Biochemistry Letters, 17(1), pages 77-107

Scientific Research & Studies Center-Faculty of Science- Zagazig University- Egypt

Biochemistry Letters

Journal home page:

Bee venom attenuates degenerative effects of diabetes associated with hyperlipidemia in rats.

Faten Zahran[1], Alaa Mohamed[1] , Nabila Zein[1]

[1]Biochemistry Division, Chemistry Department, Faculty of science, Zagazig University, Zagazig, Egypt.

ARTICLE INFO

Received : 27/7/2021
Accepted : 20/9/2021
Available online : 20/10/2021

Keywords:
Diabetes, Hyperlipidemia, Bee venom, Oxidative stress, Fetuin-A

ABSTRACT

Background: Natural products have been known as one of the most important therapeutic agents for diabetes and hyperlipidemia. Bee venom is a multipurpose agent that contains different bioactive compounds including melittin and phospholipase A2. **Aim**: The aim of this study was to investigate the effects of bee venom on biochemical and histopathological abnormalities in pancreas, liver and kidney of diabetic hyperlipidemic rats compared to synthetic drugs (Metformin and Atorvastatin).**Material and methods**: Bee venom's median lethal dose (LD_{50}) was determined, and then 50 adult male albino rats were divided into five groups: group 1 was fed standard diet and served as a negative control group, while the other four groups were received streptozotocin and nicotinamide injections to induce type 2 diabetes and after diabetes confirmation, rats were fed a high-fat diet for 28 days and then they were divided as follows: group 2 : served as a positive control group, and the remaining three groups represented the treated groups, group 3: bee venom treated group (0.5mg/kg), group 4: bee venom treated group (1/10 LD_{50}) (1.23mg/kg), and group 5: Metformin (60 mg/kg) plus Atorvastatin (10 mg/kg) treated group for 28 days, respectively. At the end of the experiment: blood samples, liver, kidney, and pancreas tissues were collected. **Results**: Treatment of diabetic hyperlipidemic rats using two doses of bee venom (0.5 and 1.23 mg/kg) and Metformin plus Atorvastatin revealed significant decrease ($p< 0.0001$) in levels of glucose, HOMA-IR, total protein, globulin, blood urea nitrogen, creatinine, MDA, Fetuin-,A, ALT and AST activities compared to the positive control group. Furthermore, Levels of insulin, HOMA-ß, Albumin, A/G ratio, and TAC were significantly increased compared to the positive control group ($p< 0.0001$). Our results were confirmed by histopathological examination of the pancreas, liver, and kidney tissues. **Conclusion**: Bee venom can be considered as a new potential therapeutic strategy for diabetes associated with hyperlipidemia.

Corresponding Author: Nabila Zein, Adress: Zagazig university post office: 29 Saad Zaghloul, Zagazig, Ash Sharqia Governorate, Postal Code: 44519, P.O.box: 10162 Tel. No: 00201093087238, Fax. No: 055-2346461, Email: dr.nabila.zein@gmail.com.

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by the absence of insulin or a reduction in the action of insulin that contributes to carbohydrates, proteins, and lipids metabolism disorders [1]. The most prevalent type of diabetes mellitus, type 2 diabetes, is characterized by insulin resistance (IR) and pancreatic beta cells dysfunction [2]. A clinical hallmark to diabetes, chronic hyperglycemia, resulting in significant pathological abnormalities and metabolic imbalances in liver and kidney [3]. Dietary intake, obesity, metabolic disorders such as diabetes and familial hypercholesterolemia (FH) are responsible for hyperlipidemia [4]. Hyperlipidemia is a complex disease refers to increased levels of lipids in the blood including cholesterol, cholesterol esters, phospholipids and triglycerides [5]. Lipid accumulation occurs and induces chronic kidney and liver diseases when the balance of lipid uptake, synthesis, and excretion is disrupted [6,7].

Oxidative stress may occur because of glucose and lipid metabolism disorders, that favor dyslipidemia and hyperglycemia [8]. An imbalance between antioxidant and reactive oxygen species causes oxidative stress [9], that can cause molecular damage, redox signaling disruption, modification in the function and structure of cellular proteins and lipids, triggering tissue injury, metabolism , impairment, disturbed cell cycle and cell signaling control [10].

Treatment with medication may induce adverse side effects, therefore, much concern is focused on natural products, which can provide minimization of harmful side effects [11]. In developing treatments for many diseases, great attention has recently been paid to the venomous products of certain species [12]. Bee venom is a complex combination of active ingredients, including phospholipase A2, peptides, including apamin, melittin, mast cell degranulating peptide, tertiapin, adolapin, procamin, cardiopep, secapin, and non-peptide (e.g., carbohydrates , lipids, biogenic amines, and histamine) [13].The main ingredients are melittin and phospholipase A2 and they were known to have a large range of medicinal effects [14].

Bee venom improves insulin secretion from β-cells and reduces the levels of glucose, as well as its lipolytic actions [15]. A study revealed that bee venom had diminishing effects on hyperglycemia and dyslipidemia [16]. The aim of this study was to investigate the effects of bee venom on biochemical and histopathological abnormalities in the pancreas, liver, and kidney of diabetic hyperlipidemic rats compared to synthetic drugs (Metformin and Atorvastatin).

Materials and Methods

Materials:

Bee venom

The bee venom sample was collected from *Apis mellifera carnica* that is kept at Plant Protection Research Institute, Agriculture Research Centre, Egypt.

Chemicals and drugs

Nicotinamide and streptozotocin were obtained from sigma chemicals Co, USA. Cholic acid, cholesterol, and Tert. Butylhydroquinone was purchased from Alpha chemical company, India. Vitamins, minerals mixtures, choline bitartrate ,and L-cystine were purchased from Acros-Organics Chemical Co, USA. Atorvastatin Calcium and Metformin Hydrochloride were kindly supplied by Amoun Pharmaceutical Co., Egypt.

Experimental Animals

Male adult albino rats, weighing 150-

170 ,g, were obtained from the Experimental Animal Care Center from Cairo University and were kept at experimental animal house of Faculty of Science, Zagazig University. The rats were maintained in a controlled environment of temperature, humidity , and light. The rats were allowed free access to tap water and fed a standard chow diet *ad libitum*. The Ethical Committee of Zagazig University was approved experimental design and animal handling (Approval number ZU-IAUUC /1/F/32/2019).

Methods

Determination of median lethal dose (LD_{50}) of bee venom

The median lethal dose (**LD_{50}**) of bee venom was determined according to a previous method [17].

Experimental diets preparation

The standard diet was prepared according to American Institute of Nutrition (AIN-93M) [18], which consisted of 14% casein, 10% sucrose, 4% soybean oil, 5% cellulose, 3.5% mineral mixture, 1% vitamin mixture,0.18% L-cystine , 0.25% choline bitartrate, 0.0008% Tert. Butylhydroquinone, 5% fats, and 57.0692% corn starch. The components of a high fat diet (HFD) were the same as those of a standard diet, with the addition of 1% Cholesterol, 0.2% cholic acid, and 23% fats [19]. The used vitamin and mineral mixtures were that recommended by the American Institute of Nutrition (AIN-93M) [18].

Experimental design

After seven days of accommodation, total number of 50 adult male albino rats were divided into 5 groups (10 rats /group). Group 1: served as negative control group, received intraperitoneal injections of saline solution and fed on a standard diet. All rats in the other groups were fasted for 18 hours before induction of type 2 diabetes mellitus. Rats were received a single intraperitoneal (i.p) injection of 120 mg/kg nicotinamide dissolved in normal saline and after 15 minutes, 60 mg/kg streptozotocin dissolved in a freshly prepared 0.1 M citrate buffer, (pH 4.5) was injected intraperitoneally [20]. 5 % glucose solution was given overnight to prevent hypoglycemia. Blood glucose levels were determined for all rats using a portable glucometer after 3^{rd} and 7^{th} day after injection and only rats with blood glucose levels higher than 250 mg/dl were considered diabetic [21]. Subsequently, diabetic rats were fed on a high fat diet (HFD) for 28 days. Upon termination of 28 days, blood samples were withdrawn from the tail vein of each diabetic rat for determination of lipid profile to confirm the induction of hyperlipidemia [22], then they were divided as follows: group 2 received no treatment, fed on a high-fat diet (HFD) and served as a positive control group, group 3, received bee venom (0.5mg/kg) [15] , group 4, received bee venom ($1/10^{th}$ of LD_{50}) (1.23 mg/kg) [23] daily for 28 days intraperitoneally along with the HFD, group 5, received metformin (60 mg/kg) and atorvastatin (10 mg/kg) daily for 28 days orally along with the HFD [24].

Bee venom, Metformin, and Atorvastatin doses were dissolved in distilled water and adjusted every week based on any changes in body weight to ensure that each group received the same dose per kilogram of body weight throughout the experimental trial.

Samples collection

The rats fasted for 10 hours at the end of the experiment, and blood samples were taken from the retroorbital venous plexus under urethane anesthesia. Blood samples were collected in three types of tubes, the first containing sodium

fluoride to get plasma for determination of glucose concentration, the second containing EDTA to obtain plasma for estimation of insulin concentration and the third is free coagulant tube to get serum by centrifugation at 4000 rpm for 20 min for determination of liver and kidney function tests. Until biochemical analysis, all of these samples were kept at -20°C. [25].Different tissues (pancreas, liver, and kidney) were excised from each rat, rinsed in saline solution, cleared off blood, and divided into two parts: The first portion (0.2 g) from each rat's liver, pancreas, and kidney tissues were homogenized in 2 ml phosphate buffer saline (pH 7.4) using Teflon homogenizer and then the homogenate was centrifuged at 3000 rpm for 10 min at 4°C. The supernatant of each tissue homogenate was stored at -20^0 C [26] for determination of total antioxidant capacity and malondialdehyde concentration, whereas the concentration of fetuin-A was measured in liver tissue homogenate only. For histopathological examination, the second portion of liver, pancreas, and kidney tissues was fixed in 10% formalin solution.

Biochemical parameters

Determination of Glucose, Insulin, HOMA-IR, and HOMA- β.

Plasma glucose was estimated according to the method [27] using a commercial kit derived from Spectrum Diagnostics Co., Egypt. Plasma insulin was estimated according to the method [28] using an ELISA kit derived from Cloud-Clone Crop, USA. Homeostatic model assessment of insulin resistance (HOMA-IR) and homeostatic model assessment of β-cell function (HOMA-β) was calculated according to the following equations [29].

HOMA-IR = [fasting plasma glucose (mg/dl) × fasting plasma insulin (μIU/mL)] /405.

HOMA- β= [360 × fasting insulin (μIU /ml)]/ [fasting glucose (mg/dl) –63].

Determination of liver functions tests

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured using commercial kits derived from Diamond Diagnostic Co., Germany according to the methods [30,31], respectively. Serum total protein and albumin concentration were measured using commercial kits derived from Diamond Diagnostic Co., Germany according to the methods [32,33], respectively. Serum globulin was calculated by subtracting the total protein value from the albumin value of each corresponding sample [34]. A/G ratio was calculated from the serum albumin present with concerning the amount of globulin [35].

Determination of Kidney functions tests

The concentration of serum blood urea nitrogen concentration was measured according to the method [36] and serum creatinine was measured according to the method [37] using commercial kits derived from (Diamond Diagnostic Co., Germany).

Determination of total antioxidant capacity and malondialdehyde

The concentrations of total antioxidant capacity and malondialdehyde were determined according to the methods [38,39] using commercial kits derived from Biodiagnostic Company, Egypt.

Determination of Fetuin-A

Fetuin-A concentration was determined according to the method described by [40] using an ELISA kit derived from Cloud-Clone Crop, USA.

Histopathological examination

A piece of pancreas, kidney, and liver samples was fixed in 10% formalin for 24h. The fixed tissues were embedded in paraffin, sectioned, deparaffinized, rehydrated, and stained with hematoxylin

and eosin for microscopic examination [41].

Statistical analysis

The Statistical analysis was performed using Package for Social Sciences (SPSS version 25) [42]. The data were expressed as mean ± SEM. The statistical significance was evaluated by ANOVA test followed by Tukey's post hoc test. $p<0.05$ is considered statistically significant.

RESULTS:

The median lethal dose (LD_{50}) of Carniolan bee venom

Median lethal dose (LD_{50}) of Carniolan bee venom was found to be 12.3 mg/kg.

Effect of bee venom (0.5 and 1.23 mg/kg) and combined therapy (Metformin and Atorvastatin) on Glucose, Insulin, HOMA-IR and HOMA- β levels.

The levels of glucose, insulin, HOMA-IR and HOMA- β in all studied groups were shown in Table (1). The mean value of plasma glucose was found to be 91.2±0.94 mg/dl in the negative control group. This value was significantly increased to 467.8±8.44 mg/dl by 412.94% in positive control group compared to the negative control group ($p<0.0001$). Meanwhile, the mean values of plasma glucose were significantly decreased to 161.6±2.12 mg/dl, 103.4±1.17 mg/dl and 96.5±1.32 mg/dl by -65.45%, -77.89% and -79.37% in bee venom treated group (0.5mg/kg), bee venom treated group (1.23 mg/kg) and Metformin and Atorvastatin treated group, respectively compared to the positive control group ($p<0.0001$).

The mean value of plasma insulin was found to be 126.0±1.16 pg/ml in the negative control group. This value was significantly decreased to 57.9±1.32 pg/ml by -54.05% in the positive control group compared to the negative control group ($p<0.0001$). Meanwhile, the mean values of plasma insulin were significantly increased to 82.7±2.03 pg/ml, 121.6±0.9 pg/ml and 123.1±1.14 pg/ml by 42.83%, 110.01%, and 112.61% in bee venom treated group (0.5mg/kg), bee venom treated group (1.23 mg/kg) and Metformin and Atorvastatin treated group, respectively compared to the positive control group ($p<0.0001$).

The mean value of homeostatic model assessment of insulin resistance (HOMA-IR) was found to be 15.0±0.19 in the negative control group. This value was significantly increased to 35.3±0.87 by 135.33% in the positive control group compared to the negative control group ($p<0.0001$). Meanwhile, the mean values of HOMA-IR were significantly decreased to 17.2±0.25, 16.4±0.12, and 15.7±0.13 by -51.27%, -53.54% and -55.52% in bee venom treated group (0.5mg/kg), bee venom treated group (1.23 mg/kg) and Metformin and Atorvastatin treated group, respectively compared to the positive control group ($p<0.0001$).

The mean value of homeostatic model assessment of β-cell function (HOMA- β) was found to be 864.1±31.57 in negative control group. This value was significantly decreased to 27.4±1.03 by -96.83% in the positive control group compared to the negative control group ($p<0.0001$). Meanwhile, the mean values of HOMA- β were significantly increased to 161.3±6.93, 579.4±19.69 and 734.4±39.83 by 488.68%, 2014.6% and 2580.29% in bee venom treated group (0.5mg/kg), bee venom treated group (1.23 mg/kg), and Metformin and Atorvastatin treated group, respectively compared to the positive control group ($p<0.0001$).

Effect of bee venom (0.5 and 1.23 mg/kg) and combined therapy

(Metformin and Atorvastatin) on serum liver function tests.

The levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin, globulin and albumin/ globulin ratio (A/G ratio) in all studied groups were represented in Table (2). The mean value of alanine aminotransferase (ALT) activity was found to be 37.2±0.81 U/L in negative control group. This value was significantly increased to 83±1.24 U/L by 123.1% in the positive control group compared to the negative control group ($p<0.0001$). Meanwhile, the mean values of ALT activities were significantly decreased to 57.4±1.55 U/ L, 46.4±0.73 U/ L, and 44.4±0.97 U/L by -30.84%, -44.09% and -46.5% in bee venom treated group (0.5mg/kg), bee venom treated group (1.23 mg/kg) and Metformin and Atorvastatin treated group, respectively compared to the positive control group ($p<0.0001$).

The mean value of aspartate aminotransferase (AST) activity was found to be 89.1±3.1 U/ L in the negative control group. This value was significantly increased to 165.7±4.86 U/ L by 85.9% in the positive control group compared to the negative control group ($p<0.0001$). Meanwhile, the mean values of AST activities were significantly decreased to 113.2±2.85 U/ L, 108±3.9 U/ L, and 105±3.09 U/L by -31.68%, -34.82% and -36.63% in bee venom treated group (0.5mg/kg), bee venom treated group (1.23 mg/kg) and Metformin and Atorvastatin treated group, respectively compared to the positive control group ($p<0.0001$).

The mean value of total protein was found to be 6.1±0.1 g/dl in the negative control group. This value was significantly increased to 7.5±0.07 g/dl by 22.95% in the positive control group compared to negative control group ($p<0.0001$). Meanwhile, the mean values of total protein were significantly decreased to 6.6±0.05g/dl, 6.4±0.09g/dl and 6.3±0.05 g/dl by -12%, 14.6% and -16% in bee venom treated group (0.5mg/kg), bee venom treated group (1.23 mg/kg) and Metformin and Atorvastatin treated group, respectively compared to the positive control group ($p<0.0001$).

The mean value of albumin was found to be 3.9±0.06 g/dl g/dl in the negative control group. This value was significantly decreased to 2.9±0.06 g/dl by -25.64% in the positive control group compared to the negative control group ($p<0.0001$). Meanwhile, the mean values of albumin were significantly increased to 3.4±0.08 g/dl, 3.7±0.11 g/dl and3.7±0.09 g/dl by 17.24%, 27.58%, and 27.58% in bee venom treated group (0.5mg/kg), bee venom treated group (1.23 mg/kg) and Metformin and Atorvastatin treated group, respectively compared to the positive control group ($p<0.0001$).

The mean value of globulin was found to be 2.2±0.05 g/dl in the negative control group. This value was significantly increased to 4.4±0.06g/dl by 100% in the positive control group compared to negative control group ($p<0.0001$). Meanwhile, the mean values of globulin were significantly decreased to 2.9±0.14 g/dl, 2.8±0.11g/dl and 2.6±0.1g/dl by -34.09%,-36.36% and -40.9% in bee venom treated group (0.5mg/kg), bee venom treated group (1.23 mg/kg) and Metformin and Atorvastatin treated group, respectively compared to the positive control group ($p<0.0001$).

The mean value of A/G ratio was found to be 1.7±0.02 in negative control group. This value was significantally decreased to 0.66±0.02 by -61.4% in positive control group compared to negative control group ($p<0.0001$). Meanwhile, the mean values of A/G ratio were significantly increased to1.1±0.06,

1.3±0.08 and 1.4±0.1 by 66.66% , 96.96% and 112.12% in bee venom treated group (0.5mg/kg), bee venom treated group (1.23 mg/kg) and Metformin and Atorvastatin treated group, respectively compared to the positive control group (p<0.0001).

Effect of bee venom (0.5 and 1.23 mg/kg) and combined therapy (Metformin and Atorvastatin) on serum kidney function tests.

The levels of serum blood urea nitrogen and creatinine in all studied groups were displayed in Table (3). The mean value of blood urea nitrogen was found to be 35.3±0.82 mg/dl in the negative control group. This value was significantly increased to 57.8±1.31 mg/dl by 63.73% in the positive control group compared to the negative control group (p<0.0001). Meanwhile, the mean values of blood urea nitrogen were significantly decreased to 42.6±1.3mg/dl, 38.5±0.86mg/dl and 36.5±1.57 mg/dl by -26.29% , -33.39% and -36.85 % in bee venom treated group (0.5mg/kg), bee venom treated group (1.23 mg/kg) and Metformin and Atorvastatin treated group, respectively compared to positive control group (p<0.0001).

The mean value of creatinine was found to be 0.38±0.01mg/dl in the negative control group. This value was significantly increased to 0.71±0.017mg/dl by 86.84 % in the positive control group compared to the negative control group (p<0.0001). Meanwhile, the mean values of creatinine were significantly decreased to0.57±0.014 mg/dl, 0.53±0.007 mg/dl and 0.5±0.011mg/dl by -19.71%,-25.35% and -29.57% in bee venom treated group (0.5mg/kg), bee venom treated group (1.23 mg/kg) and Metformin and Atorvastatin treated group, respectively compared to positive control group (p<0.0001).

Effect of bee venom (0.5 and 1.23 mg/kg) and combined therapy (Metformin and Atorvastatin) on total antioxidant capacity levels.

The levels of total antioxidant capacity (TAC) in different tissue homogenates in all studied groups were shown in Table (4). The mean value of pancreatic TAC was found to be 0.96±0.02 mM/L in the negative control group. This value was significantly decreased to 0.51±0.01mM/L by -46.87% in the positive control group compared to the negative control group (p<0.0001). Meanwhile, the mean values of pancreatic TAC were significantly increased to 0.61±0.15 mM/L, 0.79±0.05 mM/L and 0.81±0.01mM/L by 19.6%, 54.9% and 58.8% in bee venom treated group (0.5mg/kg), bee venom treated group (1.23 mg/kg) and Metformin and Atorvastatin treated group, respectively compared to the positive control group (p<0.0001).

The mean value of hepatic TAC was found to be 1.352±0.009 mM/L in the negative control group. This value was significantly decreased to 0.98±0.012 by -24.4% in the positive control group compared to the negative control group (p<0.0001). Meanwhile, the mean values of hepatic TAC were significantly increased to 1.32±0.008 mM/L, 1.347±0.01mM/L and 1.33±0.006 mM/L by 34.69%, 36.73% and 35.71% in bee venom treated group (0.5mg/kg), bee venom treated group (1.23 mg/kg) and Metformin and Atorvastatin treated group, respectively compared to the positive control group (p<0.0001).

The mean value of renal TAC was found to be 1.73±0.026mM/L in the negative control group. This value was significantly decreased to 0.96±0.013 mM/L by -44.5% in the positive control group compared to the negative control group (p<0.0001). Meanwhile, the mean values of renal TAC were significantly increased to 1.063±0.023 mM/L,

1.335±0.025 mM/L and 1.332±0.014 mM/L by 10.72%, 39.06% and 38.75% in bee venom treated group (0.5mg/kg), bee venom treated group (1.23 mg/kg) and Metformin and Atorvastatin treated group, respectively compared to positive control group ($p<0.0001$).

Effect of bee venom (0.5 and 1.23 mg/kg) and combined therapy (Metformin and Atorvastatin) on malondialdehyde levels.

The levels of malondialdehyde (MDA) in different tissue homogenates in all studied groups were summarized in Table (5). The mean value of pancreatic MDA was found to be 64.6±1.7 nmol/g tissue in the negative control group. This value was significantly increased to 204±1.94 nmol/g tissue by 215.7% in the positive control group compared to the negative control group ($p<0.0001$). Meanwhile, the mean values of pancreatic MDA were significantly decreased to 183.2±1.37 nmol/g tissue, 151.9±1.68 nmol/g tissue and 530.1±13.23 nmol/g tissue by -10.19%,-25.53% and -24.65% in bee venom treated group (0.5mg/kg), bee venom treated group (1.23 mg/kg) and Metformin and Atorvastatin treated group, respectively compared to the positive control group ($p<0.0001$).

The mean value of hepatic MDA was found to be 511.1±2.7nmol/g tissue in the negative control group. This value was significantly increased to 788.4±20.33 nmol/g by 54.25% in the positive control group compared to the negative control group ($p<0.0001$). Meanwhile, the mean values of hepatic MDA were significantly decreased to 583.5±14.29nmol/g tissue, 35.3±15.13nmol/g tissue and 530.1±13.23 nmol/g tissue by -25.98%,-32.1%, and -32.76 % in bee venom treated group (0.5mg/kg), bee venom treated group (1.23 mg/kg) and Metformin and Atorvastatin treated group, respectively compared to the positive control group ($p<0.0001$).

The mean value of renal MDA was found to be 89.5±0.5 nmol/g tissue in thenegative control group. This value was significantly increased to 184.2±0.66nmol/g by 105.8% in the positive control group compared to negative control group ($p<0.0001$). Meanwhile, the mean values of renal MDA were significantly decreased to 170.3±1.03 nmol/g tissue, 147.5±1.12 nmol/g tissue and 147.4±1.11 nmol/g tissue by 7.54%,-19.92% and -19.97 % in bee venom treated group (0.5mg/kg), bee venom treated group (1.23 mg/kg) and Metformin and Atorvastatin treated group, respectively compared to the positive control group ($p<0.0001$).

Effect of bee venom (0.5 and 1.23 mg/kg) and combined therapy (Metformin and Atorvastatin) on Fetuin-A levels.

The levels of fetuin-A in liver tissue homogenate in all studied groups were shown in Table (6). The mean value of Fetuin-A was found to be 57.2±3.5ng/ml in the negative control group. This value was significantly increased to 159.9±5.79 ng/ml by 179.5% in the positive control group compared to the negative control group ($p<0.0001$). Meanwhile, the mean values of Fetuin-A were significantly decreased to 85.2±2.12 ng/ml, 63.5±1.69 ng/ml and to 61.6±1.12 ng/ml by -46.71%, -60.28%% and -61.47% in bee venom treated group (0.5mg/kg), bee venom treated group (1.23 mg/kg) and Metformin and Atorvastatin treated group, respectively compared to positive control group ($p<0.0001$).

Histopathological results

Histopathological pancreas sections revealed normal pancreatic parenchyma, pancreatic acini, ducts, and in the negative control group (Figure. 1A). Conversely, the positive control group experienced acute pancreatitis, the congested blood vessel, and lecucocytic

cells infiltration (Figure. 1B). The pancreas sections of the bee venom-treated group (0.5 mg/kg) (Figure. 1C) as well as the bee venom-treated group (1.23 mg/kg) (Figure. 1D) displayed slight congestion in the blood vessels. Metformin and Atorvastatin-treated group exhibited congestion in the blood vessels (Figure. 1E).

Histopathological liver sections exhibited normal hepatocytes, hepatic parenchyma, portal tract, and blood sinusoids in the negative control group (Figure. 2A). In contrast, the positive control group displayed portal tract changes, congested hepatoportal blood vessels, and hyperplastic bile duct (Figure. 2B). The liver sections of the bee venom-treated group (0.5 mg/kg) revealed congestion in the central vein and blood sinusoids, (Figure. 2C) . Unlike the bee venom treated group (1.23 mg/kg) (Figure. 2D) as well as the metformin and atorvastatin-treated group (Figure. 2E) exhibited normal hepatocytes, hepatic parenchyma, portal tract, and blood sinusoids similar to the negative control group.

Histopathological kidney sections revealed normal renal parenchyma, renal glomeruli, and renal tubules in the negative control group (Figure. 3A). Conversely, the positive group experienced acute interstitial nephritis, the interstitial congested blood vessels, and leucocytic cells infiltrations (Figure. 3B). The kidney sections from of the bee venom-treated group (0.5 mg/kg) displayed slight congestion in the glomerular blood capillaries and peri-renal blood capillaries (Figure. 3C) Unlike the bee venom treated group (1.23 mg/kg) (Figure. 3D) as well as the metformin and atorvastatin-treated group (Figure. 3E) exhibited normal renal parenchyma, glomeruli and renal tubules similar to the negative control group.

DISCUSSION:

Diabetes mellitus (DM) is a life-threatening metabolic disease and characterized by chronic hyperglycemia. It develops when insulin secretion and/or action are impaired, resulting in micro- and macrovascular consequences [43]. In addition to hyperglycemia, hyperlipidemia is one of the major factors that complicate the diabetic condition and leads to death [44]. Owing to changes in lifestyle and diet practice, hyperlipidemia is a prevalent issue in society. Diet, in addition to drugs, has a significant role in controlling blood lipid and lipoprotein levels. Previous studies have already shown that excessive high-fat diet intake often contributes to insulin resistance (IR) because insulin action has interfered with saturated fatty acids (SFA) [45].

One of the most widely used diabetogenic agents is streptozotocin. It is used to induce type 2 diabetes mellitus when combined with nicotinamide [46]. It is well known that streptozotocin causes selective damage to pancreatic B-cells. Nicotinamide is administered to rats to protect insulin-secreting cells against streptozotocin cytotoxicity. streptozotocin is transported through the glucose transporter-2 (GLUT2) into B-cells and causes DNA damage that leads to increased activity of poly (ADP-ribose) polymerase (PARP-1) that act to repair DNA. However, this enzyme's excessive activity results in the reduction of intracellular NAD+ and ATP and necrosis of insulin-secreting cells. Nicotinamide inhibits PARP-1 activity, preventing NAD^+ and ATP loss in streptozotocin-treated cells [47].

Bee venom is a complex mixture of peptides, proteins, and low-molecular-weight components released by the

worker and queen bees. [48]. Anti-diabetic activity is attributed to the presence of melittin which decreases blood glucose by various mechanisms including β-cell membranes depolarization, increased calcium channels and extracellular calcium[49], cytosolic phospholipase A2 activation [50], increased glucose and lipid transport into insulin's target tissues [51] , and inhibition the inflammation of β-cells [52].The study is aimed to investigate the effects of bee venom on biochemical and histopathological abnormalities in the pancreas, liver, and kidney of diabetic hyperlipidemic rats compared to synthetic drugs (Metformin and Atorvastatin).

The results of studies to determine the median lethal dose (LD_{50}) are crucial in determining the recommended limit dose. The median lethal dose (LD_{50}) was estimated by intraperitoneal administration of the of Carniolan bee venom in rats .Our results revealed that LD_{50} is equal to 12.3 mg/kg. These results were near to those obtained in a previous study [53], reporting the LD_{50} value of Carniolan bee venom (8.47 mg/kg) when injected intravenously into mice. In a previous study, the LD_{50} of Sweet Bee Venom in rats was over 30 mg/kg through single-dose toxicity tests [54]. Phospholipase A2 and melittin resulted in the toxicity of bee venoms, which act synergistically [55]. The venom of all Apis species is similar in quality and composition, but the differences in LD_{50} values may be due to the geographical distribution of different Apis mellifera species [56], and also due to variation in their production and toxicity according to their size and physiological differences [57,58].

Streptozotocin (STZ) is a toxic agent that can cause selective damage of pancreatic β - cells, resulting in a substantial rise in blood glucose levels, and perhaps due to STZ induction, a significant reduction in insulin secretion is observed [59].

The results revealed that the positive control group had a significant increase in plasma glucose levels and a significant decrease in plasma insulin levels compared to the negative control group. Our result is in harmony with previous studies [60,61] which stated that there was a significant increase in glucose levels and a significant decrease in insulin levels in diabetic rats because of the cytotoxic influence of STZ, which leads to β cells degradation and reduced insulin secretion in diabetic rats when compared to the negative control group. Meanwhile, bee venom-treated groups using two doses (0.5 and 1.23 mg/kg) and combined therapy (metformin and atorvastatin) showed a significant decrease in the levels of glucose and a significant increase in plasma insulin levels compared to the positive control group. Our results are in agreement with previous studies[15,62,63] which showed that the anti-diabetic activity of bee venom and its ability to increase insulin secretion. The active compounds in bee venom, melittin, and phospholipase-A2, may be related to the improvements in glucose and insulin levels. They diminish Langerhans islet inflammation and cause them to release insulin. [51,53]. Bee venom also inhibits the production of proinflammatory cytokines and free

radicals, which cause beta-cell death. [64]. Moreover, melittin can depolarize β-cells plasma membranes, resulting in the opening of Ca^{2+} channels, allowing a high amount of Ca^{2+} to enter -cells and increase insulin secretion. [50].

The induction of diabetes by STZ causes dysfunction of β-cells and high-fat diet leads to insulin resistance [65]. The pathogenesis of type 2 diabetes is defined by a reduction in -cell function as well as insulin resistance (IR). [66], so an increase in HOMA-IR and a decrease in HOMA- β are exhibited in diabetic hyperlipidemic rats.

The results illustrated that the positive control group had a significant increase in HOMA-IR and a significant decrease in HOMA- β levels compared to the negative control group. Our result is in agreement with previous research [67] which reported that a significant increase in HOMA-IR and a significant decrease in HOMA- β in diabetic rats when compared with the negative control group. Also, another study illustrated that high fat diet-fed male mice exhibited an elevation in HOMA-IR index and exhibited dysfunction of β- cell which was measured by calculating HOMA- β [68]. Meanwhile, bee venom-treated groups using two doses (0.5 and 1.23 mg/kg) and combined therapy (metformin and atorvastatin) showed a significant decrease in the levels of HOMA-IR and a significant increase in HOMA- β levels compared to the positive control group.

These results are consistent with an earlier study [69] which reported that HOMR-IR significantly decreased in rats fed on high-fat diet and treated with different doses of bee venom when compared to untreated rats because bee venom can improve plasma insulin levels which meant that the bee venom increases insulin sensitivity in the peripheral tissues which revealed as decreased HOMA-IR in rats treated with bee venom. The improvement in HOMA-β levels is attributed to the ability of bee venom to restore the normal histological structure and function of pancreatic β-cells [16].

Liver enzymes such as ALT and AST are biomarkers for liver integrity and function [70]. Cellular liver damage induced by streptozotocin indicates a disturbance in cell membranes of hepatocytes resulting in leakage of enzymes into blood and elevation in the activity of serum ALT and AST [71]. The results displayed that the activities of ALT and AST were significantly elevated in the positive control when compared to the negative control. These results are supported by a previous study which revealed that there was a significant increase in ALT and AST activities in the diabetic group compared to the negative group due to liver dysfunction induced by diabetes [72]. While groups treated with bee venom using both doses (0.5 and 1.23 mg/kg) and combined therapy (metformin and atorvastatin) showed a significant reduction) in ALT and AST activities when compared to the positive control group. These results are inconsistent with other studies [73,74] which showed that because of the potent hepatoprotective effect of bee venom, increased activities of aminotransferase enzymes were reduced in diverse models of induced hepatic injury due to the presence of phospholipase A2, which is regarded as the second most abundant component of bee venom. Bee venom inhibits the

secretion of pro-inflammatory cytokines [75], which was associated with hepatic injury and reduces aminotransferases enzymes [76].

Serum total protein plays an important role in osmotic pressure. With lower osmotic pressure, extra body fluid can build up in the tissues, resulting in edema. If the serum overall protein level is abnormal, additional tests should be performed to decide which type of specific protein level is declining or rising. If the serum total protein level falls below the normal limit, it normally indicates a reduced albumin level, which may be caused by liver disease or an acute infection [77]. A high concentration of total protein is attributed to an increase in globulin concentration due to infection and inflammatory diseases [78]. A/G ratio may reduce due to an increase in globulin levels during chronic inflammatory diseases, cancer and rheumatoid diseases, and diminished albumin levels that are associated with chronic liver diseases, nephrotic syndrome, and chronic infections [79].

The results stated that the positive control group exhibited a significant increase in levels of total protein and globulin and a significant decrease in levels of albumin and A/G ratio compared to the negative control group.

These results were supported by several studies [80-83] which indicated that in the case of diabetes, elevated total protein concentration was observed due to elevation in concentrations of different acute phase proteins, globulins, and fibrinogen that contribute to the increased concentration of total proteins, hypoalbuminemia is associated with hyperglycemia due to oxidative stress, low hepatic synthesis and increased urinary excretion of albumin resulting from diabetic nephropathy, hyperglobulinemia may be present in diabetic patients due to inflammatory processes and a significant decrease in A/G ratio was observed because of hypoalbuminemia and elevated protein catabolism that detected in diabetic rats. Through our results, treated groups with bee venom using both doses (0.5 and 1.23 mg/kg) and combined therapy (metformin and atorvastatin) showed a significant reduction in total protein and globulin levels and a significant increase in albumin and A/G ratio levels compared to positive control. Our outcomes are in agreement with a previous study [84] which accredited that the improvement in the levels of total protein, albumin, globulin, and A/G ratio was attributed to the presence of phospholipase A2 in bee venom which has antioxidant, anti-inflammatory, and hepatoprotective effects.

It is well accepted that the main extrinsic factors that contributed in the pathogenesis of renal dysfunction are obesity, sepsis, hypertension, liver diseases, and diabetes [85]. Diabetes leads to the production of free radicals, which produce profibrogenic and inflammatory mediators in organs such as the kidney leading to changes in renal blood flow and glomerular function which is assessed in terms of an increase in serum blood urea nitrogen and creatinine[86].

Our results showed that blood urea nitrogen and creatinine levels were significantly increased in the positive control group compared to the negative control group. These results come in accordance with previous studies [87,88]

which showed that diabetes is associated with a significant increase in levels of blood urea nitrogen and creatinine indicating renal tissue dysfunction. Meanwhile, there was a significant decrease in levels of blood urea nitrogen and creatinine in bee venom treated groups (0.5 and 1.23 mg/kg) and in Metformin and Atorvastatin treated group compared to positive control. In accordance with our results, a previous study [89] have shown that bee venom and its active components (phospholipase A2) could have remarkable reno-protective effects on diseases-induced renal dysfunction through upregulation of regulatory T cells (Tregs) which are immunosuppressive lymphocytes that play a vital role in maintaining immune tolerance. The Treg's anti-inflammatory activities can protect against renal injury and decreased levels of serum creatinine and blood urea nitrogen were confirmed. Also, another study [90] reported that melittin induces M2 macrophage activation, which is associated with parasite infiltration, tissue remodeling, and immunoregulatory functions and eventually leads to recovery of renal function, and reductions in nitrogen levels of creatinine and blood urea nitrogen were reported.

Total antioxidant potential (TAC) is a commonly used parameter to determine the overall antioxidant status in diseases that induce the production of free radicals, including diabetes mellitus [91].

Our results stated that levels of pancreatic, hepatic, and renal total antioxidant capacity (TAC) were decreased significantly in the positive control group compared to the negative control group. These results are in harmony with previous studies [92-94] which described that total antioxidant levels in the pancreas, liver, and kidney were reduced in diabetic rats because hyperglycemia is associated with an imbalance between reactive oxygen species production and antioxidant defense mechanism. While the levels of pancreatic, hepatic, and renal total antioxidant capacity (TAC) were significantly increased (0.5 and 1.23 mg/kg) and in Metformin and Atorvastatin treated group compared to positive control. These results are in agreement with other findings [62,95] which reported that the improvement of total antioxidant capacity in treated groups with bee venom compared to the diabetic control group is due to antioxidant activity of bee venom by suppressing production of radicals and scavenging free radicals.

Lipid peroxidation is an essential element, which is closely related to the consistency of cellular membranes and assists in assessing the structure and composition of cellular lipids. The presence of free radicals in some tissues may increase the rate of lipid peroxidation in diabetics. Malondialdehyde (MDA), is a lipid peroxidation marker, which was measured in diabetic rats' tissues as a marker of a higher degree of free radicals attacking and oxidative stress [96].

Our data illustrated that the positive control group exhibited significantly elevated levels of pancreatic, hepatic, and renal malondialdehyde (MDA) compared to the negative control. These findings are in accordance with previous studies [97,98] which stated that elevated malondialdehyde levels in pancreas, liver, and kidney tissues ascribed to damage of antioxidant defense system and an increase in lipid

peroxidation products in diabetic rats compared to normal rats. While the levels of pancreatic, hepatic, and renal malondialdehyde (MDA) were significantly decreased in bee venom treated groups (0.5 and 1.23 mg/kg) and in Metformin and Atorvastatin treated group compared to positive control. These results are in line with previous studies [99-101] which stated that the decrease in MDA level attributed to the antioxidant activity of bee venom that results in a reduction in reactive oxygen species levels and lipid peroxidation.

Fetuin-A is a glycoprotein that belongs to the 2-Heremans-Schmid family, that is synthesized in the hepatocytes. Fetuin-A is an effective regulator of dysfunctional vascular calcification, but has a role in insulin resistance [102]. Fetuin-A levels are greater in type 2 diabetic patients and obesity than in healthy controls. Fetuin-A blocks the insulin receptor Tyrosine kinase in skeletal muscle and liver cells, preventing insulin signals from being transmitted and leading to insulin resistance in target tissues of insulin [103]. In insulin resistance states, there are associations between gene expression of hepatic fetuin-A and key regulatory enzymes in lipid and glucose metabolism. The expression of fetuin-A tends to parallel the expression of both key enzyme in gluconeogenesis, phosphoenolpyruvate kinase 1 (PEPCK1), and key transcription factors in lipogenesis, hepatic sterol regulatory element-binding protein (SREBP) [104]. The activity of hepatic AMP-activated kinase (AMPK) is regarded as a significant factor in metabolic syndrome. Interestingly, AMPK is directly involved in the downregulation of all proteins associated with the expression of hepatic fetuin- A [105].

The results revealed that hepatic fetuin-A levels were significantly increased in positive control compared to the negative control. These findings in covenant with a previous study [106] which reported that there was a significant increase in the expression levels of fetuin-A in the liver of diabetic rats because a disruption in lipid profiles usually occurs during type 2 diabetes and insulin resistance leading to alteration in the expression of genes associated with lipid metabolism including fetuin-A. Also, Another study [107] reported that high levels of hepatic fetuin-A in rats ascribed to high fat diet consumption that promotes production and secretion of fetuin-A by hepatocytes. While the levels of fetuin-A were significantly decreased in bee venom treated groups (0.5 and 1.23 mg/kg) and in Metformin and Atorvastatin treated groups compared to positive control. It may be hypothesized that AMP-activated kinase (AMPK) activation prevents the production of fetuin-A [108]. A study reported that bee venom exhibits anti-obesity activity which is attributed to the ability of bee venom to activate hepatic AMP-activated kinase (AMPK) by phosphorylation. phosphorylated AMPK suppresses the expression of the 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA) and acetyl CoA carboxylase (ACC) leading to the suppression of fatty acids and cholesterol synthesis which are contributed to the elevation of hepatic fetuin-A synthesis [109]. Another study found that bee venom lowers hepatic

sterol regulatory element-binding protein (SREBP) levels in fructose-induced nonalcoholic steatohepatitis (NASH) rat model, resulting in lower levels of fetuin-A [104,110]. Based on our findings, which are compatible with these previous studies [109,110], bee venom decreases fetuin-A levels by suppressing hepatic sterol regulatory element-binding protein (SREBP) expression and through the activation pathway of AMP-activated kinase (AMPK).

Histopathological Studies of Pancreas sections revealed that, the negative group showed normal pancreatic parenchyma, pancreatic acini, ducts, and islets, but Positive control rats showed acute pancreatitis, the congested blood vessel and lecucocytic cells infiltration. These findings are consistent with those of a previous study [111] which stated that diabetes resulted in reduced cellular density in pancreatic islets and widespread β- cells degranulation. Meanwhile, in bee venom treated groups (0.5 mg/kg and 1.23 mg/kg), there is an appearance of slight congestion in the blood vessels. In Metformin and Atorvastatin treated group, it showed congestion in the blood vessels. These results are supported by an earlier study [112] which reported that the ability of bee venom to restore normal structure and function of the pancreas due to its antioxidant and anti-inflammatory effects.

Histopathological Studies of liver sections revealed that, the negative group showed normal hepatic parenchyma, hepatocytes, blood sinusoids, and portal tract, but Positive control rats showed portal tract changes, congested hepatoportal blood vessel, and hyperplastic bile duct. These findings are in agreement with an earlier study [16] which stated that hepatocytes were significantly degenerated and vacuolated in diabetic rats. In contrast, bee venom treated group (0.5 mg/kg) showed congestion in the central vein and blood sinusoids, the bee venom treated group (1.23 mg/kg) as well as Metformin and Atorvastatin treated group showed normal hepatic parenchyma, hepatocytes, blood sinusoids, and portal tract. These results are supported by an earlier study [69] which confirmed the hepatoprotective effect of bee venom against liver damage is induced by oxidative stress.

Histopathological Studies of kidney sections revealed that, the negative group showed normal renal parenchyma, renal glomeruli, and renal tubules, but Positive control rats displayed acute interstitial nephritis, the interstitial congested blood vessels, and leucocytic cells infiltrations. These results are consistent with those of a previous study [113] which stated that kidney tissue of STZ-diabetic rats displayed degenerative abnormalities in both renal tubules and glomeruli ,as well as congestion in the interstitial blood vessel. Meanwhile, in the bee venom treated group (0.5 mg/kg), it showed slight congestion in the glomerular blood capillaries and peri-renal blood capillaries. In the bee venom treated group (1.23 mg/kg) and Metformin and Atorvastatin treated group, it showed normal renal parenchyma, glomeruli, and renal tubules. Our results are in agreement with other studies [90,114]. which illustrated that in various models of kidney injury and nephrotoxicity, bee venom was shown to have a significant improvement in tubular and glomerulus damage owing to its protective role against nephrotoxicity by suppressing the secretion of pro-inflammatory cytokines.

Conclusion

The current research concluded that bee venom has protective effects against diabetes associated with hyperlipidemia-induced degenerative changes at biochemical and

histopathological levels of the pancreas, liver, and kidney in rats. Bee venom can ameliorate degenerative effects of diabetes associated with hyperlipidemia in a dose-dependent manner. Therapeutic dose (1.23 mg/kg) and combined therapy (metformin and atorvastatin) revealed potential effects that normalize biochemical and histopathological changes compared to the therapeutic dose (0.5 mg/kg) of bee venom.

REFERENCES:

[1] **Patti, M. E., and Corvera, S. (2010)**: The role of mitochondria in the pathogenesis of type 2 diabetes. Endocrine reviews., 31(3): 364-395.

[2] **Asmat, U., Abad, K. and Ismail, K. (2016)**: Diabetes mellitus and oxidative stress—A concise review. Saudi pharmaceutical journal., 24(5): 547-553.

[3] **Akpan, O. U., Ikpi, D. E. and Etim, B. E. (2013)**: Ocimum gratissimum alleviates derangements in serum and biliary bilirubin, cholesterol and electrolytes in streptozotocin-induced diabetic rats. International Journal of Biochemistry Research & Review., 3(3): 171-189.

[4] **Sudhakaran, S., Bottiglieri, T., Tecson, K. M., Kluger, A. Y. and McCullough, P. A. (2018)**: Alteration of lipid metabolism in chronic kidney disease, the role of novel antihyperlipidemic agents, and future directions. Reviews in cardiovascular medicine., 19(3):77-88.

[5] **Ibrahim, S. R., Mohamed, G. A., Banjar, Z. M. and Kamal, H. K. (2013)**: Natural antihyperlipidemic agents: Current status and future perspectives. Phytoarmacology.,4 (3):492-531.

[6] **Su, H., Wan, C., Lei, C. T., Zhang, C. Y., Ye, C., Tang, H.and Zhang, C. (2018)**: Lipid deposition in kidney diseases: interplay among redox, lipid mediators, and renal impairment. Antioxidants & redox signaling., 28(10): 1027-1043.

[7] **Lee, H. S., Nam, Y., Chung, Y. H., Kim, H. R., Park, E. S., Chung, S. J. and Jeong, J. H. (2014)**: Beneficial effects of phosphatidylcholine on high-fat diet-induced obesity, hyperlipidemia and fatty liver in mice. Life sciences., 118(1):7-14.

[8] **Almeida, D. A. T. D., Braga, C. P., Novelli, E. L. B. and Fernandes, A. A. H. (2012)**: Evaluation of lipid profile and oxidative stress in STZ-induced rats treated with antioxidant vitamin. Brazilian Archives of Biology and Technology., 55(4):527-536.

[9] **Sies, H. (2015)**: Oxidative stress: a concept in redox biology and medicine. Redox biology., 4: 180-183.

[10] **Newsholme, P., Cruzat, V. F., Keane, K. N., Carlessi, R. and de Bittencourt Jr, P. I. H. (2016)**: Molecular mechanisms of ROS production and oxidative stress in diabetes. Biochem J., 473(24): 4527-4550.

[11] **Apostolidis, E., Li, L., Lee, C. and Seeram, N. P. (2011)**: In vitro evaluation of phenolic-enriched maple syrup extracts for inhibition of carbohydrate hydrolyzing enzymes relevant to type 2 diabetes management. Journal of Functional Foods., 3(2): 100-106.

[12] **Roudbari, L. and Imani, S. (2012)**: The effects of Androctonus

crassicauda scorpion venom in the treatment of diabetes mellitus type 1 in animal models. Ann Biol Res., 3: 5782-5785.

[13]**Silva, J., Monge-Fuentes, V., Gomes, F., Lopes, K., dos Anjos, L., Campos, G., Arenas,C., Biolchi, A., Gonçalves, J., Galante, P., Campos, L. and Mortari, M. (2015)**:Pharmacological alternatives for the treatment of neurodegenerative disorders: wasp and bee venoms and their components as new neuroactive tools. Toxins (Basel)., 7 (8): 3179-3209.

[14] **Junior, R. S. F., Sciani, J. M., Marques-Porto, R., Junior, A. L., Orsi, R. D. O., Barraviera, B. and Pimenta, D. C. (2010)**:Africanized honey bee (Apis mellifera) venom profiling: Seasonal variation of melittin and phospholipase A2 levels. Toxicon., 56(3): 355-362.

[15] **Mousavi, S. M., Imani, S., Haghighi, S., Mousavi, S. E. and Karimi, A. (2012)**: Effect of Iranian honeybee (Apis mellifera) venom on blood glucose and insulin in diabetic rats. Journal of arthropod-borne diseases.,6(2):136-143.

[16] **Gawad, S. A., Fikry, H., Amin, M. M., Elmahdi, A. R. and Elaziz, D. A. (2016)**: Effect of apitherapy on the pancreas and liver of streptozotacin induced diabetic rats: a biochemical and histological study. European Journal of Pharmaceutical and Medical Research.,3: 555-565.

[17] **Reed, L. J. and Muench, H. (1938)**: A simple method of estimating 50 percent end points. American Journal of Epidemiology., 27(3): 493-497.

[18] **Reeves, P. G., Nielsen, F. H. and Fahey Jr, G. C. (1993)**: AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr., 123(11):1939-1951.

[19] **Yuan, Y., Liu, Q., Zhao, F., Cao, J., Shen, X. and Li, C. (2019)**: Holothuria leucospilota polysaccharides ameliorate hyperlipidemia in high-fat diet-induced rats via short-chain fatty acids production and lipid metabolism regulation. International journal of molecular sciences., 20(19): 4738-4751.

[20] **Punitha, I. R., Rajendran, K., Shirwaikar, A. and Shirwaikar, A. (2005)**: Alcoholic stem extract of Coscinium fenestratum regulates carbohydrate metabolism and improves antioxidant status in streptozotocin–nicotinamide induced diabetic rats. Evidence-Based Complementary and Alternative Medicine.,2(3): 375–381.

[21] **Deeds, M. C., Anderson, J. M., Armstrong, A. S., Gastineau, D. A., Hiddinga, H. J., Jahangir, A. and Kudva, Y. C. (2011):** Single dose streptozotocin-induced diabetes: considerations for study design in islet transplantation models. Laboratory animals ., 45(3):131-140.

[22] **Sunder, A. S., Reddy, A. R. N., Kiran, G. and Thirumurugu, S. (2010)**: Antihyperlipidemic and antioxidant activity of methanolic extract of Trianthema portulacastrum in rats fed a high-fat diet. Journal of herbs, spices & medicinal plants., 16(3-4): 193-202.

[23] **Phatak, R., Khanwelkar,C., Matule ,S., Datkhile ,K. and Hendre, A. (2019)**:

Antihyperglycemic Activity of Murraya koenigii Leaves Extract on Blood Sugar Level in Streptozotocin-Nicotinamide Induced Diabetes in Rats. Biomedical & Pharmacology Journal., 12(2): 597-602.

[24] **Matafome, P., Louro, T., Rodrigues, L., Crisostomo, J., Nunes, E., Amaral, C. and Seica, R. (2011)**: Metformin and Atorvastatin combination further protect the liver in type 2 diabetes with hyperlipidaemia. Diabetes/metabolis m research and reviews., 27(1):54-62.

[25] **Thavasu, P.W., Longhurst, S., Joel, S. P., Slevin, M. L. and Balkwill.F.R. (1992)**: Measuring cytokine levels in blood. Importance of anticoagulants, processing, and storage conditions. Journal of immunological methods, 153(1-2):115-124.

[26] **Badawy, S. M., Hammad, S. A., Amine, S. A., El-Seidy, A. M. and Slima, S. R. A. (2017)**: Biochemical and histopathological changes in the brain of albino rats treated with profenofos and the possible protective effect of vitamins C and E. Menoufia. Medical Journal ., 30(1): 278-285.

[27] **Trinder, P. (1969)**: Ann. Clin. Biochem. 6:24-26.

[28] **Morgan, C. R. and Lazarow, A. (1963)**: Immunoassay of insulin: two antibody system: plasma insulin levels of normal, subdiabetic and diabetic rats. Diabetes.,12(2):115-126.

[29] **Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F. and Turner, R. C. (1985)**: Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia., 28(7): 412-419.

[30] **Young, D.S. (1990)**: Effect of drug on clinical laboratory tests. 3th Edition.3:6-12.

[31] **Karmen, A., Wroblewski, F. and Ladue, J.S. (1955)**: Transaminase activity in human blood. J. Clin. Invest., 34(1):126-133.

[32] **Gornall, A.G., Bardawill, C.J. and David, M.M. (1949)**: Determination of serum proteins by means of the biuret reaction. Journal of biological chemistry., 177(2):751-766.

[33] **Doumas, B. T., Watson, W. A. and Biggs, H. G. (1971)**: Albumin standards and the measurement of serum albumin with bromcresol green. Clinica chimica acta., *31*(1): 87-96.

[34] **Janice, T. B. (1990):** Clinical methods: the history, physical, and laboratory examinations. 3rd edition, chapter101: serum albumin and globulin.,497-499.

[35] **Pillbmer, L. and Hutohinson, M. C. (1946)**: The determination of the albumin and globulin contents of human serum by methanol precipitation. Journal of Biological Chemistry., 168: 299-301.

[36] **Patton, C. J. and Crouch, S. R. (1977)**: Determination of blood urea . *Analytical Chemistry*., *49*: 464-469.

[37] **Murray, R. (1984)**: Creatinine. Kaplan A *et al*. Clin Chem the C.V. Mosby Co. St Louis. Toronto. Princeton; 1261-1266 and 418.

[38] **Koracevic, D., Koracevic, G., Djordjevic, V., Andrejevic, S. and Cosic, V. (2001)**: Method for the measurement of antioxidant activity in human fluids. JClin Pathol., 54(5):356– 361.

[39] **Satoh, K. (1978)**: Serum Lipid Peroxide in cerebrovascular disorders determined by a new colorimetric method. Clinic. Chimic. Acta., 90(1):37-43.

[40] **Axelsson, J., Wang, X., Ketteler, M., Qureshi, A. R., Heimbürger, O., Bárány, P. and Stenvinkel, P. (2008)**: Is fetuin-A/α2-Heremans-Schmid glycoprotein associated with the metabolic syndrome in patients with chronic kidney disease?. American journal of nephrology., 28(4): 669-676.

[41] **Bancroft JD, Gamble M(2008):** Theory and practice of histological techniques 6th edition. Elsevier health sciences,London; 2008;83-134

[42] **Abu-Bader, S. H. (2021)**: Using statistical methods in social science research: With a complete SPSS guide. Oxford University Press, USA,297-302.

[43] **Fernandes, J. D. R., Ogurtsova, K., Linnenkamp, U., Guariguata, L., Seuring, T., Zhang, P. and Makaroff, L. E. (2016)**: IDF Diabetes Atlas estimates of 2014 global health expenditures on diabetes. Diabetes Research and Clinical Practice., 117:48-54.

[44] **Nabel, E. G. (2003)**: Cardiovascular disease. New England Journal of medicine., 349 (1): 60-72.

[45] **Park, S., Kim, D. S. and Kang, S. (2011)**: Gastrodia elata Blume water extracts improve insulin resistance by decreasing body fat in diet-induced obese rats: vanillin and 4-hydroxybenzaldehyde are the bioactive candidates. European Journal of Nutrition., 50(2): 107-118.

[46] **Aboonabi, A., Rahmat, A. and Othman, F. (2014):** Antioxidant effect of pomegranate against streptozotocin-nicotinamide generated oxidative stress induced diabetic rats. Toxicology reports, 1: 915-922.

[47] **Szkudelski, T. (2012)**: Streptozotocin–nicotinamide-induced diabetes in the rat. Characteristics of the experimental model. Experimental biology and medicine., 237(5): 481-490.

[48] **Bogdanov, S. (2012)**: Bee venom: composition, health, medicine: a review. Bee Prod. Sci., 1-17.

[49] **Morgan, N. G. and Montague, W. (1984)**: Stimulation of insulin secretion from isolated rat islets of Langerhansby melittin. Bioscience reports., 4(8): 665-671.

[50] **Simonsson, E., Karlsson, S. and Ahrén, B. (2000)**: Islet phospholipase A2 activation is potentiated in insulin resistant mice. Biochemical and biophysical research communications., 272(2): 539-543.

[51] **Khulan, T. S., Ambaga, M. and Chimedragcha, C.H. (2015)**: Effect of HoneyBee Venom (Apis mellifera) on Hyperglycemia and Hyperlipidemia in Alloxan Induced Diabetic Rabbits. Journal of Diabetes Metabolism. 6(3):3-6.

[52] **Park, H. J., Lee, H. J., Choi, M. S., Son, D. J., Song, H. S., Song, M. J. and Hong, J. T. (2008)**: JNK pathway is involved in the inhibition of inflammatory target gene expression and NF-kappaB

activation by melittin. Journal of inflammation., 5(1): 1-13.

[53] **Zidan, H. A. E. G., Mostafa, Z. K., Ibrahim, M. A., Haggag, S. I., Darwish, D. A. and Elfiky, A. A. (2018):** Venom Composition of Egyptian and Carniolan Honeybee, Apis mellifera L. Affected by Collection Methods. Egyptian Academic Journal of Biological Sciences. A, Entomology., 11(4): 59-71.

[54] **Kim, Y. J., Lim, C. S. and Kwon, K. R. (2009)**: Study of single dose test of Sweet Bee Venom in rats. Journal of Pharmacopuncture ., 12(4): 5-32.

[55] **Gary, B. Q., Wayne, S.S. and David, A. S. (1988)**: Venoms of social hymenoptera toxicity to lepidoteran, Manduca sexta. Insect Biochemistry., 18(6): 511-514.

[56] **Habermann, E. (1972):** Bee and waspvenoms. Science., 177(4046) : 314-322.

[57] **Devi, A., Sangeeta, B., Kumar, N.R. and Kaur, J. (2016)**: HoneyBee Venom and Its Composition: Focusing on Different Apis Species-A Review. Journal of Basic and Applied Engineering Research., 3 (1): 96-98.

[58] **Adl, M.B.F., Gençer, H.V., Firatli, C. and Bahreini, R. (2007)**: characterization of Iranian (Apis mellifera meda), Central Anatolian (Apis mellifera anatoliaca) and Caucasian (Apis mellifera caucasica) honeybee populations. Journal of Apicultural Research., 46 (4):225-231.

[59] **Malini, P., Kanchana, G.and Rajadurai, M. U. R. U. (2011)**: Antibiabetic efficacy of ellagic acid in streptozotocin-induced diabetes mellitus in albino wistar rats. Asian J Pharm Clin Res., 4(3): 124-128.

[60] **Asokan, S. M., Wang, R. Y., Hung, T. H. and Lin, W. T. (2019)**: Hepato-protective effects of Glossogyne tenuifolia in Streptozotocin- nicotinamide-induced diabetic rats on high fat diet. BMC complementary and alternative medicine., 19(1): 1-10.

[61]**Mohammed, F. Z., Gurigis, A. A., Abdel-Mageed, W. S. and Nassr, A. (2018)**: Improvement of Insulin Sensitivity and Maintenance of Glucose Homeostasis in Insulin-sensitive Tissues via PPAR-γ and Through Activation of PI3K/p-Akt Signaling Pathway by Resveratrol in Type 2 Diabetic Rats. *J Mol Immunol*., *3*(119): 2:10.

[62] **Hassan, A. K., El-kotby, D. A., Tawfik, M. M., Badr, R. E.and Bahgat, I. M. (2019)**: Antidiabetic effect of the Egyptian honeybee (Apis mellifera) venom in alloxan-induced diabetic rats. The Journal of Basic and Applied Zoology., 80(1): 1-9.

[63] **Raafat, M. and Hamam, G. G. (2020)**: The Possible Role of Bee Venom on Gastric Fundic Mucosa in Streptozotocin Induced Diabetes Mellitus in Rats. A Histological Study. Egyptian Journal of Histology., 42(4): 1029-1043.

[64] **Badr, G., Hozzein, W. N., Badr, B. M., Al Ghamdi, A., Saad Eldien, H. M. and Garraud, O. (2016)**: Bee venom accelerates wound healing in diabetic mice by suppressing activating transcription factor- 3 (ATF- 3)

and inducible nitric oxide synthase (iNOS)- mediated oxidative stress and recruiting bone marrow- derived endothelial progenitor cells. Journal of Cellular Physiology., 231(10): 2159-2171.

[65] **Skovsø, S. (2014)**: Modeling type 2 diabetes in rats using high fat diet and streptozotocin. Journal of diabetes investigation., 5(4): 349-358.

[66] **Bonora, E. and DeFronzo, R. A. (Eds.). (2018)**: Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment., Springer, (pp. 181-253).

[67] Kotha, P., Badri, K. R., Nagalapuram, R., Allagadda, R. and Chippada, A. R. (2017): Anti-diabetic potential of the leaves of Anisomeles malabarica in streptozotocin induced diabetic rats. Cellular Physiology and Biochemistry., 43(4):1689-1702.

[68] **Avtanski, D., Pavlov, V. A., Tracey, K. J. and Poretsky, L. (2019)**: Characterization of inflammation and insulin resistance in high- fat diet-induced male C57BL/6J mouse model of obesity. Animal models and experimental medicine., 2(4): 252-258.

[69] **Hanafi, M. Y., Zaher, E. L., El-Adely, S. E., Sakr, A., Ghobashi, A. H., Hemly, M. H. and Kamel, M. A. (2018)**: The therapeutic effects of bee venom on some metabolic and antioxidant parameters associated with HFD-induced non-alcoholic fatty liver in rats. Experimental and therapeutic medicine., 15(6): 5091-5099.

[70] **Zafar, M., Naqvi, S. N. U. H., Ahmed, M. and Kaimkhani, Z. A. (2009)**: Altered Liver Morphology and Enzymes in Streptozotocin Induced Diabetic Rats. International Journal of Morphology., 27(3): 719-725.

[71] **Mohamed, J., Nazratun Nafizah, A. H., Zarivantev, A. H. and Budin, S. B. (2016)**: Mechanisms of Diabetes-Induced Liver Damage: The role of oxidative stress and inflammation. Sultan Qaboos University medical journal., 16(2): e132–e141.

[72] **Yazdi, H. B., Hojati, V., Shiravi, A., Hosseinian, S., Vaezi, G. and Hadjzadeh, M. A. R. (2019)**: Liver Dysfunction and Oxidative Stress in Streptozotocin-Induced Diabetic Rats: Protective Role of Artemisia Turanica. Journal of pharmacopuncture., 22(2): 109-114.

[73]**Darwish, S. F., El-Bakly, W. M., Arafa, H. M. and El-Demerdash, E. (2013)**: Targeting TNF-a and NF-kB Activation by Bee Venom: Role in Suppressing Adjuvant Induced. PLoS ONE.,8(11): 1-13.

[74]**Kim, H., Keum, D. J., won Kwak, J., Chung, H. S. and Bae, H. (2014)**: Bee venom phospholipase A2 protects against acetaminophen-induced acute liver injury by modulating regulatory T cells and IL-10 in mice. PLoS One., 9(12): 1-12.

[75] **Park, J. H., Kim, K. H., Kim, S. J., Lee, W. R., Lee, K. G. and Park, K. K. (2010)**: Bee venom protects hepatocytes from tumor necrosis factor-α and actinomycin D. Archives of Pharmacal Research., 33(2): 215-223.

[76] **Amra, E. S. A., Lashein, F. E. D. M., Seleem, A. A. and Badr, A. H. (2018).** Counter effect of bee venom and its extracted bradykinin-potentiating factor on acrylamide and chips administration-induced complications in the liver and kidney of male mice. The Journal of Basic and Applied *Zoology*, *79*(1): 1-15.

[77] **Arthur, G. and John, H. (2006)**: Chapter 16: The Microcirculation and the Lymphatic System. Gruliow, Rebecca. Textbook of Medical Physiology (Book) (11th ed.). Philadelphia, Pennsylvania: Elsevier Inc, 187-188.

[78] **Lindsay, R. S., Krakoff, J., Hanson, R. L., Bennett, P. H. and Knowler, W. C. (2001**): Gamma globulin levels predict type 2 diabetes in the Pima Indian population. Diabetes., 50(7): 1598-1603.

[79] **Suh, B., Park, S., Shin, D. W., Yun, J. M., Keam, B., Yang, H. K.and Cho, B. (2014)**:Low albumin-to-globulin ratio associated with cancer incidence and mortality in generally healthy adults. Annals of oncology., 25(11): 2260-2266.

[80] **Adiga, U. and Kathyayani, P. (2019)**: Association of Insulin Resistance with Liver Biomarkers in Type 2 Diabetes Mellitus. International Journal of Pharmaceutical and Phytopharmacological Research., 9(1): 88-91.

[81] **Chang, D. C., Xu, X., Ferrante, A. W.and Krakoff, J. (2019)**: Reduced plasma albumin predicts type 2 diabetes and is associated with greater adipose tissue macrophage content and activation. Diabetology & metabolic syndrome., 11(1), 1-8.

[82] **Hassan, H. R.and Abdul Sattar, A. (2015)**: Influence of diabetes disease on concentration of total protein, albumin and globulins in saliva and serum: A comparative study. Iraqi National of Chemistry., 15(1):1-11.

[83] **Preetha, P. P., Devi, V. G. and Rajamohan, T. (2013)**: Comparative effects of mature coconut water (Cocos nucifera) and glibenclamide on some biochemical parameters in alloxan induced diabetic rats. Revista Brasileira de Farmacognosia., 23(3): 481-487.

[84] **Radwan, O.K., El-Nabarawy, S.K., El-Sayed., R.A., El-Sisi,S.F. and Abd El-Salam,F.F. (2019)**: The Protective Effect of Bee Venom and Echinacea Purpblood blood urea nitrogen nitrogen against Liver Injury Induced by Dexamethasone. The Pharmaceutical and Chemical Journal., 6(2):25-38.

[85] **Barnett, L. M. and Cummings, B. S. (2018)**: Nephrotoxicity and renal pathophysiology: a contemporary perspective. Toxicological Sciences ., 164(2): 379-390.

[86] **Tang, Y., Wang, C., Wang, Y., Zhang, J., Wang, F., Li, L. and Wang, L. (2018)**: Isoliquiritigenin attenuates LPS-induced AKI by suppression of inflammation involving NF-κB pathway. American journal of translational research., 10(12): 4141-4151.

[87] **Kaur, R., Sodhi, R. K., Aggarwal, N., Kaur, J. and Jain, U. K. (2016)**: Renoprotective effect of lansoprazole in streptozotocin-induced diabetic nephropathy in wistar rats. Naunyn-Schmiedeberg's archives of pharmacology., 389(1): 73-85.

[88] **Ebaid, H., Bashandy, S. A., Abdel-Mageed, A. M., Al-Tamimi, J., Hassan, I. and Alhazza, I. M. (2020)**: Folic acid and melatonin mitigate diabetic nephropathy in rats via inhibition of oxidative stress. Nutrition&Metabolism., 7(1):1-14.

[89]**Kim, H., Lee, H., Lee, G., Jang, H., Kim, S. S., Yoon, H. and Bae, H. (2015)**: Phospholipase A2 inhibits cisplatin-induced acute kidney injury by modulating regulatory T cells by the CD206 mannose receptor. Kidney international., 88(3): 550-559.

[90]**Kim, H., Hong, J. Y., Jeon, W. J., Baek, S. H. and Ha, I. H. (2020)**: Bee venom melittin protects against cisplatin-induced acute kidney injury in mice via the regulation of M2 macrophageactivation. Toxins., 12(9), 574-587.

[91] **Rubio, C. P., Hernández-Ruiz, J., Martinez-Subiela, S. Tvarijonaviciute, A. and Ceron, J. J. (2016)**: Spectrophotometric assays for total antioxidant capacity (TAC) in dog serum: an update. BMC veterinary research., *12*(1): 1-7.

[92] **Ragy, M. M. and Ahmed, S. M. (2019)**: Protective effects of either C- peptide or l- arginine on pancreatic β- cell function, proliferation, and oxidative stress in streptozotocin- induced diabetic rats. Journal of cellular physiology.m 234(7): 11500-11510.

[93] **Thomson, M., Al-Qattan, K. K., Divya, J. S. and Ali, M. (2015)**: Anti-diabetic and anti-oxidant potential of aged garlic extract (AGE) in streptozotocin-induced diabetic rats. BMC Complementary and Alternative Medicine., 16(1):1-9.

[94] **Samadi, M., Aziz, S. G. G. and Naderi, R. (2021)**: The effect of tropisetron on oxidative stress, SIRT1, FOXO3a, and claudin-1 in the renal tissue of STZ-induced diabetic rats. Cell Stress and Chaperones., 26(1): 217-227.

[95]**Ahmed, S.B., Ibrahim, N.A., Hassan, A.A., Sadek, A.N. and Abdelwahed, M.K (2020)**: Evaluation of the possible apitherapeutic value of bee venom and bee propolis on L-arginine-induced acute pancreatitis and lung injury in albino rats. Int J Mol Biol Open Access.,5(1):1-12.

[96] **Tsikas, D. (2017)**: Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. Analytical biochemistry., 1(524): 13-30.

[97] **Paudel, Y. N., Ali, M. R., Shah, S., Adil, M., Akhtar, M. S., Wadhwa, R. and Sharma, M. (2017)**: 2-[(4-Chlorobenzyl) amino]-4-methyl-1, 3-thiazole-5-carboxylic acid exhibits antidiabetic potential and raises insulin sensitivity via amelioration of oxidative enzymes and inflammatory

cytokines in streptozotocin-induced diabetic rats. Biomedicine & Pharmacotherapy., 89: 651-659.

[98] **Masood, S., ur Rehman, A., Bashir, S., El Shazly, M., Imran, M., Khalil, P.and Khursheed, T. (2021)**: Investigation of the anti-hyperglycemic and antioxidant effects of wheat bread supplemented with onion peel extract and onion powder in diabetic rats. Journal of Diabetes & Metabolic Disorders., 20: 485–495

[99] **Hamza, R.G., Mounir, A.M. and El shahat, A.N. (2019)**: Studying the Ameliorative Effect of Bee Venom Against Damage and Inflammation Induced in Gamma-Irradiated Rats. Arab Journal of Nuclear Sciences and Applications., 52(1): 178-184.

[100] **Abd El-Rahim, A. H., Abd-El-Moneim, O. M., Abd El-Kader, H. A. and Abd El Raouf, A. (2018)**: Inhibitory effect of bee venom against potassium bromate causing genetic toxicity and biochemical alterations in mice. Journal of The Arab Society for Medical Research., 13(2): 89-98.

[101] **Kim, J. Y., Lee, S. J., Maeng, Y. I., Leem, J. and Park, K. K. (2020)**: Protective effects of bee venom against endotoxemia-related acute kidney injury in mice. Biology., 9(7):154-164.

[102] **Fatima, F., Ahsan, N., Nasim, A. and Alam, F. (2020)**: Association of fetuin-A with dyslipidemia and insulin resistance in type-II Diabetics of Pakistani population. Pakistan journal of medical sciences., 36(2): 64-68.

[103] **Zhang, L. Y., Qu, X. N., Sun, Z. Y. and Zhang, Y. (2020)**: Effect of liraglutide therapy on serum fetuin A in patients with type 2 diabetes and non-alcoholic fatty liver disease. Clinics and research in hepatology and gastroenterology., 44(5): 674-680.

[104] **Haukeland, J. W., Dahl, T. B., Yndestad, A., Gladhaug, I. P., Løberg, E. M., Haaland, T. and Birkeland, K. I. (2012)**: Fetuin A in nonalcoholic fatty liver disease: in vivo and in vitro studies. European journal of endocrinology., 166(3): 503-510.

[105] **Viollet, B., Guigas, B., Leclerc, J., Hébrard, S., Lantier, L., Mounier, R. and Foretz, M. (2009)**: AMP- activated protein kinase in the regulation of hepatic energy metabolism: from physiology to therapeutic perspectives. Acta physiologica ., 196(1): 81-98.

[106] **Ismail, T. A., Soliman, M. M. and Nassan, M. A. (2015)**: Molecular and immunohistochemical effects of metformin in a rat model of type 2 diabetes mellitus. Experimental and therapeutic medicine., 9(5): 1921-1930.

[107] **Hosseini, S. D., Rahimi, M. R. and Abbaspoor, M. (2021)**: Chronic Caffeine Ingestion Down-Regulates Liver and Visceral

Adipose Tissue Inflammatory Gene Expression in High-Fat Diet-Induced Obesity. Journal of Advances in Medical and Biomedical Research.,29(135): 189-196.

[108] **Öner-İyidoğan, Y., Kocak, H., Seyidhanoğlu, M., Gürdöl, F., Gülçubuk, A., Yildirim, F. and Uysal, M. (2013)**: Curcumin prevents liver fat accumulation and serum fetuin-A increase in rats fed a high-fat diet. Journal of physiology and biochemistry., 69(4): 677-686.

[109] **Cheon, S. Y., Chung, K. S., Roh, S. S., Cha, Y. Y. and An, H. J. (2018)**: Bee venom suppresses the differentiation of Preadipocytes and high fat diet-induced obesity by inhibiting Adipogenesis . Toxins., 10(1), 9-22.

[110] **Abd El-Haleim, E. A. (2020)**: Molecular Study on the Potential Protective Effects of Bee Venom against Fructose-Induced Non-alcoholic Steatohepatitis inRats. Pharmacology.,105(1 1-12): 692-704.

[111] **El-Demerdash, R. F., Hammad, L. N., Kamal, M. M. and El Mesallamy, H. O. (2015)**:A comparison of Wharton's jelly and cord blood as a source of mesenchymal stem cells for diabetes cell therapy. RegenerativeMedicine. 10(7): 841-855.

[112] **El Senosi, Y. A., Omayma, A. R., Elmaged, A. D. A. and Ali, M. A. (2018):** BIOCHEMICAL STUDY ON THE REGENERATIVE EFFECT OF BEE VENOM ON EXPERIMENTALLY INDUCED DIABETES. WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES. 7(10):209-225.

[113] **AL-SHIMAA, M. A., AHMED, F. E. and NEHAL, W. A. (2019)**: RUTIN RESTORE BIOCHEMICAL CHANGES, OXIDATIVE STRESS AND BETATROPHIN LEVEL IN STZ-INDUCED DIABETIC RATS. Int J Curr Pharm Res., 11(6): 62-70.

[114] **Amra, E. S. A., Lashein, F. E. D. M., Seleem, A. A. and Badr, A. H. (2018)**:Counter effect of bee venom and its extracted bradykinin-potentiatingfactor on acrylamide and chips administration-induced complications in the liver and kidney of male mice. The Journal of Basic and Applied Zoology., 79(1): 1-15.

Table (1): Effect of bee venom (0.5 and 1.23 mg/kg) and combined therapy (Metformin and Atorvastatin) on the levels of Glucose, Insulin, HOMA-IR and HOMA- β.

Groups	Negative control	Positive control	Bee venom treated group (0.5 mg/kg)	Bee venom treated group (1.23 mg/kg)	Metformin and Atorvastatin treated group	P Value
Glucose (mg/dl)	91.2±0.94[c]	467.8±8.44	161.6±2.12[c]	103.4±1.17[c]	96.5±1.32[c]	< 0.0001
% change	---------	412.94%	-65.45%	-77.89%	-79.37%	
Insulin (pg/ml)	126.0±1.16[c]	57.9±1.32	82.7±2.03[c]	121.6±0.96[c]	123.1±1.14[c]	
% change	---------	-54.05%	42.83%	110.01%	112.61%	
HOMA-IR	15.0±0.19[c]	35.3±0.87	17.2±0.25[c]	16.4±0.12[c]	15.7±0.13[c]	
% change	-------	135.33%	-51.27%	-53.54%	-55.52%	
HOMA-β	864.1±31.57[c]	27.4±1.03	161.3±6.93[b]	579.4±19.69[c]	734.4±39.83[c]	
% change	------	-96.83%	488.68%	2014.6%	2580.29%	

Values are expressed in Mean ± SEM. Values with different superscript letters represent significant differences ($p < 0.05$). [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$ compared to positive control group. % change of positive control was calculated according to negative control. % change of all treated groups was calculated according to positive control.

Table (2): Effect of bee venom (0.5 and 1.23 mg/kg) and combined therapy (Metformin and Atorvastatin) on the levels of serum liver function tests.

Groups	Negative control	Positive control	Bee venom treated group (0.5 mg/kg)	Bee venom treated group (1.23 mg/kg)	Metformin and Atorvastatin treated group	P Value
ALT (U/L)	37.2±0.81[c]	83±1.24	57.4±1.55[c]	46.4±0.73[c]	44.4±0.97[c]	< 0.0001
% change	-----	123.1%	-30.84%	-44.09%	-46.5%	
AST (U/L)	89.1±3.1[c]	165.7±4.86	113.2±2.85[c]	108±3.9[c]	105±3.09[c]	
% change	-----	85.9%	-31.68%	-34.82%	-36.63%	
Total protein (g/dl)	6.1±0.1[c]	7.5±0.07	6.6±0.05[c]	6.4±0.09[c]	6.3±0.05[c]	
% change	------	22.95%	-12%	-14.6%	-16%	
Albumin (g/dl)	3.9±0.06[c]	2.9±0.06	3.4±0.08[a]	3.7±0.11[c]	3.7±0.09[c]	
% change	-----	-25.64%	17.24%	27.58%	27.58%	
Globulin (g/dl)	2.2±0.05[c]	4.4±0.06	2.9±0.14[c]	2.8±0.11[c]	2.6±0.1[c]	
% change	-----	100%	-34.09%	-36.36%	-40.9%	
A/G ratio	1.7±0.02[c]	0.66±0.02	1.1±0.06[c]	1.3±0.08[c]	1.4±0.1[c]	
% change	-----	-61.4%	66.66%	96.96%	112.12%	

Values are expressed in Mean ± SEM. Values with different superscript letters represent significant differences ($p < 0.05$). [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$ compared to positive control group. % change of positive control was calculated according to negative control. % change of all treated groups was calculated according to positive control.

Table (3): Effect of bee venom (0.5 and 1.23 mg/kg) and combined therapy (Metformin and Atorvastatin) on the levels of serum kidney function tests.

Groups	Negative control	Positive control	Bee venom treated group (0.5 mg/kg)	Bee venom treated group (1.23 mg/kg)	Metformin and Atorvastatin treated group	P Value
Blood blood urea nitrogen nitrogen (mg/dl)	35.3±0.82[c]	57.8±1.31	42.6±1.3[c]	38.5±0.86[c]	36.5±1.57[c]	< 0.0001
% change	-----	63.73%	-26.29%	-33.39%	-36.85%	
Creatinine (mg/dl)	0.38±0.01[c]	0.71±0.017	0.57±0.014[c]	0.53±0.007[c]	0.5±0.011[c]	
% change	-----	86.84%	-19.71%	-25.35%	-29.57%	

Values are expressed in Mean ± SEM. Values with different superscript letters represent significant differences ($p < 0.05$). [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$ compared to positive control group. % change of positive control was calculated according to negative control. % change of all treated groups was calculated according to positive control.

Table (4): Effect of bee venom (0.5 and 1.23 mg/kg) and combined therapy (Metformin and Atorvastatin) on the levels of total antioxidant capacity (TAC) .

Groups	Negative control	Positive control	Bee venom treated group (0.5 mg/kg)	Bee venom treated group (1.23 mg/kg)	Metformin and Atorvastatin treated group	P Value
Pancreatic TAC (mM/L)	0.96±0.02 [c]	0.51±0.01	0.61±0.15 [b]	0.79±0.05 [c]	0.81±0.01 [c]	< 0.0001
% change	------	-46.87%	19.6%	54.9%	58.8%	
Hepatic TAC (mM/L)	1.352±0.009[c]	0.98±0.012	1.32±0.008[c]	1.347±0.01[c]	1.33±0.006[c]	
% change	-----	-24.4%	34.69%	36.73%	35.71%	
Renal TAC (mM/L)	1.73±0.026 [c]	0.96±0.013	1.063±0.023[b]	1.335±0.025[c]	1.332±0.014 [c]	
% change	------	-44.5%	10.72%	39.06%	38.75%	

Values are expressed in Mean ± SEM. Values with different superscript letters represent significant differences ($p < 0.05$). [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$ compared to positive control group. % change of positive control was calculated according to negative control. % change of all treated groups was calculated according to positive control.

Table (5): Effect of bee venom (0.5 and 1.23 mg/kg) and combined therapy (Metformin and Atorvastatin) on the levels of malondialdehyde (MDA).

Groups	Negative control	Positive control	Bee venom treated group (0.5 mg/kg)	Bee venom treated group (1.23 mg/kg)	Metformin and Atorvastatin treated group	P Value
Pancreatic MDA (nmol/g tissue)	64.6±1.7^{c}	204±1.94	183.2±1.37^{c}	151.9±1.68^{c}	153.7±1.7^{c}	< 0.0001
% change	------	215.7%	-10.19%	-25.53%	-24.65%	
Hepatic MDA (nmol/g tissue)	511.1±2.7^{c}	788.4±20.33	583.5±14.29^{c}	535.3±15.13^{c}	530.1±13.23^{c}	
% change	-----	54.25%	-25.98%	-32.1%	-32.76%	
Renal MDA (nmol/g tissue)	89.5±0.5 ^{c}	184.2±0.66	170.3±1.03 ^{c}	147.5±1.12 ^{c}	147.4±1.11 ^{c}	
% change	-----	105.8%	-7.54%	-19.92%	-19.97%	

Values are expressed in Mean ± SEM. Values with different superscript letters represent significant differences ($p < 0.05$). $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ compared to positive control group. % change of positive control was calculated according to negative control. % change of all treated groups was calculated according to positive control.

Table (6): Effect of bee venom (0.5 and 1.23 mg/kg) and combined therapy (Metformin and Atorvastatin) on the levels of Fetuin-A.

Groups	Negative control	Positive control	Bee venom treated group (0.5 mg/kg)	Bee venom treated group (1.23 mg/kg)	Metformin and Atorvastatin treated group	P Value
***Fetuin-A* (ng/ml)**	57.2±3.5^{c}	159.9±5.79	85.2±2.12^{c}	63.5±1.69^{c}	61.6±1.12^{c}	< 0.0001
% change	-----	179.5%	-46.71%	-60.28%	-61.47%	

Values are expressed in Mean ± SEM. Values with different superscript letters represent significant differences ($p < 0.05$). $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ compared to positive control group. % change of positive control was calculated according to negative control. % change of all treated groups was calculated according to positive control.

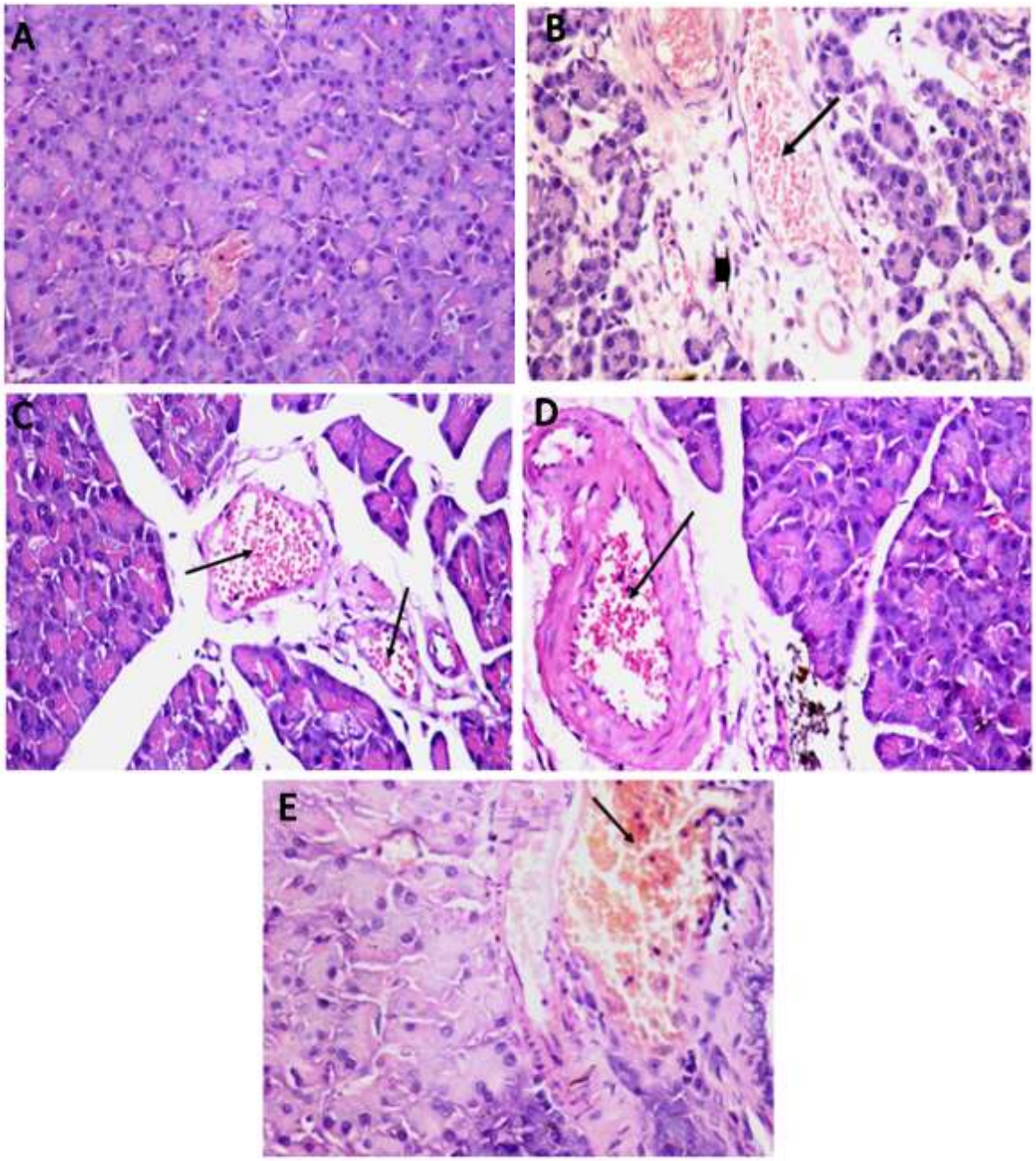

Figure (1): Histopathological examination of pancreas tissue. **A** (negative control): showed normal pancreatic parenchyma, pancreatic acini, ducts and islets. **B** (positive control): showed acute pancreatitis, the congested blood vessel (arrow) and lecucocytic cells infiltration (arrowhead). **C** (bee venom treated group (0.5 mg/kg) , **D** (bee venom treated group (1.23 mg/kg)): showed slight congestion in the blood vessels (arrows) and **E** (Metformin and Atorvastatin treated group): showed congestion in the blood vessels (arrow).

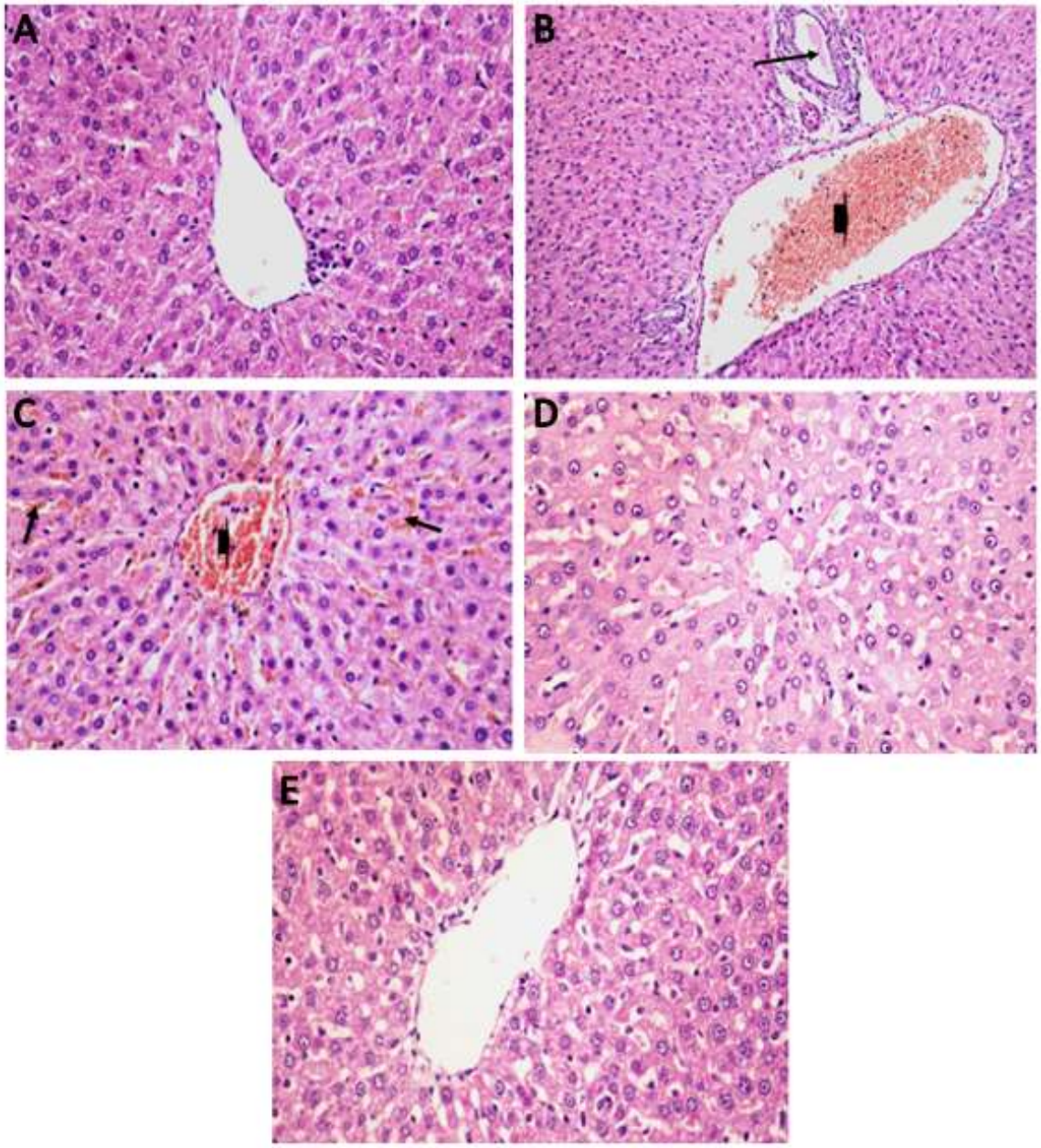

Figure (2): Histopathological examination of liver tissue. **A** (negative control),**D** (bee venom treated group (1.23 mg/kg)),**E** (Metformin and Atorvastatin treated group) : showed normal normal hepatocytes, hepatic parenchyma, portal tract and blood sinusoids, **B** (positive control): showed portal tract changes, the congested hepatoportal blood vessel (arrow head), and the hyperplastic bile duct (arrow), **C** (bee venom treated group (0.5 mg/kg)): showed congestion in the central vein (arrow head) and blood sinusoids (arrows).

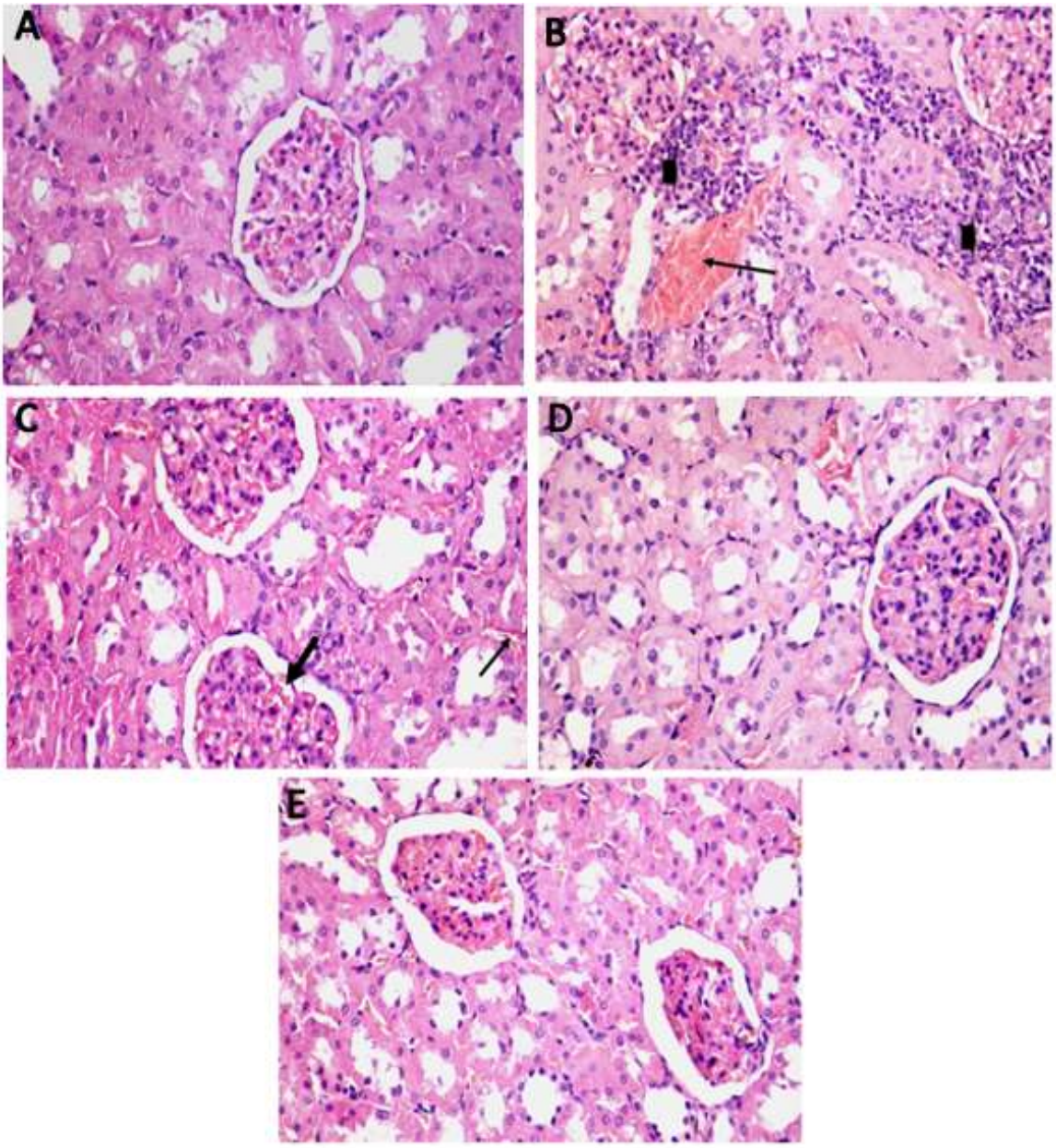

Figure (3): Histopathological examination of kidney tissue. **A** (negative control),**D** (bee venom treated group (1.23 mg/kg)),**E** (Metformin and Atorvastatin treated group) : showed normal renal parenchyma, renal glomeruli and renal tubules, **B** (positive control):showed acute interstitial nephritis, the interstitial congested blood vessels (arrow) and leucocytic cells infiltrations (arrow heads), **C** (bee venom treated group (0.5 mg/kg)): showed slight congestion in the glomerular blood capillaries (thick arrow) and peri-renal blood capillaries (thin arrow).