A photomicrographs of an islet of Langerhans (IL) from adult male albino rat in group I (control rat) showing strongly positive immunostained β-cells (arrow). **Fig. 6:** A photomicrographs of an islet of Langerhans (IL) from adult male albino rats in Group II (diabetic rat) showing weekly positive immunostained β-cells (arrow). **Fig. 7:** A photomicrographs of an islet of Langerhans (IL) from adult male albino rat in group III (diabetic rat + GLB) showing mild positive (dark arrow) in some cells and strong positive (white arrow) immunostained β-cells in other cells. **Fig. 8:** A photomicrographs of an islet of Langerhans (IL) from adult male albino rat in group IV (diabetic rat + MECM) showing strongly positive immunostained β-cells (arrow).

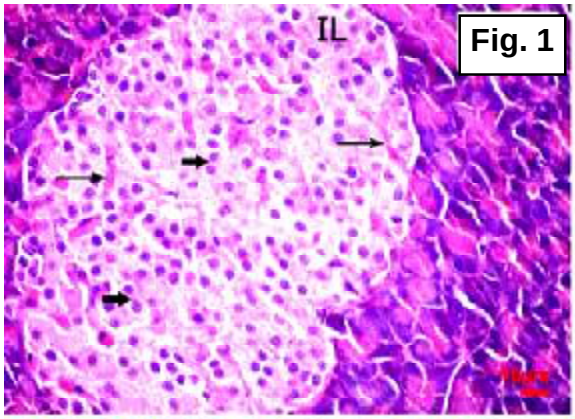

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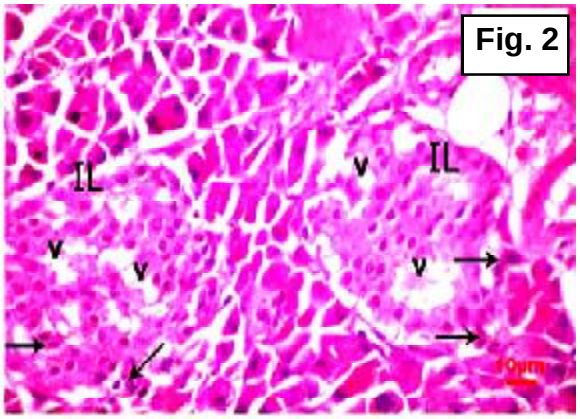
Fig. 1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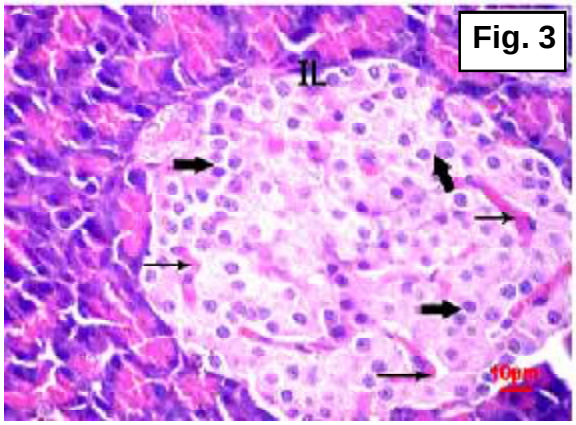
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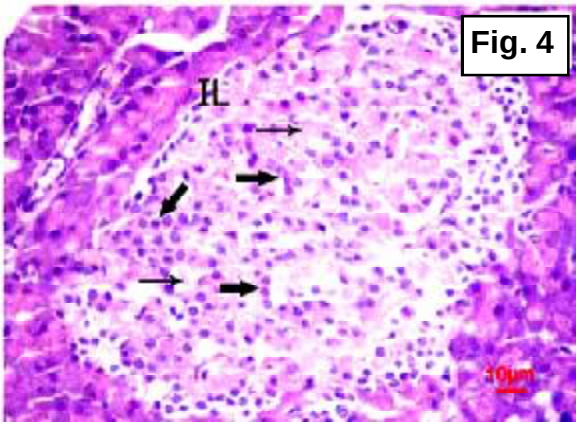
Fig. 3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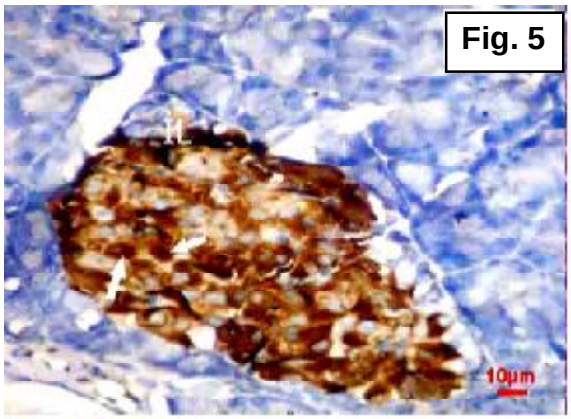
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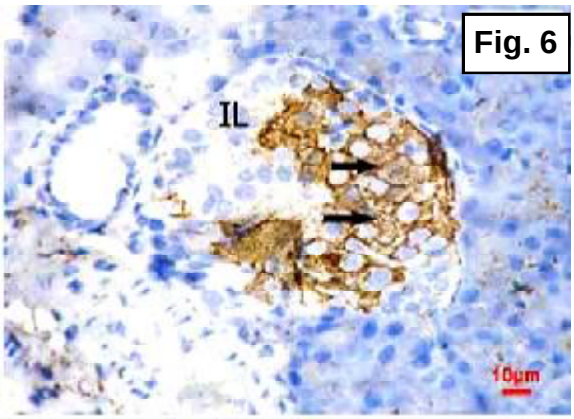
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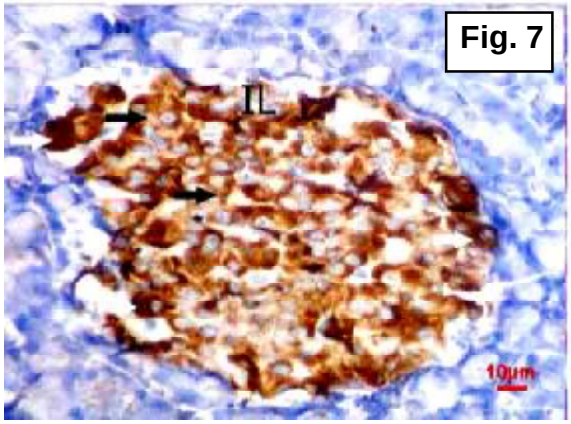
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Fig. 7

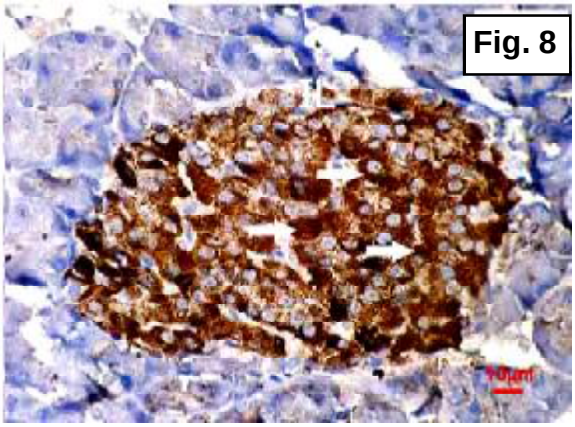
Fig. 8

**Histological methods:** At the time of sacrifice, Parts of tail of pancreas and liver tissues were collected for the histological study. For light microscope**,** the hepatic and pancreatic tissues were preserved in 10% formalin. These tissues were embedded in paraffin blocks and sections of 4 - 6 μm were cut. Some of these sections were stained with H&E [15].

**For immunohistochemical staining**: Thin paraffin sections were mounted on positively charged glass slides with polylysine. These sections were used for immunohistochemical staining for the following antibodies: anticleaved caspase-3 [16] and monoclonal mouse antisera against human insulin protein [17]. In brief, sections were deparaffinized and rehydrated; to retrieve antigen, sections were incubated with 0.1% trypsin and 0.1% $CaCl_2$ $2H_2O$ in Tris buffer (50 mmol/l) at pH 7.4 at $37^0$C for 120 min. Sections were soaked in absolute methanol containing 0.3% hydrogen peroxide for 30 min at room temperature, to eliminate endogenous peroxidase activity. The sections were then incubated with 1.5% nonimmunized goat serum for 30 min at room temperature, then incubated with the diluted primary antibodies (1:500) for cleaved caspase-3 and diluted monoclonal mouse antisera against human insulin protein (1:100) for 30 minutes at room temperature, and washed three times with phosphate-buffered saline for 30 min. The slides were rinsed in PBS and then incubated with the secondary antibody (biotinylated anti-mouse IgG, DAKO LSAB 2 Kit; Dako, Denmark) for 1 h at room temperature and rinsed again in PBS. The immunoreactivity was visualized by using 0.05% diaminobenzidine (DAP). Finally, all the sections were counterstained with Mayer's hematoxylin, dehydrated and mounted BY DPX. Brown cytoplasmic staining was scored as a positive reaction.

## Explanation of figures

**Fig. 9:** A photomicrograph of section of liver from adult male albino rat group I (control group) showing hepatic normal architecture, central vein (CV), radiating cords of acidophilic hepatocytes (H) with rounded vesicular nuclei and also blood sinusoids (S). Few hepatocytes appeared bi-nucleated (h) and others with few vacuoles in their cytoplasm (V). **Fig. 10:** A photomicrograph of section of liver from adult male albino rat group I (control group) showing normal portal tract with its content, a bile duct (Bd) with simple cuboidal epithelial lining and also hepatic artery (HA). Some hepatocytes (H) with vesicular nuclei and acidophilic cytoplasm, others with few vacuoles in their cytoplasm (V) and few of them with bi-vesicular nuclei (h) are also appeared. **Fig. 11:** A photomicrographs of sections of liver from adult male albino rat group II (diabetic group) showing loss of normal hepatic architecture. Extensively dilated central vein (CV), some hepatocytes (H) with pale vacuolated cytoplasm and others with deeply stained shrunken nuclei (arrow) are observed. **Fig. 12**: A photomicrograph of section of liver from adult male albino rat group II (diabetic group) showing congested central vein (CV), rupture of its endothelial cell lining (thick arrow) and also congested blood sinusoids (S). Numerous bi-nucleated hepatocytes (thin arrow) are present. **Fig. 13:** A photomicrograph of section of liver from adult male albino rat group II (diabetic group) showing disorganized cords of hepatocytes radiating from the central vein (CV) and fatty cellular infiltration (*) among hepatocyts. Hypertrophied or ballooned hepatocyte with multiple cytoplasmic vacuoles (H) is observed. **Fig. 14:** A photomicrograph of section of liver from adult male albino rats group II (diabetic group) showing dilated portal vein (PV) and two bile duct or proliferated bile duct (Bd) with stratified epithelial lining (thick arrow). Perivascular mononuclear cellular infiltration (F) is also seen. Fig 9 to 14 are H&E stained section (x 400).

**Fig. 15:** A photomicrographs of sections of liver from adult male albino rat in group III (diabetic rat + GLB) showing dilated congested central vein (CV), radiating cords of hepatocytes (H) with bi-vesicular nuclei and vacuolated acidophilic cytoplasm. Some dilated (S) while other congested (cs) blood sinusoids in between the hepatocytes are observed. **Fig. 16:** A photomicrograph of section of male albino rat in group III (diabetic rat + GLB) showing the portal area contains the portal vein (PV) and a bile duct with simple cuboidal epithelial lining (Bd). Note, some hepatocytes with acidophilic cytoplasm and vesicular nuclei (H) while, other hepatocytes with vacuolated cytoplasm (h). Few inflammatory cells (F) is seen in the portal area. **Fig. 17:** A photomicrographs of sections of liver from adult male albino rat in group IV (diabetic rat + MECM) showing an apparently normal hepatic architecture and a dilated central vein (CV) with radiating hepatic cords. Some dilated sinusoids (S) and hepatocytes (H) with vesicular nuclei and acidophilic cytoplasm are also present. **Fig. 18:** A photomicrograph of section of liver from adult male albino rat in group IV (diabetic rat + MECM) Portal area contains the portal vein (PV), hepatic artery (HA) and also a bile duct with simple cuboidal epithelium. Some hepatocytes with vesicular nuclei and acidophilic cytoplasm (H), others with few vacuoles in their cytoplasm (V) and few of them with bi-vesicular nuclei (h) are appeared. Note, few perivascular inflammatory cells (thick arrow). Fig 15 to 18 are H & E stained section (x 400).

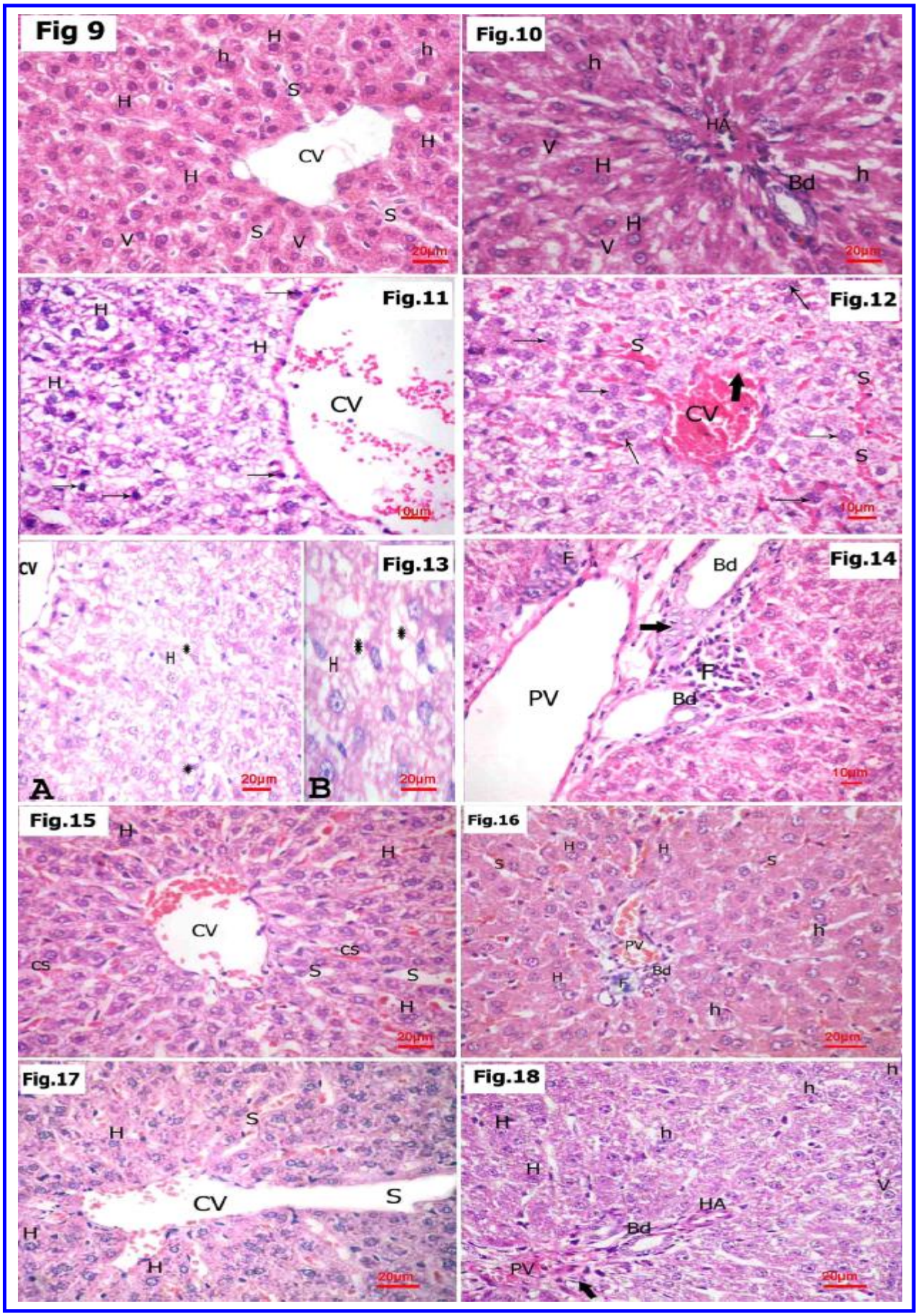
Fig 9
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## RESULTS

Comparison between mean values of body weight (gm) in different studied groups (histogram 1), comparison between mean values of blood glucose (mg/dl) in different studied groups (histogram 2), comparison between mean values of serum ALT & AST levels (IU/L) in different studied group (histograms 3a and 3b), comparison between mean values of serum TC & VLDL levels (mg/dl) in different studied group (histograms 4a and 4b) and comparison between mean values of size and number of islets (micron) in different studied group (histograms 5a and 5b) are quite distinct, hence need no further explanation.

**Haematoxline and eosin staining:** Pancreas of control rat (group I) showed the typical architecture of an Islets of Langerhans. It contained lightly stained acidophilic cells arranged in branching and anastomosing cords intermingled with blood capillaries (Fig. 1). Diabetic group (group II) showed marked changes in numerous endocrine cells having deeply stained nuclei and many vacuoles (Fig. 2). In groups III (diabetic rat + GLB) and IV (diabetic rat + MECM), islets of Langerhans preserved the general architecture. Numerous endocrinal cells with rounded basophilic nuclei and multiple blood capillaries scattered in between these cells (Fig. 3, 4).

**Immunostained sections** of the control group (I) for insulin protein showed a strongly positive reaction in β-cells of most pancreatic islets (Fig. 5). Marked observed changes appeared in group II; it exhibited weekly positive reaction in β-cells (Fig. 6). In group III, showed mild positive reaction in some cells and strong positive reaction in other cells of Islets of Langerhans (Fig. 7). While in group IV, showed strongly positive reaction in β-cells of Islets of Langerhans (Fig. 8).

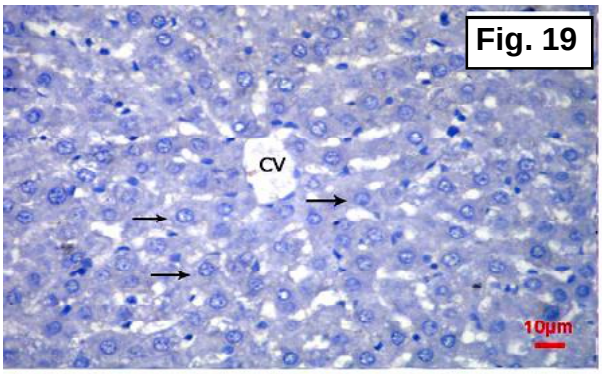
**H&E stained sections**, from the control rat liver (group **I**) presented a normal hepatic architecture. The hepatocytes radiated from the central vein and separated from each other by irregular hepatic sinusoids. The hepatocytes appeared polyhedral with acidophilic cytoplasm and vesicular, central, rounded nuclei. Some hepatocytes appeared bi-nucleated and others appeared vacuolated (Fig. 9). Portal tracts contained normal many structures including branches of hepatic artery with small diameter and thick wall and also a bile duct with simple cuboidal epithelial lining. Some hepatocytes with vesicular nuclei and acidophilic cytoplasm, others with few vacuoles in their cytoplasm and few of them with bi-vesicular nuclei were observed (Fig. 10).

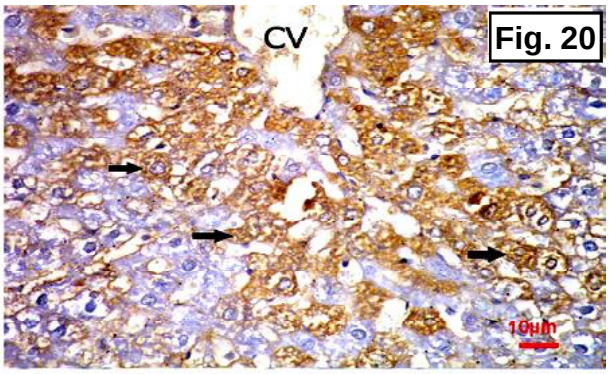
Marked observed changes appeared in diabetic group (group II); it exhibited disrupted hepatic architecture with dilated congested central vein, rupture in its endothelial lining an also dilated congested blood sinusoids in some areas. Numerous bi-nucleated hepatocytes and others with deeply stained shrunken nuclei were observed were seen (Figs. 11,12). There was marked hepatic injury, especially in the peri-central region, characterized by hepatoceytes with minimal vacuolated cytoplasm or extensively vacuolated (ballooned hepatocyte) with apoptotic or shrunken nuclei (Fig. 13). The peri-portal injury; the portal vein as well seemed to be dilated, proliferation of the bile duct and also mononuclear cellular infiltration was observed (Fig. 14).

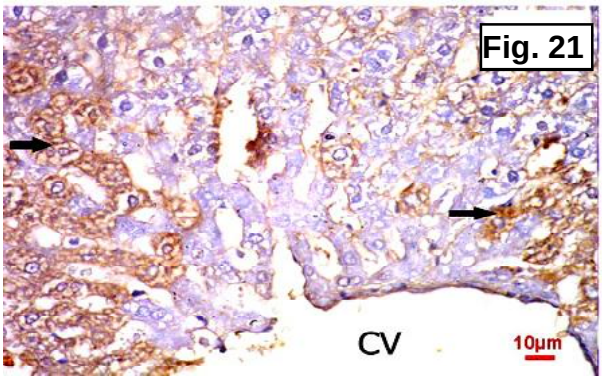
In group III (diabetic rat + GLB), livers preserved the general architecture and lacked evidence of major damages. Dilated central vein and few dilated or congested blood sinusoids with radiating cords of hepatocytes were observed. Affected hepatocyte appeared to be reduced. Scared cells with bi-vesicular nuclei and vacuolated acidophilic cytoplasm were demonstrated (Figs. 15,16). Also, the portal area includi-ng portal vein, a bile duct with simple cuboidal lining and some inflammatory cell were seen (Fig. 16).

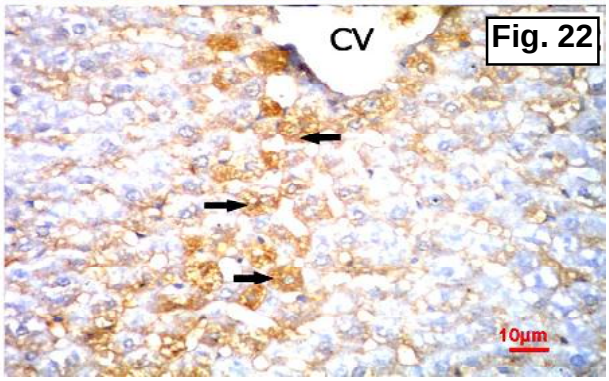
In group IV (diabetic rat + MECM), livers also preserved the general architecture and lacked the major injury but more pronounced than previous group (group III). Hepatocytes retained their normal acidophilic cytoplasm with vesicular nuclei and few vacuoles (Fig. 17). Portal area including the portal vein, a bile duct with simple epithelial lining and also branch of hepatic artery were observed. Also, few hepatocytes with bi-vesicular nuclei were also seen. Minimal perivascular mononuclear inflammatory cells were observed (Fig. 18).

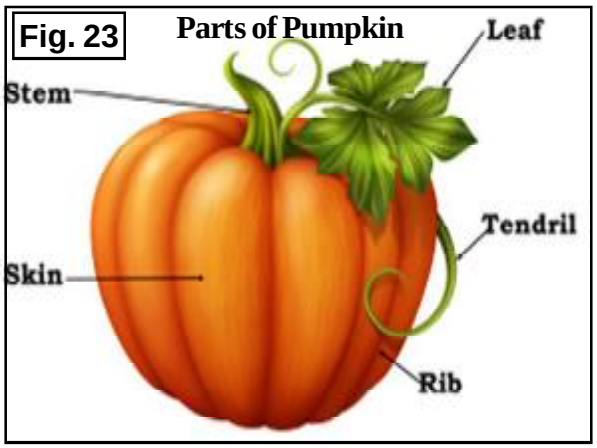
Immunostaining of the liver sections from the control rats (group **I**) displayed normal lobular architecture with no detectable immunolabeling for activated caspase-3 (apoptotic marker) in hepatocytes around the central vein (Fig. 19). Group II showed obvious high immunolabeling for activated caspase-3 in numerous hepatocytes especially around the central




**Figures 19 to 22** represent sections revealing Caspase-3 immunoreactivity (x 400). **Fig. 19:** A photomicrograph of section of liver from adult male albino rat in group I (control rat) showing no detectable immunostaining for activated caspase-3 in the cytoplasm of hepatocytes (arrow) around the central vein (CV). **Fig. 20:** A photomicrographs of section of liver from adult male albino rat in group II (diabetic rat) showing positively immunostained (brown colour) in numerous hepatocytes (arrow) around the central vein (CV). **Fig. 21:** A photomicrograph of section of liver from adult male albino rat in group III (diabetic rat + GLB) showing many positively immunostained hepatocytes for activated caspase -3 (arrow) around the central vein (CV). **Fig. 22:** A photomicrographs of section of liver from adult male albino rat in group IV (diabetic rat + MECM) showing few positively immunostained hepatocyes for activated caspase-3 (arrow) around the central vein (CV). **Fig. 23:** Pumpkin and its parts.

**Morphometrical study:** Measurement of size and number of Langerhans islets using computerized image analyzer system software Leica Qwin 500 (Cambridge, UK, Leica Microsystems Imaging Solutions Ltd) connected to a camera attached to a Leica universal microscope in the image analyzing unit) The measurement was carried out using an objective lens of ×10. At least 6 islets were analyzed per animal from five animals in each group.

**Statistical analysis:** The different data (body weight, blood glucose level, liver enzymes, lipid profile, size and number of Langerhans islets) are shown as means ±SEM. The statistical significance of the changes in the previous data was evaluated by using the one-way analysis of variance (ANOVA), followed by least significance difference test (LSD) for comparison between groups. The differences were regarded as statistically significant if the (P value was < 0.05).

vein (Fig. 20). Treatment of rats with MECM (group IV) showed some positive immunolabeling cells for activated caspase-3 (Fig. 22) than that showed in rats treated with GLB (group III) (Fig. 21) around the central vein.

## DISCUSSION

Diabetes mellitus (DM) is a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins attributed to diminished production of insulin or resistance to its action, as well as an increased risk of complications from vascular diseases. Many aspects of diabetes needs to be explored with respect to physiological actions of insulin and the various clinical features of this disease such as tissue complication, since this is life style disease, so proper treatment in relation to diet and anti-diabetic agents is emphasized [18,19].

Medicinal plants and herbal preparations have recently received considerable attention and have been found to be promising choice over modern synthetic medicines, in a number of studies. In developing countries, all over the world, 80% of population continues to use traditional medicine in primary medical problems. Research carried out in last few decades has validated several such claims of use of traditional medicinal plants [20].

The pumpkin, *Cucurbita maxima* Duchesne (C. maxima) belongs to the family Cucurbitaceae is widely cultivated throughout the world for use as vegetable. Both, its fruits and aerial parts are commonly consumed as vegetable. The plant has been used traditionally as medicine in many countries as antidiabetic [21], anti-tumor, anti-hypertensive, anti-inflammatory, immunomodulatory and anti-bacterial agents [22,23]. However, pharmacology of its aerial parts has not yet been explored scientifically.

The methanol extract of *C. maxima* (MECM) aerial parts as well as for long term therapeutic application in case of various chronic diseases, without producing any toxic effects [7]. Sharma et al. [10] reported that the maximum protective results were obtained from this extract. In the present study, STZ was used in a dose of 45mg/kg, while [24] used 90 mg/kg and [25] used 70 mg/kg in rats for producing hyperglycemia. The selection of lower dose was adopted as present strain of rats could not tolerate and survive with the dose used by previous investigators. STZ is a potent diabetogenic agent and widely used for inducing diabetes in a variety of animals by selectively destroying pancreatic insulin secreting β-cells, resulting in a disease in endogenous insulin release, which paves the ways for the decreased utilization of glucose by the tissues [10]. Also, other studies recorded that STZ induces severe diabetes, with a decrease in insulin level, to produce a cytotoxic model of diabetes very similar to type I DM [26].

Saeed et al. [27] reported that STZ-induced DM leads to increased blood glucose and association between diabetes and disturbances in various tissues, such as diabetic nephropathy and cardiovascular diseases. Interestingly a limited data is available on the possible association between DM and liver structures and functions [28]. In absence of reliable liver protective drugs in modern medicine, folk remedies from plant source are therefore evaluated for their potential hepato-protective effects [29]. Therefore, the present investigation was carried out to evaluate the possible protective effect of the methanol extract of aerial parts of pumpkin on liver and pancreatic islets in experimentally induced diabetes.

In the current work, the morphometrical histological analysis of diabetic pancreas showed, the mean diameter and number of Langerhans islets was significantly decreased as compared to that of the control, which was consistent with other studies [30,31]. Our results are similar to earlier findings [7, 32] that both parameters increased in MECM treated group as compared to diabetic one which illustrates the effects of pumpkin on repair and restoration of pancreatic tissue. MECM has been found to possess flavonoids, polyphenolics, steroids and triterpenes that have antioxidant activity. Anti-oxidants are substances that protect cell membranes and other components against damage caused by oxidants by collecting free radicals, transferring electron to them and ultimately rendering them inactive. Further Zhu et al. [33] found that pumpkin polysaccharides can decrease the blood glucose level of diabetic rats, enhance the activity of superoxide dismutase (SOD), reduce malondial-dehyde (MAD) and improve the structural features of islet cells.

In the current study, insulin-secreting β cells showed strong positive immunoreactivity in the supplemented group with MECM. Nugent et al. [34] reported that the islet as an endocrine cellular mass is reactive to

changes in demand. Therefore, the strong positive immunoreactivity observed in β cells might be because of the increase in their activity to produce insulin. Also, our results are in agreement with Rauter et al. [35] data who illustrated that the administration of antioxidants to diabetic rats significantly increases the number of β-cells. Therefore, the protective effect of pumpkin on pancreas and its hypoglycemic properties should be attributable to antioxidant activity and its flavonoid compounds of this fruit.

In the present study, a significant decrease was observed in the body weight of diabetic rats compared with control. This loss of their weights may be due to injurious effects of STZ which caused alkylation of DNA, hyperglycaemia and necrotic lesions [36]. Treatment with MECM and glibenclamide, improved the weight gain and more pronounced in MECM treated rats. Sharma et al. [10] and ] Zafar et al [36] also demonstrated the same results.

The fundamental mechanism underlying hyperglycemia involves overproduction of glucose by excessive hepatic glycogenolysis and gluconeoge-nesis, and decreased utilization of glucose by tissues [37]. Pumpkin contains a higher level of D-chiroinositol which decreases glucose, increases hepatic glycogen and insulin. Therefore, it is considered as a good treatment for diabetes, as it composed of polysaccharide and protein. Protein bound polysaccharides extracted from pumpkin significantly increased levels of insulin, reduced blood glucose and improved glucose tolerance [38]. Moreover, the presence of pectin itself can serve as a hypoglycemic agent in pumpkin [6,8].

Marked elevation in serum ALT and AST of the diabetic rats as compared to control is also shown by Saha et al. [7] and Sharma et al. [10]. Serum ALT and AST are the most sensitive markers used in the diagnosis of hepatic damage. They are cytoplasmic enzymes released into circulation after hepato-cellular damage. These enzymes are in conformity with the extent and type of hepatic damage [39**]** and inflammatory disorders [12]. This change in the enzymes is directly related to the release of aminotransferases by damaged hepatocytes [8]. Restoration of these levels to/towards near normal values in the MECM and glibenclamide treated rats is a clear manifestation of anti-hepatotoxic effect of them [7].

In the current work, GLB significantly decreased the blood glucose level. It has been used for many years to treat diabetes to stimulate insulin secretion from β-cells [40]. Oral administration of pumpkin significantly reduced the elevated blood glucose level which was comparable to it. It is supposed that pumpkin concentrate enhance the activity of β-cells resulting in secretion of large amount of insulin [37 ]. In previous studies, the obtained data indicated that different extracts of *C. maxima* significantly reduced the elevated blood glucose but the maximum result obtained from its methanolic extract. The possible mechanism by which maxima brings about its hypoglycemic action may be potentiating the insulin effect by increasing either insulin from β-cells or its release from bound insulin or increased peripheral utilization of glucose [6].

Present study revealed that pumpkin and GLB significantly reduce the levels of Tc and VLDL when compared with diabetic group. In accordance to others authors [41] the hypolipedemic effects of pumpkin is probably due to its fibres, they inhibit the absorption of bile acids and cholesterol as also suggested by Lecumberri et al. [41]. Furthermore, a fiber-rich diet reduces triglyceride levels by suppressing lipogenesis in the liver. Also, the presence of unsaturated fatty acids in pumpkin reduces cholesterol levels in rats. While, Fernandez et al.[42] reported that this effect is partly attributed to the presences of pectin in pumpkin. The diets rich in pectin facilitate excretion of bile acids which lead to their synthesis increase from cholesterol in the liver and ultimately reduction of blood cholesterol levels. Also, pectin enhances the activity of lipoprotein lipase in fat tissue and heart, resulting in higher absorption of VLDL in tissues other than liver to promote their breakdown and therefore reducing triglyceride levels.

In the current work, structure disarrangement of normal hepatocytes, vacuolization of cytoplasm and also proliferation of bile duct were observed in the diabetic livers also shown by other workers [11,12, 43]. Such alterations could be due to the stress of the diabetic injury and agree with those delivered by several authors.

Powell et al. [44] declared that diabetes is one of the metabolic causes of steatosis (fat droplets in the hepatocytes). In addition, they emphasized steatosis could take one of two forms either multiple small vesicles or a single large vesicle that may cause ballooning of the hepatocyte, so that it resembles

a mat mature adipocyte. While Nagle et al. [45] assumed that the potential sources of fat include, the increased lipolysis in the white adipose tissue and newly formed fatty acids within the liver through de novo lipo-genesis. The affected sinusoids, vascular congestion and inflammatory cellular infiltration encountered in the diabetic liver of this work. These results were in consistent with other studies [11,43]. They stressed on that, may be complicated by adhesion of leucocytes to sinusoidal endothelium followed by leucocytic infiltration into the hepatic parenchyma to form inflammatory foci. According to the findings in other studies [46], hyperglycemia is the main offending factor in the onset of the micro-vascular diabetic complications. Also, an apparent increase in the bi-nucleated hepatocytes was observed in the present work. It is may be a sign of regeneration. Ozdemir et al. [47] found an increase in large cytomegalic hepatocytes in diabetic rats. It was suggested that this increase may be because of early hyperplasia and decreased apoptosis in the STZ-induced diabetic rat.

STZ administration markedly increased the expression of active caspase-3 in rat liver as also demonstrated by Green [48], who mentioned that caspase-3 activation is a hallmark of almost all apoptotic systems. The results of the current study provide noticeable evidence that administration of 200mg/kg b.wt. MECM orally for 30 days showed hepatoprotective effect on STZ- induced diabetic rats. This is clearly evidenced by decreased number of hepatocytes with positve activated caspase-3.

All these changes arise from chronic hyperglcaemia in concert with hyperlipidemia, is mediated to a significant extent via oxidative stress, carbonylic stress and inflammation **[10]**. Normally, the body has an effective defense mechanism, consisting of a set of endogenous antioxidant enzymes. In hepatocellular damage or hepatotoxicity, the balance between ROS production and these antioxidant defenses may be lost, consequently oxidative stress may result which finally may lead to hepatic apoptosis or necrosis **[7]**. Free radicals are implicated in diabetes. Hyperglycemia lead to the non-enzymatic glycation of circulating proteins (collagen & hemoglobin) and DNA to produce advanced glycated end products [49]. Also, the free radicals in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation, also disturbs Ca2+ homeostasis, and finally result in cell death [7].

Following treatment of the diabetic rats with MECM, in the current investigation, their livers showed, more or less, an improvement in the histological architecture with persistence of the cytoplasmic vacuoles in some hepatocytes that could be attributed to the residual adverse effect of the diabetic affliction. But the noticed apparent general improvement signifies that MECM could possess cyto-protective ability on the hepatocytes. The present findings are supported by Abou Seif [50] who confirmed the pretreatment with pumpkin oil protect the liver from the hepat-otoxic effect and oxidative stress caused by alcohol.

The present investigation thus provides evidence for the total safety profile of the methanol extract of the aerial parts of *C. maxima*, suggesting its safe use in treatment as well as for long term therapeutic application in case of various chronic diseases, without producing any toxic effects. In conclusion, in light of the beneficial hepatoprotective effects of MECM detected in the current investigation, it is advisable to widen the scale of its use, after further purification procedures, for patients at high risk of diabetes mellitus in a trial to alleviate the diabetic undesirable hepatic hazards. Further work is in progress to reveal the details of the mechanism of its potent hepato-protection as well as to isolate and purify the bioactive principle (s) from the methanol extract of *C. maxima* aerial parts.

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