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# EFFECT OF APITHERAPY ON THE PANCREAS & LIVER OF STREPTOZOTACIN INDUCED DIABETIC RATS. A BIOCHEMICAL AND HISTOLOGICAL STUDY

**Sara Abdel Gawad[1], Heba Fikry*[1], Mariam Maged Amin[2], Amira Ramadan Elmahdi[2] and Doaa Abd Elaziz[3]**

[1]*Lecturer, Department of Histology, Faculty of Medicine, Ain Shams University Khalifa El-Maamon st, Abbasiya sq. Cairo, Egypt.

[2]Lecturer, Department of Internal Medicine, Allergy and Clinical Immunology, Faculty of Medicine, Ain Shams University Khalifa El-Maamon st, Abbasiya sq. Cairo, Egypt.

[2]Department of Internal Medicine, Allergy and Clinical Immunology, Faculty of Medicine, Ain Shams University, Cairo, Egypt. Khalifa El-Maamon st, Abbasiya sq. Cairo, Egypt.

[3]lecturer, Department of Clinical Pathology, Faculty of Medicine, Ain Shams University Khalifa El-Maamon st, Abbasiya sq. Cairo, Egypt.

***Correspondence for Author: Heba Fikry**

Lecturer, Department of Histology, Faculty of Medicine, Ain Shams University Khalifa El-Maamon st, Abbasiya sq. Cairo, Egypt.

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## ABSTRACT

**Objective:** The main issue in management of diabetes mellitus (DM) is to control blood glucose level and lipid profile using medical agents including natural toxins. Bee venom (BV) which had been used to treat various diseases is of a great importance in this regard. So the aim of the work was to evaluate the therapeutic effects of honey BV (apitoxin) on blood glucose level, lipid profile as well as on the histological changes in pancreas and liver of Streptozotacin (STZ) induced diabetic rats. **Design and methods:** This study assessed serum glucose level, C.peptide, C- Reactive protein (CRP), triglyceride (TG) and total cholesterol in fifty adult male albino rats divided into four randomly groups: control, BV group, STZ induced diabetic group and BV treated group. Then they were sacrificed for pancreatic and liver biopsy. **Results**: Serum glucose, CRP, TG and total cholesterol levels was significantly decreased (P≤ 0.05) in BV treated group in comparison with diabetic group. Moreover, BV treated group showed significant increase in serum C.peptide level and area percentage of anti-insulin antibodies in the pancreatic tissue (P≤ 0.05) as well as restoration of normal histological structure of pancreatic cells and hepatocytes in comparison with diabetic group. **Conclusion**: Administration of BV improves blood glucose and lipid profile as well as histological features of the pancreas and liver in STZ induced diabetic rats, therefore it can be considered as a novel therapeutic agent for DM.

**KEYWORDS:** Bee venom, Streptozotacin, Diabetes mellitus, Insulin.

## INTRODUCTION

The estimated prevalence of DM was about 9% among adults[1] and about 1.5 million deaths were caused directly by diabetes in 2012[2] in which more than 80% of them occur in low and middle income countries and WHO projects that the 7th leading cause of death in 2030 will be DM.[3]

DM is a metabolic disorder that is characterized by chronic high blood glucose level that causes complications in multiple organs as well as abnormal lipid profile. Hence, the potential remedy for DM not only needs the blood glucose levels controlling action, but also lipid regulating effect. Hyperglycaemia leads to increased protein glycation resulting in structural and functional alterations in proteins[4] leading to long-term complications of DM, such as retinopathy[5], atherosclerosis[6] nephropathy[7] and incomplete wound healing.[8] Protein glycation is the most important factor in the development of these complications and it is the main reason of morbidity and mortality.[9]

BV therapy used to treat various diseases. It has been used since ancient times in traditional medicine for humans and animals.[10] It is synthesized by the venom glands in the sting apparatus of workers and queens, stored in the venom reservoir, and injected through the sting apparatus during the stinging process.[11]

Various peptides including mellitin, apamin, adolapin and mast cell degranulation peptide, as well as enzymes e.g. phospholipase A2 and non-peptide components e.g. histamine, lipids and carbohydrates are the main components of BV and was found to have a wide variety of pharmaceutical actions.[12] Phospholipase A2 and melittin are the major ingredients of BV.[13] Melittin has been reported to contain proinflammatory[14], anti-inflammatory[15], anti-nociceptive[15] and anticancer

effects.[16] So BV can act as analgesic, antiarthritic and anti-inflammatory effects attributable to its bioactive compounds.[17] Therefore, BV is effective for the treatment of multiple chronic and autoimmune conditions[18, 19] It is not only providing symptoms relief, but can resolve the underlying condition.[20]

The two major components of BV increase insulin secretion from β-cells and lowers blood glucose level[21], in addition to its lipolytic properties. BV also was found to have a significant antiglycation effect and it can prevent glycation-induced alteration in the structure and function of hemoglobin, thus could also treat associated complications in diabetes. Moreover, BV has the potential to not only up regulate healing in 'normal wounds' but also to promote healing in diabetic wounds.[22, 23] So the aim of the work was to evaluate the therapeutic effects of BV on blood glucose level, lipid profile as well as on the histological changes in pancreas and liver of STZ induced diabetic rats.

## MATERIALS AND METHODS

In the present study adult male albino rats (weighing 180-200 gm) were purchased and maintained in medical research center in Ain Shams University. The animals were maintained on standard laboratory chow and housed in individual wire-bottom cages. The protocol was performed according to the Ethical Committee recommendations of Ain Shams University for the use of experimental animals.

Fifty male rats were divided randomly into four groups. **Group I** (control group, n=20) was further subdivided as follow: Subgroup IA: 10 rats were used as a negative control group. Animals were left without intervention. Subgroup IB: 10 rats received single intraperitoneal (I.P.) of 0.5 ml citrate buffer. Five rats were sacrificed after four weeks and the other 5 rats were sacrificed after 8 weeks. **Group II** (BV group, n= 10); each rat was injected by single I.P. injection of 0.5 mg/kg BV twice per week (The drug was purchased from Department of Allergy and Clinical Immunology, Ain Shams University) at fasting condition for four consecutive weeks. **Group III** (diabetic group, n=10); The rats were fasted for 18 hours prior to the induction of DM. Diabetes was induced by a single I.P. of STZ (N-(methyl nitroso carbamoyl) alpha-D-glucosamine, Sigma, St. Louis, MO, USA) at the dose of 60 mg/kg body weight.[24] STZ dissolved in 0.1 M citrate buffer (pH 4.5) was immediately prepared 10 minutes prior to injection, on account of the instability of STZ in solution. STZ diabetes was confirmed by measuring the blood glucose level 3 days after the induction. Diabetes was verified by a serum glucose level > 250 mg/dL.[25] The animals were sacrificed after 4 weeks from the induction of diabetes. **Group IV** (treated group with BV, n=10); in each rat induction of diabetes was done by STZ and rats were left for 4 weaks then BV was injected by I.P. injection of 0.5 mg/kg twice per week. The animals were sacrificed 4 weeks after the onset of BV treatment.[26]

### Biochemical analysis

Blood samples were drawn from each group twice per week. After over night fasting, a small incision was made on the animal's tail using a lancet and a drop of fresh blood was taken from the distal end of the tail, applied to a test strip, and analyzed immediately with a blood glucose monitoring device (Accu-Check Active, Roche Diagnostics, Mannheim, Germany).[24]

Blood samples were collected at the end of the experiment from the heart to measure the level of: serum C.peptide, CRP, cholesterol and TG. All blood samples were allowed to clot and the serum was separated by centrifugation and stored at -20°C until analysis. Total cholesterol and TG were done on Synchron CX-9 PRO autoanalyzer (Beckman Coulter, Inc. Fullerton, CA 92835-3100, USA). CRP measurement was done by latex immunoassay (Plasmatec, Dhanmondi, Dhaka - Bangladesh). Assay of c-peptide was performed by means of sandwich enzyme immunoassay ELISA technique using ST AIA-PACK C-Peptide ELISA Kit (TOSOH CORPORATION, MINATO, TOKYO). The assay procedures were followed as per the manufacturer's instructions.

### Histological and Immuno-histochemical study

Pancreas and Liver specimens were collected and fixed overnight in 10% buffered formalin. Serial 5 µm paraffin sections of the pancreas were stained with H&E and Masson's trichrome stain and the right lobes of the liver were stained with H&E and Periodic Acid Schiff Stain (PAS).[27]

Immunohistochemical staining for anti-insulin antibody detection was done in pancreas specimens using Avidin-Biotin detection system (Ventana, Tucson, AZ, USA), following the manufacturer's instructions. Sections were counterstained with hematoxylin. Slides were examined and photographed using a light microscope (BX51, Olympus, Tokyo, Japan) fitted with an Olympus digital camera (DP20).[24]

### Morphometric & statistical analysis

Quantification of the following parameters was determined visually in a microscopic study using the Image Pro plus image analyzer computer system (Media Cybernetics, Rockville, MD, USA): area percentage of collagen content using Masson's trichrome pancreatic stained sections and area percentage of anti-insulin antibody immunoreaction expression. All parameters were measured in randomly chosen five fields/ section in five sections in ten rats in each group at magnification 400.[24]

**Statistical analysis** was using SPSS statistical software, version 17.0 (SPSS Inc., Chicago, IL, USA). Data were analyzed and presented as means $\pm$ SD. Differences between continuous data were analyzed using one-way ANOVA. $P < 0.05$ was considered significant.[24]

## RESULTS AND DISCUSSION

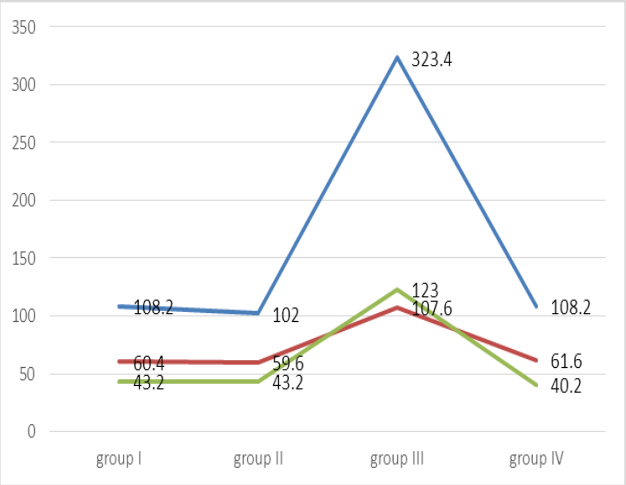
### Biochemical findings

There was a significant increase in blood glucose level, cholesterol and TG (Table 1, Histogram 1) of rats of group III as compared to that seen in the group I, group II and group IV (P=0.000). Moreover, there was a significant decrease in C-peptide level (Table 1, Histogram 2) in group III as compared to that seen in the group I, group II and group IV (P=0.000). CRP level was also decreased among group IV in comparison to the other groups.

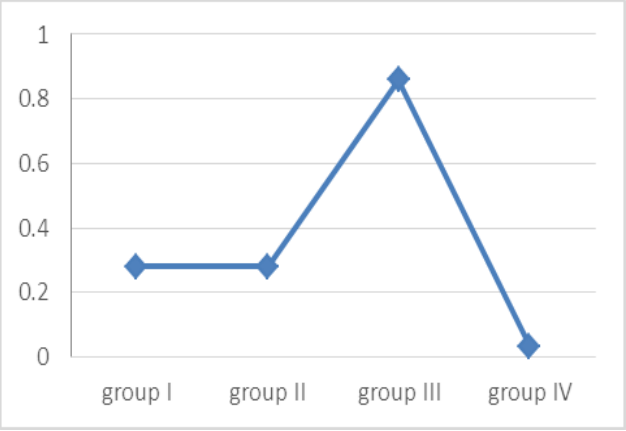
**Table 1: Mean± SD of biochemical and histological data in different groups**

| | Group I | Group II | Group III | Group IV |
|---|---|---|---|---|
| **Blood glucose level mg/dl** | 108±23.6 **(▲)** | 102±10.58 **(▲)** | 323.4±23.64 **(*■O)** | 108.2±27.4 **(▲)** |
| **Cholesterol level mg/dl** | 60.4±8.38 **(▲)** | 59.6±8,26 **(▲)** | 107.6+ 8.14 **(*■O)** | 61.6+5.63 **(▲)** |
| **Triglycerides level mg/dl** | 43.2+ 2.9 **(▲)** | 43.2+ 2.9 **(▲)** | 123+ 6.7 **(*■O)** | 40.2 +5.01 **(▲)** |
| **C-peptide ng/ml** | 0.28 +0.044 **(▲)** | 0.28±0.044 **(▲)** | 0.086±0.15 **(*■O)** | 0.32+ 0.044 **(▲)** |
| **Area percentage Collagen fibers** | 2.85±0.23 **(▲)** | 2.30±045 **(▲)** | 9.24±2.41 **(*■O)** | 3.73±0.79 **(▲)** |
| **Area percentage Anti insulin Antibodies** | 11.32±1.7 **(▲)** | 10.2±1.8 **(▲)** | 1.55±1.2 **(*■O)** | 4.82+1.35 **(▲)** |

*****Significant difference from group I.
■ Significant difference from group II.
▲ Significant difference from group III.
**O** Significant difference from group IV.




**Histogram (1): Blood glucose level (blue), cholesterol (red) and TG (green) in different groups.**




**Histogram (2): C-peptide level in different groups.**

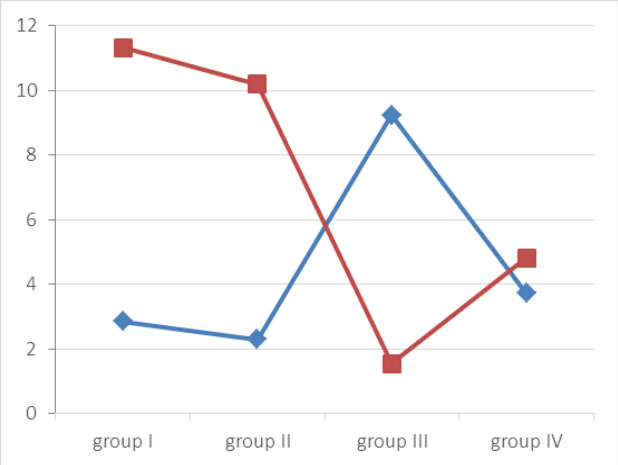
### Histological results of pancreas & liver

H&E stained sections of all subgroups of the group I revealed the normal architecture of pancreas. The predominant exocrine component of pancreas consisted of closely packed secretory acini. Each acinus was made up of an irregular cluster of pyramidal shaped cells with indistinct cell boundaries surrounding a narrow lumen. The cells showed intense basal basophilia and apical acidophilia. The nuclei of cells were vesicular and basally situated. Most of the acini contained numerous apical closely packed zymogen granules. Islets appeared as pale oval areas surrounded by a delicate capsule inside pancreatic lobules. They appeared formed of irregular branching and anastomosing cords of cells separated by blood capillaries (Fig. 1A). Group II was nearly similar to group I (Fig. 1B). Group III pancreatic sections showed disorganization of the endocrine structure illustrated in apparent decrease in the number of Langerhans cells which appeared vacuolated and degenerated. Some cells showed acidophilic degeneration with deeply stained pyknotic nuclei. Mild congestion could be seen (Fig.1C). Sections from group IV showed nearly restoration of normal morphological structure of Islets as number of Islets cells were apparently increased as compared to diabetic group and appeared healthy (Fig. 1D).

In Masson's trichrome stained sections, few collagen fibres deposition appeared in group I (Fig. 2A) and group II (Fig. 2B). In group III, there was significant increase ($P<0.05$) of area percentage of collagen fibers which deposited in-between the exocrine and endocrine portions of pancreas and around blood vessels (Fig.2C) (Table 1, Histogram 3). However, group IV showed

significant decrease (P<0.05) in the area percentage of collagen fibres as compared to group III (Fig. 2D) (Table 1, Histogram 3).

Immunohistochemical staining of the pancreas with anti-insulin antibodies showed intense positive cytoplasmic immuno-reaction in islets cells in both control and BV groups (Fig. 3A & Fig. 3B) respectively. However, the sections of group III showed significant decrease (P<0.05) in the area percentage of anti-insulin antibodies immune expression as compared to control group (Fig. 3C) (Table 1, Histogram 3). Group IV showed significant increase (P<0.05) in the area percentage of anti-insulin antibodies immune expression as compared to group III (Fig. 3D) (Table 1, Histogram 3).




**Histogram (3): Area percentage of collagen fibers (blue) and anti-Insulin antibody (red) in different groups.**

Examination of H&E stained liver sections of the groups I and II showed normal hepatocytes structure with central rounded nuclei while some were binucleated. The blood sinusoids were present in between the hepatocytes cords (Fig. 4A, Fig. 4B) respectively. Liver sections from group III showed extensively degenerated and vacuolated hepatocytes while other few hepatocytes showed acidophilic degeneration. Some hepatocytes nuclei varied from being deeply stained pyknotic nuclei to karyorrhectic and karyolytic nuclei. Moreover, there was mild congestion in the central vein and blood sinusoids with mild mononuclear cell infiltrate (Fig. 4C). Examined sections of group IV showed restoration of normal appearance of hepatocytes (Fig. 4D).

PAS stained liver sections of group I & II showed normal distribution of glycogen granules in the hepatocytes (Fig. 5A & Fig. 5B) respectively. Liver sections of group III showed depletion in the glycogen granules in hepatocytes as compared with group I (Fig. 5C). Meanwhile this depletion was apparently decreased in group IV (Fig. 5D).

**Fig.1**

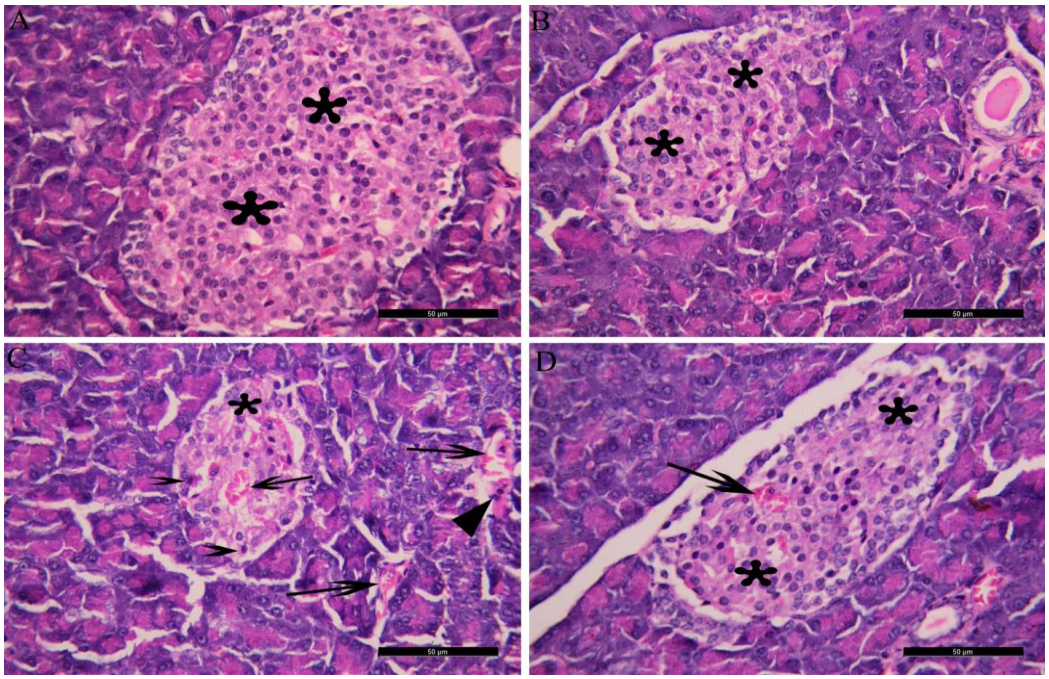



**Fig.1. Photomicrographs of sections of the pancreas stained by H&E. A. Section from control group showing the islet (*) is surrounded by a delicate connective tissue capsule. It appears formed of irregular branching and anastomosing cords of cells separated by blood capillaries. B. Section from a BV group II showing picture similar to control group. C. Section from a diabetic group III showing disorganization of the Islet (*) with paucity of cells which appear vacuolated and degenerated. Some cells show acidophilic degeneration with deeply stained pyknotic nuclei (<). Mild congestion (↑) and mononuclear inflammatory cells infiltration (▲) could be seen. D. Section from a group IV shows nearly regular outline of an islet (*) with healthy appearance. Mild congestion (↑) could be seen. H&E staining, scale bar = 50µm**

**Fig.2**

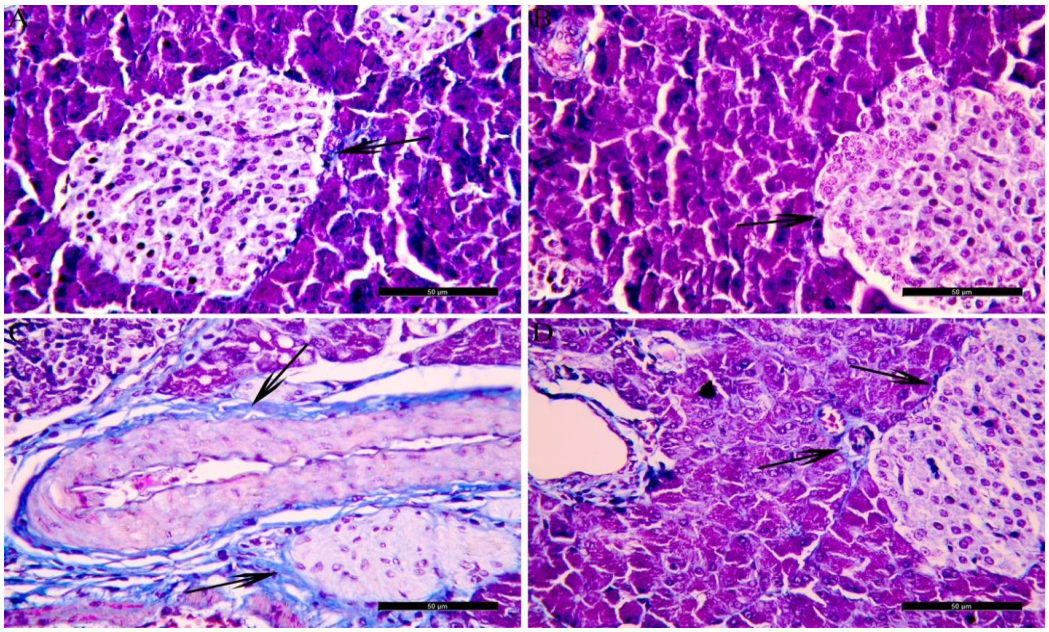



**Fig.2. Photomicrographs of sections of the pancreas stained by Masson trichrome. A. Section from control group showing few collagen fibers (↑) in between the closely packed pancreatic acini and around the islets. B. Section from a BV group II showing picture similar to control group. C. Section from a diabetic group III showing the apparent increase in the collagen fibers deposition (↑) in-between the exocrine and endocrine portions of pancreas and around blood vessels. D. Section from a rat of group IV showing minimal collagen fibers (↑) deposition in-between the exocrine and endocrine portions of the pancreas and around blood vessels. Masson Trichrome stain, scale bar = 50µm.**

**Fig.3**

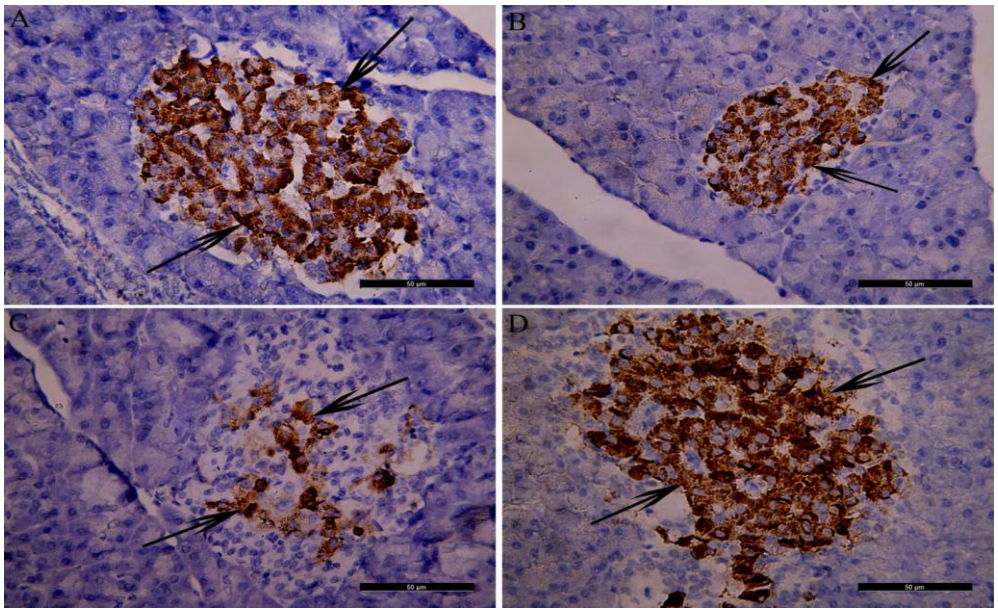



**Fig.3. Photomicrographs of insulin immunohistochemical staining of pancreatic islets. A. Section of the control group showing intense positive brownish reaction (↑) in the cytoplasm of islets cells. B. Section from a BV group II showing picture similar to control group. C. Pancreas of a diabetic group III showing weak brownish reaction (↑) in the cytoplasm of islets of Langerhans cells. D. Section from a rat of group IV showing intense positive brownish reaction (↑) in the cytoplasm of islets cells. immunohistochemical by anti-insulin antibodies, scale bar = 50 µm.**

**Fig. 4**

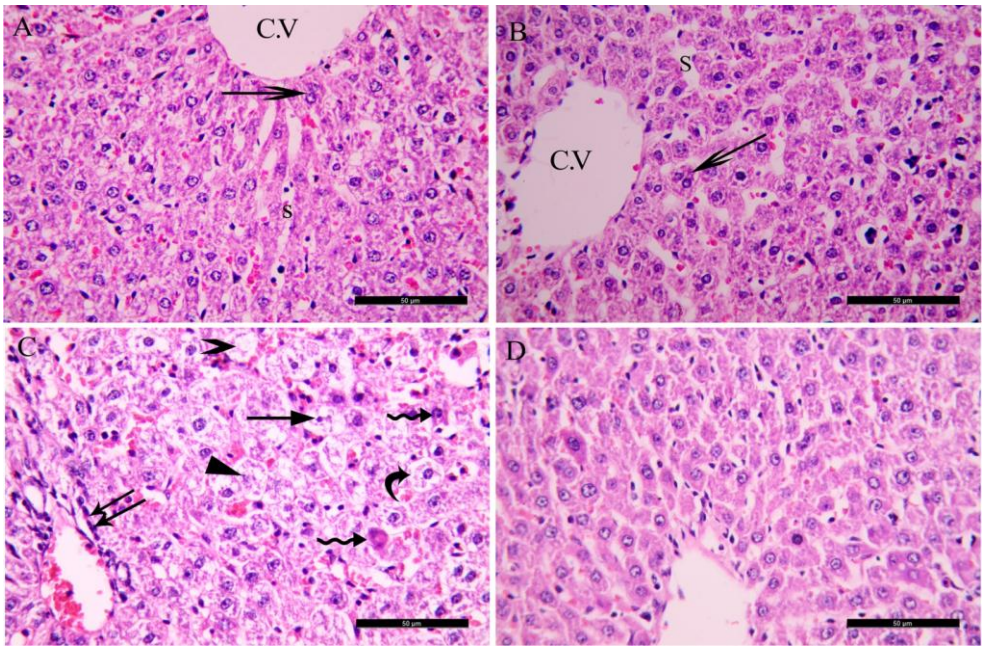



**Fig.4. Photomicrographs of sections of the liver stained by H&E. A. Section of the control group showing radiating cords of hepatocytes from the central vein (C.V.). The hepatocytes have central, rounded, vesicular nuclei and acidophilic cytoplasm. Some of the cells appear binucleated (↑). Blood sinusoids (S) appear in-between hepatocytes cords. B. Section from a BV group II showing picture similar to control group. C. Liver of a diabetic group III showing extensively degenerated and vacuolated hepatocytes (curved arrow). Few hepatocytes show acidophilic degeneration (zigzag arrow). Some hepatocytes nuclei appear deeply stained pyknotic (↑) while other nuclei appear karyorrhectic (▲) and karyolytic (＜). Moreover, there was mild congestion in the central vein and blood sinusoids with mild mononuclear cell infiltrate (↑↑) in the portal area. D. Section from a rat of group IV showing hepatocytes cords appear radiating from the central vein (C.V.) with central, rounded, vesicular nuclei and acidophilic cytoplasm. H&E staining, scale bar = 50 µm.**

**Fig. 5**

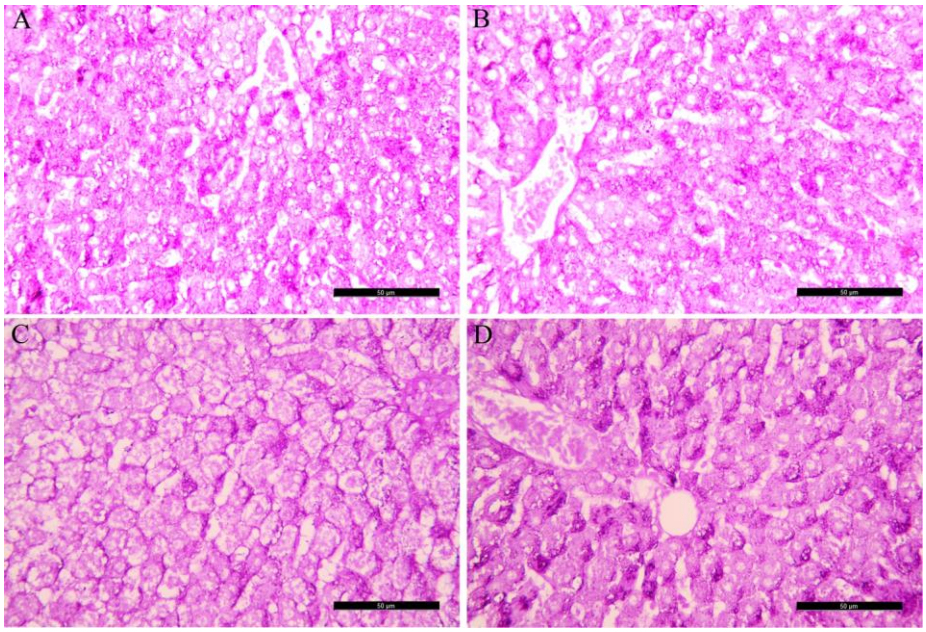



**Fig.5. Photomicrographs of sections of the liver stained by PAS. A. Section of the control group showing PAS positive granules (↑) in the cytoplasm of the hepatocytes. B. Section from a BV group II showing picture similar to control group. C. Liver of a diabetic group III showing apparent decreases of PAS positive granules (↑) in the cytoplasm of the hepatocytes. D. Section from a rat of group IV showing apparent increase of PAS positive granules (↑) in the cytoplasm of the hepatocytes as compared to group III. PAS staining, scale bar =50 µm.**

## DISCUSSION

DM is considered a chronic, systemic, metabolic disease defined by hyperglycemia and characterized by alterations in the metabolism of carbohydrate, protein and lipid.[28] The majority of islets cells of pancreas are formed of β cells which are responsible for producing insulin. Depletion of β cells will results in insulin deficiency which will lead to a disorder in carbohydrate, protein and fat metabolism with a resultant hyperglycaemia.[29] STZ was chosen in this study because it was previously proved to induce type I DM by selective destruction of the DNA of β cells of the islets by cell mediated anti-beta immune response.[30]

Type 1 diabetes occurs due to autoimmune destruction of insulin-producing β-cells in the pancreatic islets of Langerhans. This autoimmune process results from immune dysregulation, in which T helper 1 (Th1) cells cytokines as interleukin 2, interferon gamma and tumor necrosis factor beta dominate over an immunoregulatory (suppressor) Th2 subset cells cytokines as IL-4 and IL-10. These Type 1 cytokines initiate a cascade of immune/inflammatory processes in the islets (insulitis) through activation of cytotoxic T cells that interact specifically with β-cells and macrophages to produce proinflammatory cytokines (IL-1 and TNFα) which together with oxygen and nitrogen free radicals are highly toxic to islet β-cells. Therefore, stimulating Type 2 cytokines, inhibiting Type 1 cytokines and inhibiting oxygen and nitrogen free radicals in the pancreatic islets is the aim for prevention of type 1 diabetes.[31]

Several mechanisms have been postulated in humans for explanation of this selective beta cell destruction; however the precise details are poorly understood. Due to late appearance of symptoms in the process of beta cell destruction, it is difficult to study the early phases of disease in humans. For these reasons, animal models have been studied as a means of gaining insight into the human disease.[32]

In our study, BV therapy revealed decrease in plasma glucose level in group IV (treated group) in comparison to group III (diabetic group) with statistically significant difference. These changes are in line with **Mousavi et al**.[21] who confirmed hypoglycaemic activity of BV in diabetic mice. Our results were also consistent with **Ivas**[33] study in which BV reduced glycaemia and cholesterolemia in rabbits. Regarding the histological examination in our experiment, group III showed disorganization of the endocrine portions of pancreas with apparent decrease in the number of Langerhans cells in islets which contain residual β cells. These results supports the possibility of a specific destruction of β cells as the cause of type I DM[34] and this in harmony with **Abdel Aziz et al**.[29] who mentioned that massive deposits of a homogenous eosinophilic material largely occupying the islets and around blood vessels was seen in the pancreas of many diabetic rats and when present in large amount induce pressure atrophy on the surrounding structures.

However, group IV showed great improvement of the histological structure of the pancreas. This improvement could be attributed to melittin and phospholipase $A_2$

contained in the BV through suppression of β cells inflammation[35] and thus elevating insulin secretion[36] which was documented in our study by significant increase in C.pepetide in group IV in comparison to group III as well as negative CRP detection in group IV in comparison to group III with positive CRP detection. Thus, our findings are in agreement with **Nam et al**.[37] who explained that the anti-inflammatory activity of honey BV is mediated through suppression of the NF-κB signaling pathway. So, increased levels of CRP can be adjusted by treating rats with honey BV for 14 days.

Moreover, significant increase in the area percentage of anti-insulin antibody was noticed in group IV as compared to group III. These findings are in agreement with **Abdel Aziz et al**.[29] who mentioned that melittin polypeptide promotes insulin secretion from islets β cells through depolarization of plasma membranes of β-cells and acts as a calcium transporter in the cell, which in turn promotes insulin granules secretion.[38] **Simonson et al**.[36] suggested another explanation by which mellitin promotes insulin secretion via activating phospholipase $A_2$ in Islets of Langerhans which has a vital role in compensating insulin resistance response in Islets of Langerhans. Treatment with exogenous phospholipase $A_2$ or mellitin promote arachidonic acid and lysophospholipids production[36] and this Arachidonic acid which produced by phospholipase $A_2$ induction may acts as a calcium transporter in to β -cells and promotes insulin secretion.[39] Another mechanism was reported by **Abdel Aziz et al**.[29] who proved immune-modulating effect of BV which can inhibit the onset of type I diabetes in diabetic rat. At different levels, in human innate and adaptive immune responses, bee venom suppresses DNA synthesis, decreases pro-inflammatory cytokines (IL-2, IL-12 and IL-4), inactivates both the classical and alternative complement pathway, decreases superoxide anion production in neutrophils and promotes CD4+CD25+ regulatory T-cell differentiation which further can suppress the development of autoimmune diseases, such as rheumatoid arthritis and multiple sclerosis[40] but these were not assessed in the present study.

In the current study, Masson's trichrome stained sections of pancreas showed significant increase in collagen content in group III as compared to group IV. This finding is in agreement with **Siham et al**.[24] who found that the collagen fibers were seen around the blood capillaries and between the endocrine and the exocrine portion of the pancreas causing insufficient oxygen to reach the tissue, which resulted in degenerative changes and necrosis.[41]

According to the obtained results in our study, BV decreased plasma TG and cholesterol levels in group IV. **Park et al**.[35] related these results to phospholipase A2 enzymatic action which plays the central role in reducing cholesterol, TG, LDL and in increasing HDL and regulating the lipid profile. Phospholipase A2 partially lyses cell membrane through its enzymatic action on the plasmatic lipoproteins.[42] This activity increases glucose transport and lipid take-up into adipose tissue through partial lyses of adipocytes membrane and binding of higher number of insulin molecules.[39] Other studies suggest that through phospholipase A2 affinity to the plasmatic lipoproteins, it exerts its cytotoxic effect by generating free fatty acids and lisophospholipids, thus free cholesterol in HDL is esterified.[39] Also **Mousavi et al**.[21] explained that BV improves glycemic control and increased glucose consumption is instead of lipids. Acetyl coA derived from pyruvic acid enters Krebs cycle which finally leads to glucose metabolism, however Acetyl coA can enter TG synthesis pathway in usual condition while decreased cholesterol level in group IV most probably is due to inhibition of its absorption in small intestine and promoting its hepatic release.[43] in which the liver plays a critical role in discharging cholesterol via bile secretion. According to **Khulan et al**.[39] another possible strategy is improving insulin action in fat cells which results in lowering plasma LDL, triglyceride and in increased plasma HDL levels by activation of lipoprotein lipase enzyme and hydrolysis of triglycerides.

Attention has long centered on the liver in DM because of the importance of this organ in carbohydrate metabolism and regulation of blood sugar. In our study, H&E stained liver sections of diabetic rats showed loss of structural integrity of the liver. These findings are in line with **Ali Akbar et al.**[44] who mentioned that oxidative stress is currently suggested as mechanism underlying diabetes and diabetic complications which results from an imbalance between radical-generating and radical-scavenging systems. They added that, in diabetes, protein glycation and glucose autoxidation may generate free radicals, which in turn catalyse lipid peroxidation. On the other hand, it was established that hyperglycemia increases mitochondrial reactive oxygen species production, which could represent a key event in the development of diabetes complications.[45] In group IV, BV prevented these pathologic changes as hepatocytes appeared healthy with no considerable vacuolation were observed. These findings agreed with **Mousavi et al**.[21] who suggested that BV significantly inhibits enzymatic lipid peroxidation and also possesses a considerable hydroxyl radical scavenging activity which indicates its antioxidant activity.

Early studies on glycogen metabolism in type I diabetic patients using liver biopsies revealed controversial results, reporting either increased or decreased liver glycogen concentrations.[46]

In our study, PAS stained liver sections revealed that the glycogen content of the hepatocytes in diabetic animals was markedly decreased and this finding was in harmony with **Noman et al**.[47] who attributed this glycogen reduction to the displacement of glycogen in the cytoplasm of hepatocytes as a consequence of

accumulation of lipid droplets. **Afrin et al.**[48] also noticed that while liver sections of control rats showed the normal distribution pattern of glycogen granules, liver sections of diabetic rats showed depletion in these glycogen granules. On the other hand **Waer and Helmy**[49] used antioxidant drugs in diabetic rats and they noticed degradation of liver glycogen and increase gluconeogenesis, with increase in Glucose 6-phosphatase in the liver facilitating glucose release into the blood.

## CONCLUSION

All these previous findings revealed that BV may have a protective role on the biochemical and histological changes of β cells of Islets of Langerhans and liver in STZ induced diabetic rats either through suppression of pancreatic beta cell inflammation, antioxidant activity, promotion of insulin secretion or promotion of glucose uptake in adipose tissue with hypolipidemic activity through improvement of lipid uptake into adipose tissue and hydrolysis of triglyceride. However, further biochemical and pharmacological studies are necessary to provide more detailed understanding of the underlying mechanisms and to determine the most appropriate BV dose with the best therapeutic effect.

### Conflict of interest

The authors **Sara Abdel Gawad, Heba Fikry, Mariam Maged Amin, Amira Ramadan Elmahdi, and Doaa Abd Elaziz** declare that no funding or grant was received for the study and that they have no conflict of interest, financial or personal relationship related to the study.

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