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Original Article

Serological and Histological Evaluation of the Effect of Honeybee Venom on Pancreas and Liver in Diabetic Mice

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Abstract

Natural toxins have been traditionally used to trigger several diseases among which bee venom (HBV) is of great importance. The present study aimed to investigate the therapeutic effects of honeybee venom (HBV) on alloxan and glucose fluid-induced Type 2 diabetes mellitus (T2DM). Therefore, a total of 20 adult laboratory male mice (*Mus musculus*) were selected, acclimated, and divided into four equal groups (n=5). Initially, 15 mice were fasted for 12 hrs and injected with alloxan at a single dose of 150 mg/kg of body weight. The animals were exposed to drinking glucose fluid in the morning for 4 days. Then, the blood glucose was measured. The studied animals having blood glucose of ≤200 mg/dl were considered non-diabetic and re-subjected to injecting alloxan (150 mg/kg body weight) and drinking glucose fluid for another 4 days. Four groups of mice population included, Group 1: non-diabetic and untreated with HBV, Group 2: diabetic and received no HBV as the potential therapeutic agent, Group 3: diabetic and received a low level of HBV at a dose of 0.5 mg/kg, Group 4: diabetic and received a high level of HBV at a dose of 1 mg/kg. At the end of the 35-day testing period, blood samples were tested to determine the levels of insulin, glucose, and lipid profiles [cholesterol, triglyceride (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL)] using Sandwich ELISA kits. The results indicated a significant increase in blood glucose in the diabetic group compared to that of the control one, while both concentrations of HBV significantly reduced the level of blood glucose compared to that of the diabetic group. Furthermore, the level of insulin was significantly decreased in the diabetic group compared to that of the controls, while HBV significantly increased the level of insulin compared to that of the diabetic group. Moreover, the diabetic mice demonstrated a significant increase in the concentration of cholesterol and TG compared to that of control mice which were significantly reversed in response to HBV treatment. The level of HDL was significantly decreased in the diabetic group compared to that of the control group which was modulated by treatment, while no significant differences were seen between all the studied groups regarding the level of LDL. Histological examination of diabetic mice revealed a significant alteration in acinar cells and destruction of β-cells of pancreatic sections with marked lacerations in the liver extended to all structures of the organ. The present study concluded that HBV could be a potential therapeutic agent to prevent and manage diabetes and its complication.

Keywords: Cholesterol, Diabetes Mellitus, Iraq, HDL, LDL, Triglyceride

1. Introduction

Type 2 Diabetes mellitus (T2DM) is one of the fastest growing diseases worldwide resulting in abnormally high levels of glucose (hyperglycemia) due to a metabolic disorder in which the body fails to produce enough insulin or responds normally to insulin (1). Although different forms of DM are diagnosed based on their initial onset, poorly controlled diabetes can cause various consequences such as macrovascular (cardiovascular diseases) and microvascular (neuropathy, retinopathy, and

nephropathy) complications that decrease quality of the life and increase mortality (2). Abnormal lipid metabolism is one of the most dangerous conditions throughout T2DM pathogenesis which is characterized by higher concentrations of cholesterol, triglyceride (TG), low-density lipoprotein (LDL), and decreased levels of high-density lipoprotein (HDL) (3).

Although many different paths driven by various genetic and environmental factors lead to gradual loss of β-cell mass and/or function, early identifying T2DM and preserving glucose levels close to normal could change the natural history of the disease (4). Several therapies have been suggested to enhance insulin secretion and function, reduce hepatic and endogenous glucose production, and affect glycemia through other mechanisms (5). Natural toxins have been traditionally used to trigger several diseases among which honeybee venom (HBV) is of great importance (6). The major components of HBV are melittin, apamin, MCD peptide, histamine, hyaluronidase, and phospholipase-A2 which increases the secretion of insulin from the pancreas by depolarizing the membrane of β-cells (7). Limited studies, to the best of our knowledge, have confirmed the therapeutic effect of HBV on the levels of metabolic and antioxidant parameters by reducing the actions of cholesterol and lipolysis (8).

In Iraq, recent limited studies were conducted to detect therapeutic effects of HBV on glucocorticoid receptor β, blood/biochemical parameters, and antioxidant activity of experimentally induced arthritis in rats (9). Therefore, the present study aimed to evaluate the therapeutic effects of HBV on levels of insulin, glucose, and lipid metabolism including cholesterol, TG, HDL, and LDL in experimentally induced DM mice.

2. Materials and Methods

2.1. Animal Preparation and *DM* Induction

A total of 20 adult laboratory male mice (*Mus musculus*) aged 50 days with a weight of 25-36 g were selected and subjected to acclimation in their cages for a week. Initially, 15 mice were fasted for 12 hrs and were injected with alloxan (Sigma-Aldrich, UK) at a single dose of 150 mg/kg of body weight. The study animals were exposed to drinking the glucose fluid in the morning for 4 days, and the blood glucose was then evaluated by measuring the samples of blood taken from the tail. The studied animals having blood glucose of ≤200 mg/dl were considered non-diabetic and re-subjected to injecting alloxan (150 mg/kg body weight), drinking glucose fluid for another 4 days, and testing of blood glucose to confirm diabetes.

2.2. HBV Preparation

The lyophilized HBV was purchased from a local market in Wasit, Iraq, dissolved in purified distilled water, packaged in sterilized Eppendorf tubes, and kept frozen at -20 ºC for use.

2.3. Study Design

After induction DM, the studied mice were divided into 4 groups as follows:

1. Control group: Five normal mice received no alloxan and drenched glucose fluid without being treated with HBV.
2. Diabetic group: Five diabetic mice received alloxan and drenched glucose fluid without being treated with HBV.
3. Low-HBV group: Five diabetic mice were intraperitoneally treated with 0.5 mg/kg of HBV for 35 days.
4. High-HBV group: Five diabetic mice were intraperitoneally treated with 1 mg/kg of HBV for 35 days.

2.4. Laboratory Measurement

According to instructions, Sandwich ELISA kits are used for measuring serum glucose (Agappe, India), insulin (Monobind Inc., USA), total cholesterol (Agappe, India), TG (Agappe, India), and HDL (Agappe, India). The levels of LDL were measured based on the data of cholesterol, TG, and HDL following the previously described formula (LDL= Cholesterol - HDL - TG / 5) (10, 11).

2.5. Histological Evaluation

At the end of the 35-day testing period, the pancreas and liver were collected and preserved in 10% formalin. The samples were then subjected to a routine histological procedure and stained with hematoxylin and eosin stain (12). Prolonged storage of the pancreas led to the inability of processing the pancreas for the

histological observations and most of the samples were damaged due to the sensitivity and delicate structure. Also, using no standard method in preserving liver samples results in hug artifacts in histological procedure and the inability to generate histological sections. Therefore, the study confirmed this issue, and only limited samples were correctly processed to produce histology slides.

2.6. Statistical Analysis

All obtained data were documented and categorized using Microsoft Office Excel (version 2016) and statistically analyzed in GraphPad Prism (version 7). One-Way ANOVA and Tukey's HSD test were used to detect significant differences between study groups at *P*-value <0.05 (*), <0.01 (**), <0.001 (***), and <0.0001 (****). The analyzed data were presented as Mean ± Standard Error of Mean.

3. Results

Among study groups, a significant variation ($P<0.05$) was observed in the results of the studied parameters. For serum glucose, values in the diabetic group significantly increased (291.2 ± 6.4 mg/dl) compared to that of the controls (90 ± 1.5 mg/dl). While both low and high HBV significantly decreased the level of glucose by 183.8 ± 3.2 mg/dl, and 136 ± 3.1 mg/dl, respectively compared to that of the diabetic group (Figure 1).

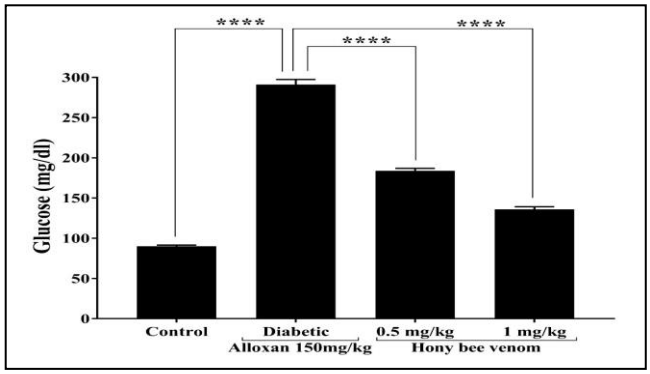

Figure 1. Blood glucose levels in mice of the studied groups

Induced diabetes significantly reduced the level of insulin (16.69 ± 0.8 IU/dl) compared to that of controls (28.59 ± 0.5 IU/dl). While the level of insulin significantly increased (19.41 ± 0.2 IU/dl, and 24.25 ± 0.6 IU/dl) in response to low and high HBV groups, respectively compared to that of the diabetic group (Figure 2). Furthermore, the concentration of cholesterol (151.4 ± 5.2 mg/dl) significantly increased in the diabetic mice ($P<0.0001$) compared to that of the control mice (90.11 ± 4.2 mg/dl). While both concentrations of HBV significantly reduced the level of cholesterol (129.2 ± 4.9 mg/dl and 117.7 ± 5.4 mg/dl), respectively compared to that of diabetic mice (Figure 3). In addition, the level of HDL was decreased (19.75 ± 1 mg/dl) in diabetic mice compared to that of controls (26.9 ± 2 mg/dl), and only a high level of HBV significantly increased HDL level (27.57 ± 1.2 mg/dl) compared to that of the diabetic mice (Figure 4).

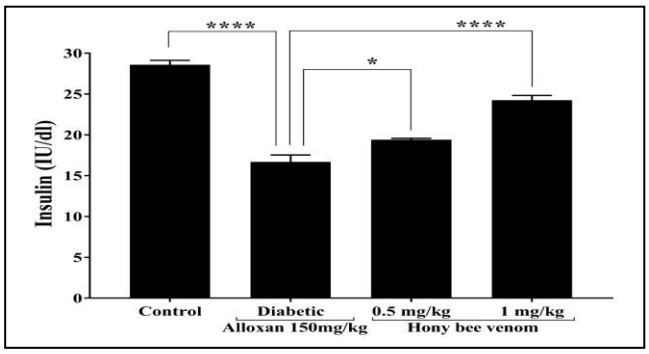

Figure 2. Serum insulin levels in mice of the studied groups

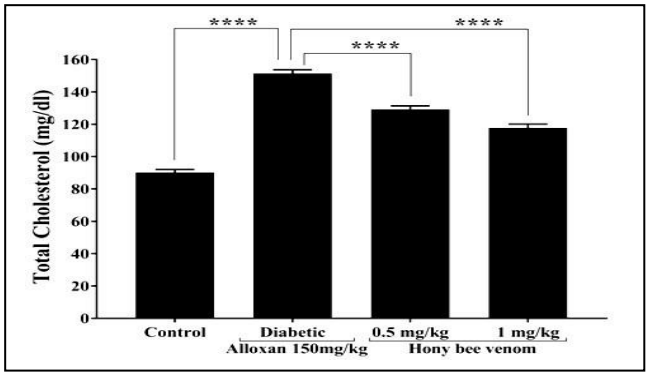

Figure 3. Serum cholesterol levels in mice of the studied groups

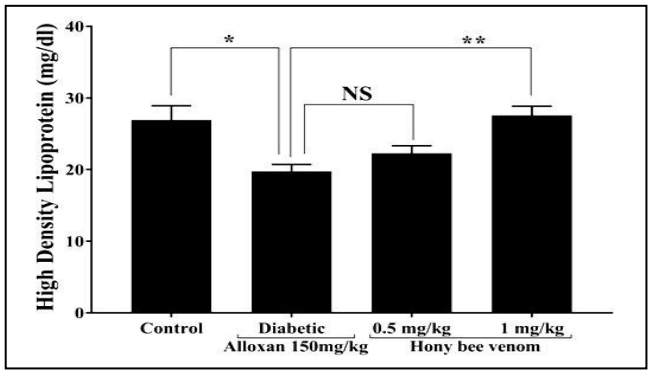

Figure 4. Serum HDL levels in mice of the studied groups

Concerning the concentration of TG, the diabetic mice indicated the highest value (128.5 ± 1.8 mg/dl) compared to the values of the control group (59.82 ± 2 mg/dl). High and low HBV significantly reversed this increase (116.4 ± 0.7 mg/dl and 88.97 ± 2 mg/dl), respectively compared to that of diabetic mice (Figure 5). No significant differences (P>0.05) were noticed in values of LDL among the mice of all studied groups including control (0.6776 ± 0.06 mg/dl), diabetic (0.6308 ± 0.07 mg/dl), low-HBV (-1.902 ± 0.7 mg/dl) and high-HBV (0.236 ± 0.06 mg/dl) (Figure 6).

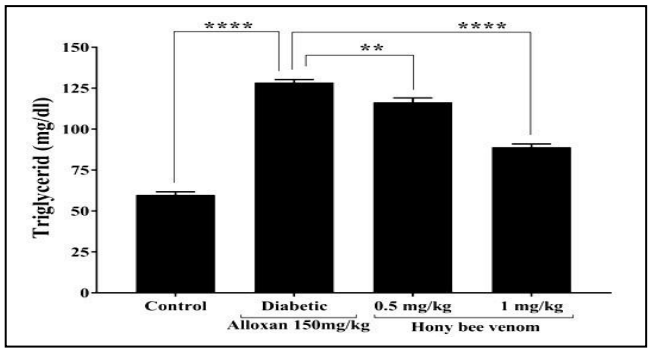

Figure 5. Serum TG levels in mice of the studied groups

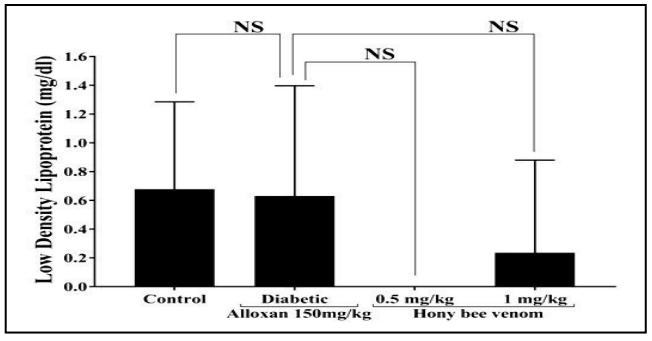

Figure 6. Serum LDL levels in mice of the studied groups

The findings of pancreatic histology of diabetic mice demonstrated that the acinar cells formed pyramidal cells with basal nuclei and apical acidophilic cytoplasm.

Islets of Langerhans show irregularly shaped vacuolation and reduced beta cells in diabetic mice (Figure 7).

Microscopic examination of stained sections in the liver reveals marked lacerations in diabetic animals extended to all structures of the liver including steatohepatitis, macro- and micro-vascular fatty degeneration, periportal fibrosis, progressive enlargement of sinusoids, congestion in the portal vein with thrombus formation, hyperplasia, vacuolation in the wall of blood vessels and focal areas of hemorrhage and necrotic lesions (Figures 8, 9).

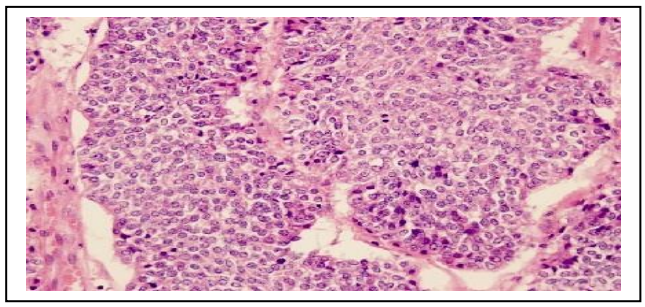

Figure 7. Pancreatic section of diabetic mice shows pyramidal cells of acinar cells and acidophilic cytoplasm (H and E stain, 100×)

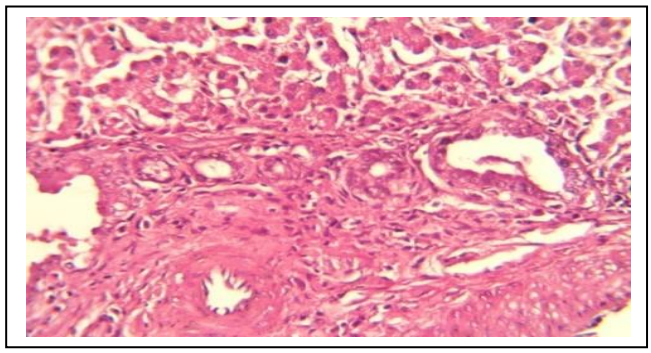

Figure 8. The hepatic section of diabetic mice shows ductal proliferation with mild portal fibrosis (H and E stain, 100×)

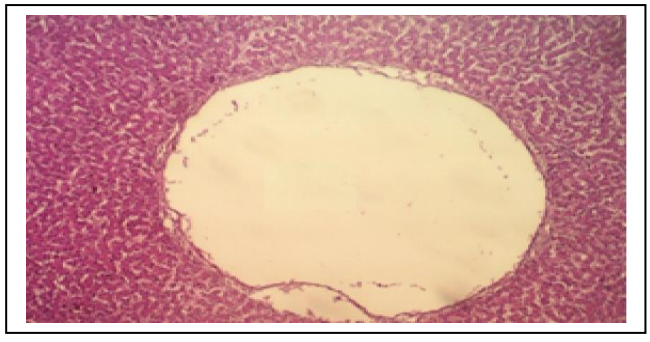

Figure 9. The hepatic section of diabetic mice shows marked dilation and congestion of portal and central veins (H and E stain, 100×)

4. Discussion

The well-documented effect of insulin in mediating the storage of carbohydrates, proteins, and lipids is vital to highlight components which affect a host's susceptibility to developing T2DM. Researchers rely on diverse animal models from multiple species and strategic scientific approaches to tackle these goals. For the past two decades, studies using small rodents, especially mice, have been at the forefront of scientific advances in T2DM (13). Evidence-based studies concerning HBV therapy in medical practice are still insufficient and the exact mechanism of action is still unknown, although a cascade of reactions is produced once the HBV enters the human body. The potential adverse effects of HBV therapy range from trivial skin reactions to severe life-threatening immunological responses (14). The result of the present study indicated a significant increase in glucose concentration and decrease in insulin level in the diabetic group compared to those of the control group. In addition, the study demonstrated that administrating HBV at a high dose (1 mg/kg) significantly contributed to decreasing the concentration of blood glucose and increasing the levels of insulin as reported by Mousavi, Imani (6), and El Senosi, Omayma (15). These results may be attributed to active constitutes in the HBV such as melittin and phospholipase A2, which may be involved in repairing damaged pancreatic β-cells and diminishing inflamed Islets of Langerhans (16). Subsequently, this may actively contribute to increasing the secretion of insulin, and therefore, decreasing the blood glucose content. Fujimoto and METZ (17) studied agonistic properties of phospholipase and melittin which indicated the existence of these substances inducing the monophasic depolarization β-cells membranes to the secret of insulin. Other studies found that melittin can initiate membrane depolarization leading to increase in the flow of Ca^{2+} ions for β-cells by the channels of Caindependence on extracellular Ca (18). The study suggests that HBV may be able to control the level of circulating glucose through stimulating insulin secretion and therefore can prevent inducing diabetes and manging progression of diabetes.

Abnormal glucose and lipid metabolism is a fundamental feature of diabetes. In the present study, inducing diabetes decreased lipid metabolism and therefore the findings indicated an increase in the cholesterol and TG as well as a reduction in HDL concentration. Administration of HBV ameliorated this deregulation in lipid metabolism without observing a significant difference in the LDL values among the mice of all studied groups. This result was expected as reported by other researchers (15, 19). In an experimental study, Tsutsumi, Hagi (20) suggested that the main cause of dyslipidemia in six animal species was decreasing activity of lipoprotein lipase which is an insulin-sensitive enzyme. Ivas, Solcan (21) mentioned that parameters of the lipid profile are modified by two plasmatic enzymes, lipoprotein lipase (LPL) and lecithin-cholesterol acyltransferase (LCAT) and that the functional specificity of these enzymes is the key to the understanding lipid metabolism. Also, they confirmed that the A2 phospholipases in HBV have an enzymatic activity three times higher than that of the plasmatic LCAT, and these activities increase transporting glucose and uptaking lipid into adipose tissues by partial lyses for membranes of adipocyte with binding large numbers of insulin molecules. Other researchers reported the role of HBV substances having great affinity for plasmatic lipoproteins by exerting their cytotoxic effects through generating lisophospholipids and free fatty acids (22). Finally, Khulan, Ambaga (23) suggested that HBV has an immune-modulating effect that inhibits the onset of DM by underlying autoimmune responses that damage pancreatic β-cells. Based on the data on serum cholesterol, Kader, Azmy (14) concluded that HBV treatment provides a simple and available means of prophylaxis against the myopathic effects induced by statins in an animal model. According to the result of the present along with findings of previous studies,

HBV could possibly be a potential anti-diabetic and anti-obesity agent; however, more qualitative and quantitative studies are required to identify possible mechanisms of action as anti-diabetic and anti-obesity.

The histological results were previously acknowledged, therefore only limited histopathological results with a limited scale were attributed to diabetic samples. Therefore, the histopathological effect of bee venom failed to be evaluated due to the lack of histological sections of the pancreas and liver were generated which mentioned in the methods and materials section. The findings of pancreatic histology were consistent with the result reported by Nurdiana, Goh (24); while, the results of hepatic sections were similar to that observed by Bilal, Riaz (25).

The finding indicated that administrating HBV to diabetic mice actively contributed to increasing the levels of insulin and HDL by reducing the concentration of circulating glucose, cholesterol, and TG. Hence, the study suggested that HBV can support health status, control the complications of diabetes, and protect the body against different biochemical and histological changes when given at precisely measured doses for healthy individuals and diabetic patients. However, further studies are required to demonstrate the role of HBV in treating T2DM and other metabolic and infectious diseases focusing on its histopathological role in addition to various impacts.

Authors' Contribution

A. M. E. and E. A. N. A. were responsible for preparing the studied animals by collecting data and blood samples; whereas, S. J. J. A. was responsible for inducing DM in study animals, administrating honeybee venom, and statistically analyzing obtained results. All authors contributed to writing and approving the final versions of this article.

Ethics

The present study was approved by the Ethics Committees of the College of Veterinary Medicine at Wasit University, Wasit, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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