Metformin and Bee Venom Enhanced Histological Changes of the Pancreas in Diabetic Mice

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Abstract

Metformin is an oral anti-diabetic medication that is used to manage type 2 diabetes mellitus (T2DM). Bee venom (BV) is recently showing several health properties including pain relief, antimicrobial, and antineoplastic with limited evidence as antidiabetic effect. Thus, the current experiment is performed to identify the proper activities of BV to regulate the level of glucose and insulin, and curative role on the histological structure of the panaceas. Totally, 20 adult male mice were used, 15 of them were subjected to diabetes induction by alloxan and the remaining 5 mice were considered as control. These15 diabetic mice were further divided into diabetic control group, the second is diabetic treated with oral metformin and the third group is diabetic treated with intraperitoneal BV. At the end of 30 days, the concentrations of glucose and insulin were detected, and the pancreata were extracted for histological evaluation. The result showed that the diabetic mice showed a significant elevation of glucose concentration associated with reduction insulin concentration. Administration of metformin and BV modulated these impairments and caused significant reduction of glucose concentration and elevation of insulin concentration. Furthermore, alloxan induced several histological alterations of the pancreas including impairments in the shape and size of the islet of langerhans, vacuolization of the islet and beta cells, irregular arrangement of alpha and beta cells, bleeding, initial degeneration of beta cells. All these impairments were returned to almost normal in response to metformin and BV. To conclude BV could be potential curative toxin that mange diabetic condition like metformin.

Keywords—Diabetes mellitus, Metformin, Bee Venom, Pancreas

1. Introduction

Diabetes is very frequent chronic disorder of the carbohydrate metabolism which eventually caused reduction of insulin generation and release and hyposensitivity of the tissues to respond to normal concentration of insulin. These alterations lead to a high level of glucose in the plasma, which ultimately causes many serious consequences on various organs. [1]. Accordingly, International Diabetes Federation in 2019 stated that there are about 463 million people all over the world suffering from diabetes, and 90% of them developed T2DM [2]. Several factors are believed to involve in raising the prevalence of T2DM including ageing, urbanization, environmental factors like lifestyle and eating behavior [2]. Furthermore, development of resistance to insulin in sensitive organs and a relative decrease in insulin production from the pancreatic β-cells are the main properties of T2DM [3].

Metformin is the only remaining member of the biguanide family that frequently prescribed for people with T2DM. Metformin perform its action by increasing the sensitivity of the peripheral tissues (muscles) and reducing the capacity of the hepatic cells to generate

glucose by inhibiting the process of gluconeogenesis [4]. This effect is achieved through stimulating AMP-activated protein kinase (AMPK) and protein kinase A (PKA) [5].

Bee venom (BV)is natural product of honey bees [6] that contains numerous substances including numerous polypeptides compounds (melittin, apamin, and mast cell degranulating peptide), bioamines (histamine, serotonin, dopamine, and norepinephrine), and many enzymes (phospholipase, hyaluronidase, histidine decarboxylase) [7]. These bioactive substances are believed to be responsible for treated several disorders including musculoskeletal and neurological disorders like arthritis and rheumatism, chronic recalcitrant neuralgia, arthralgia, and immune-related diseases. Furthermore, BV shows ability to reduce blood glucose and lipids in diabetic rodents [8] and prevent diabetes complications [9]. With limited studies investigation the proper effect of BV as antidiabetic agent, the present study is performed to illustrate the precise impact of BV to regulate glucose and insulin concentrations in the induced diabetic mice, and modulating effect on impaired histological structure of the pancreas.

2. Materials and Methods

2.1 Study Ethic approval

This study is approved and performed according to regulation of Ethical Committees/ College of Pure Sciences at Wasit University (Wasit, Iraq).

2.2 Animal housing and diabetes induction

Twenty adult male mice with a mean weight of 26.55 ± 2.70 g were purchased from the animal house of the Ministry of Sciences and Technology, Iraq. After acclimatization period, 15 mice were subjected to induce diabetes using 120 mg/kg body weight alloxan (Sigma-Aldrich, UK). The mice were supplemented with glucose fluid for 3 days. At the 4^{th} day, the blood glucose was measure through tail snipping, and the diabetes was confirmed when the concentration of blood glucose exceeded 200 mg/dl.

2.3 Preparation of Metformin and Bee Venom

The metformin powder 3 gram (Mylan Pharma, France) was dissolved in 80 ml distilled water and administered as 150 mg/kg body weight. Lyophilized BV (local market, Wasit) was dissolved in distilled water, the prepared metformin preserved at refrigerator until needed. Whereas, prepared BV was moved to sterile eppendorf tubes which preserved at -20◦C◦ until needed.

2.4 Animal study design

After the diabetes is induced, the mice were divided as following:

Control group: consisting of five mice that received only water and chew.

Diabetic group: including five mice that received only alloxan.

Metformin group: consisting of five diabetic mice treated with a single oral dose of 150 mg/kg metformin for 30 days.

Bee Venom group: consisting of five diabetic mice treated with a single intraperitoneal dose of 1 mg/kg Bee Venom for 30 days.

At the end of experiment, the mice were anaesthetized by using 0.3 mg/kg ketamine and 0.1 mg/kg lidocaine. The blood was taken directly from the heart and immediately placed in centrifuge at 3000 cycle/minute for 15 minutes to obtain the serum. The serum was preserved at -20◦C until required. Furthermore, the pancreas of all mice was extracted and preserved in 10% neutral buffered formalin for short time.

2.5 Serological evaluation

Specific ELISA kits were used to estimation the level of glucose (Spinreact, Spain) and insulin ((Monobind, USA)in serum samples based on manufacturer procedures.

2.6 Histological examination

All mice pancreas was subjected to the normal histological processing series including dehydration with ethanol, clearing with using xylene, infiltration and embedding in a liquid paraffin wax, and cutting the blocks by microtome into sections. The obtained histological

sections were stained with hematoxylin and eosin routine stain, and gomori special beta and alpha cells stain. The slides were examined under the light microscope ((Novel) China) and the images were taken using (camera type and resolution) (Optica, Italy).

2.7 Statistical analysis

Microsoft Office Excel version 2019 (Microsoft, USA) was used to process all data obtained. GraphPad Prism version 6 (GraphPad Software Inc., USA) was used to analyze data through applying One-Way ANOVA with multiple comparison Turkey's post hock. The values of P were set as <0.05 (*), <0.01 (**), <0.001 (***) and <0.0001 (****) between groups. The displayed data were represented as Mean ± Standard Error of Mean.

3. Results and Discussion

3.1 Metformin and Bee Venom reduced hyperglycemia

The level of glucose was elevated in mice that received alloxan by 174% ± 4% compared to mice that received only water and chew. This elevation in the glucose concentration was significantly decreased by 28% ± 5.4% and 29% ± 6.3% in response to metformin and Bee Venom respectively compared to diabetic (Figure 1).

3.2 Metformin and Bee Venom elevated hypoinsulinemia

Mice that administered alloxan showed significant reduction in the level of insulin by 49.5% ± 4.6% compared to control mice. This hypoinsulinemia was significantly improved by increasing the insulin concentration by 52.5% ± 2.3% and 53.5% ± 5.4% in mice received metformin and bee venom respectively compared to diabetic mice (Figure 2).

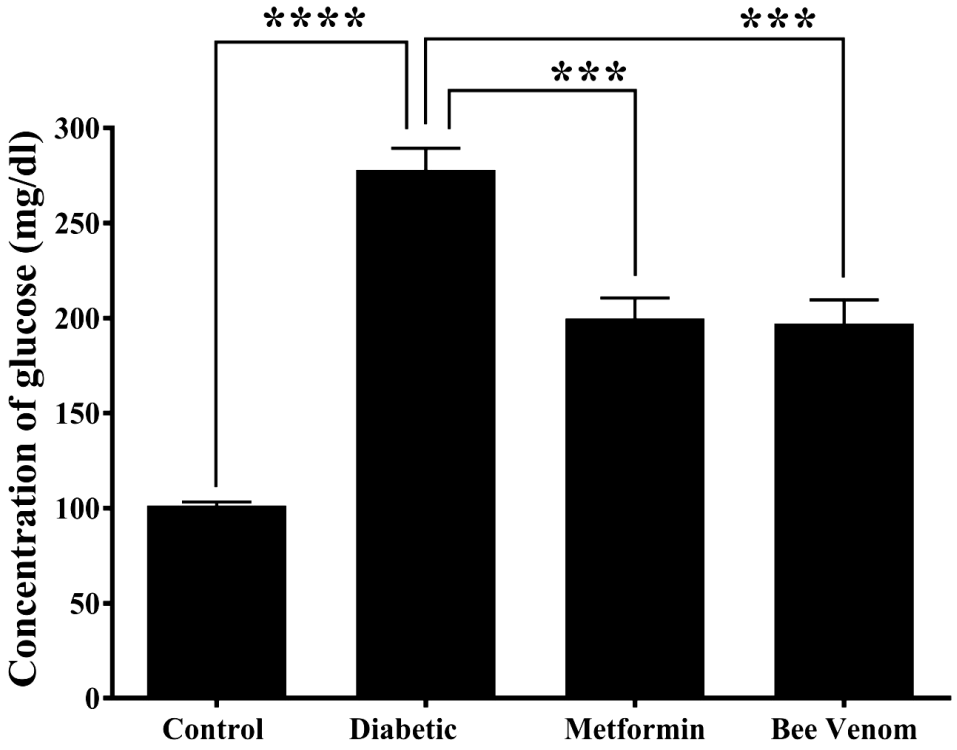

Figure 1. Metformin and BV reduced the glucose level in diabetic mice.

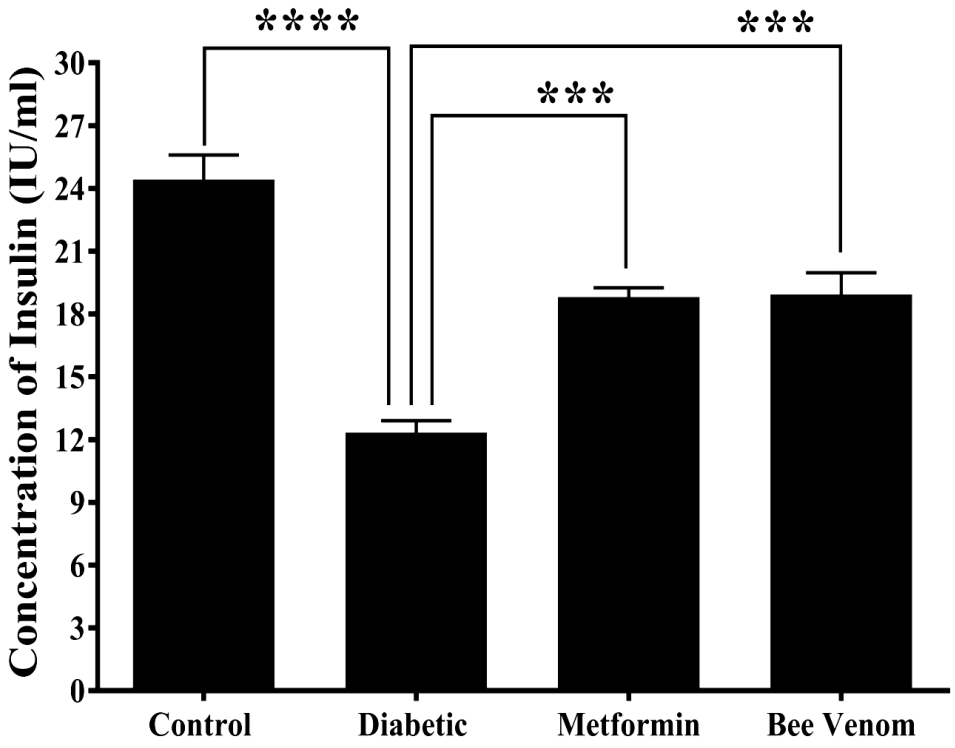

Figure 2. Metformin and BV elevated the level of insulin in diabetic mice.

The use of alloxan intraperitoneally caused a remarkable elevation of blood glucose and a reduction in the insulin concentration [10] which is consistent with our results .Since its discovery, alloxan was utilized to explore any recent substances that are expected to have a glucose lowering action with the less undesirable effect [11].Alloxan is able to destroy the pancreatic beta cells by accumulation inside them and the formation of free radicals at the end which are very toxic and leads to cell death[12].

Previous research showed that metformin possesses the ability to reduce glucose concentrations in the blood associated with elevated insulin secretion [13] that comes in agreement with our results. The hypothesized mechanism by which metformin can reduce glucose levels is by reducing hepatic gluconeogenesis, enhancing peripheral tissues sensitivity to insulin and hence increase glucose uptake by them and to some extent reduce the absorption of glucose from the gut [14].Another research study explained that metformin is able to enhance incretin pathways that eventually ends in the production of the incretin substances that comprise glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) from cells of the intestinal epithelium [15].

It has been found that administration of BV to experimental animals caused an apparent declination in the glucose levels with reciprocal effects on the insulin concentrations [16].BV has the capacity to reduce high concentrations of glucose [8] and this is concomitant with our results. This capacity occurs due to many active substances in its composition, particularly melittin and phospholipase A2 [17]. Melittin is able to stimulate insulin production by its effect to lowers the pancreatic Islets inflammatory process [18].Furthermore, melittin has ability to depolarize the cell membranes of the pancreatic β-cells which leads to the opening of Ca2+ channels allowing for the influx of huge quantities of calcium which ultimately activate them to produce insulin [19]. Based on the evidence mentioned above, BV could be potent antidiabetic agent which could aid the diabetic individuals [20], [21].

3.3 Metformin and Bee Venom enhanced the architecture of pancreas

The histological structure of the pancreas was investigated in all study mice. The mice that only received water and chew showed normal histological structure of both end and exocrine parts of pancreas. These regular structures including regular acinar cells organization that surrounded normal structure of Islet of Langerhans with dedicated connective tissue separated between them. Furthermore, the pancreatic islet cells exhibited regular organization pattern (Figures 3, 4 A). Also, Gomori stain showed similar structure with clearly distinguish of beta cells that the cytoplasm is stained in dark blue and alpha cells that the cytoplasm stained in pink color (Figure 5, 6 A).

Induction of diabetes showed several and clear histological changes in the pancreas particularly in the islet of langerhans. These alterations including reduction in the size and irregular shape of the islets, reduced the capsular tissue that surrounded the islets, islets vacuolization, blood vessels bleeding, irregular pattern of islet cells organization (Figures 3, 4, 5 B). Furthermore, initial degeneration of beta cells with reduction in the number (Figure 6 B). This result is in accordance with several studies results that showed variable amounts of alterations in the histological structure of the pancreas including changes in the islets shape, size, and number, islet vacuolization, beta cell vacuolization, hemorrhage, and initial degeneration of the pancreatic beta cells [22], [23].

The pancreatic architectures of mice that received metformin and BV showed significant enhancement compared to diabetic mice. Both study agents caused reduction of bleeding and vacuolization in the pancreatic structure, improved the capsular connective tissue around the islets, and enhanced the islets size and shape (Figures 3, 4, 5 C and D). Also, the beta cells size, shape, organization returned to nearly normal with diminished beta cells vacuolization and degeneration (Figure 6 C and D). A previous study showed that pancreatic tissue was ameliorated in response to metformin. The improvements are including clear connective tissue between the exocrine part and endocrine, increase cellular density with a declination in the inflammatory cell's invasion inside the pancreatic islets[24]. Also, metformin caused approximately cellular organization and returned cells morphology to nearly normal associated with increase in thickness of connective tissue wall that separated between exocrine and endocrine tissues [25].Furthermore, metformin acts to renew the cells of the pancreatic islet with an increasing of the β-cell mass and healing of the necrotic β-cells in diabetic animals [26]. Moreover, metformin has ability to regenerate pancreatic tissues more than the corresponding group who was treated with insulin [27].

Administration of BV displayed markedly improvement in the architecture of the pancreas in diabetic conditions which including enhanced the shape and size of islets of Langerhans, and normal cellular shape, size, cytoplasm and nucleus [28].Similar result was appeared in [29].Administering BV caused a huge transformation in the morphological features of pancreatic islets with retaining of their size and organized shape. Furthermore, β-cells were obviously up regulated their regeneration and the major portion of these cells regained their sphere border and few of cells remained anomalous with serrated border[21].It has been suggested that the ability of bee venom to restore normal anatomy and physiology of the pancreas due to its anti-oxidant and anti-inflammatory impacts[22].It is also mentioned that the mechanism that leads to the enhancement of the histological architecture of the pancreas is due to the action of melittin and phospholipase A2that present in the BV through reducing the β cells inflammation[30].

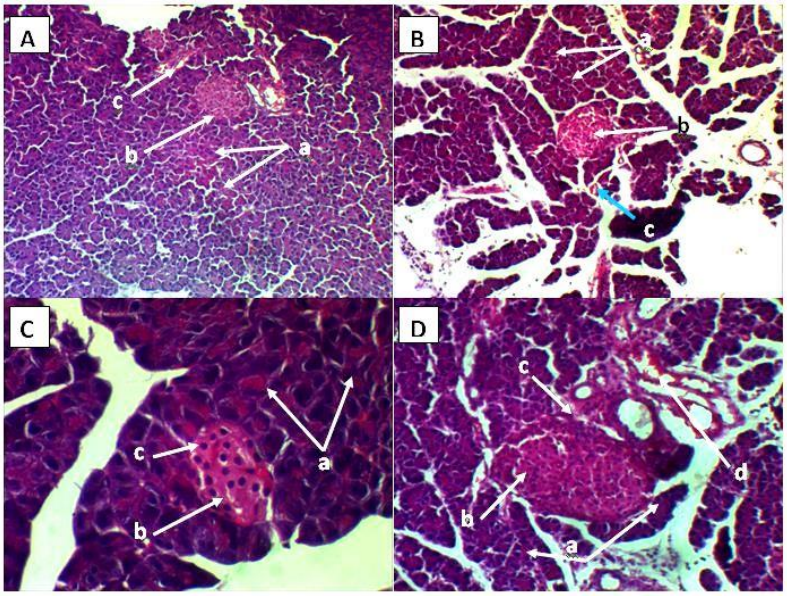

Figure 3. Metformin and BV enhanced the histological structure of the pancreas in mice.

A. Control mice. a. endocrine acinus. b. islet of langerhans. c. blood vessel.

B. Diabetic mice. a. endocrine acinus. b. islet of langerhans. c. blood vessel.

C. Metformin treatment. a. endocrine acinus. b. islet of langerhans. c. islet cells.

D. BV treatment. a. endocrine acinus. b. islet of langerhans. c. capsular connective tissue.d. blood vessels. H & E stain, 10X.

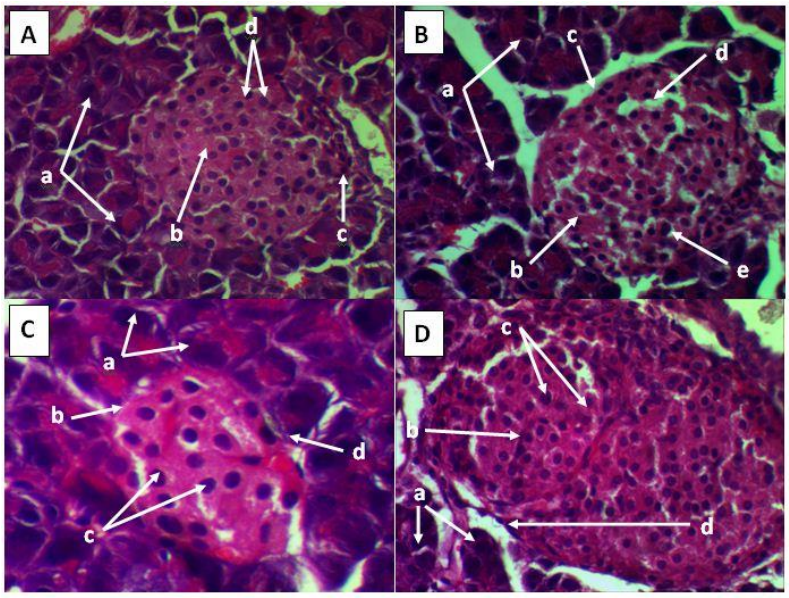

Figure 4. Metformin and BV enhanced the histological structure of the pancreas in mice.

A. Control mice. a. acinar cells. b. islet of langerhans. c. connective tissue capsule. d. islet cells.

B. Diabetic mice. a. acinar cells. b. islet of langerhans. c. delicate capsular tissue. d. cell vacuolization. e. islet vacuolization

C. Metformin treatment. a. acinar cells. b. islet of langerhans. c. islet cells. d. capsular connective tissue.

D. BV treatment. a. acinar cells. b. islet of langerhans. c. islet cells. d. capsular connective tissue. H & E stain, 40X.

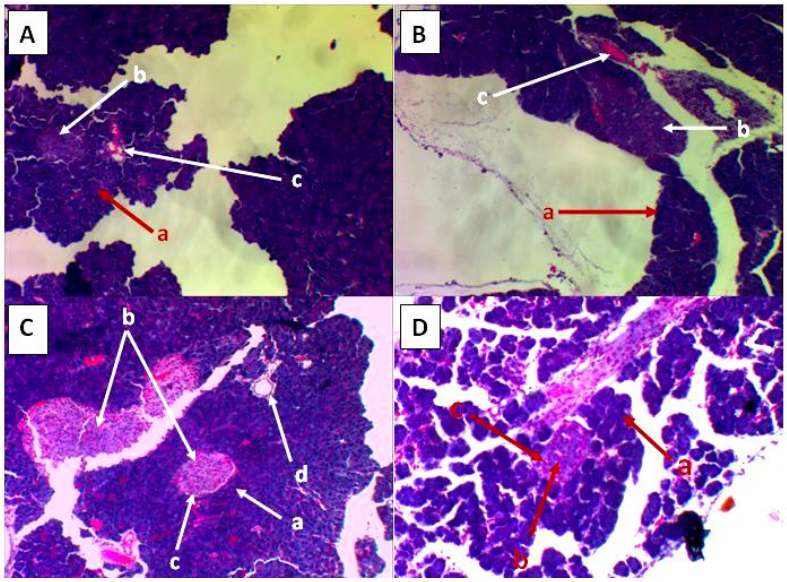

Figure 5. Metformin and BV enhanced the histological structure of the pancreas in mice.

A. Control mice. a. endocrine acinus. b. islet of langerhans. c. capsular connective tissue.

B. Diabetic mice. a. endocrine acinus. b. islet of langerhans. c. blood vessel bleeding.

C. Metformin treatment. a. endocrine acinus. b. islet of langerhans. c. capsular connective tissue. d. blood vessel.

D. BV treatment. a. endocrine acinus. b. islet of langerhans. c. capsular connective tissue. Gomori stain, 10X.

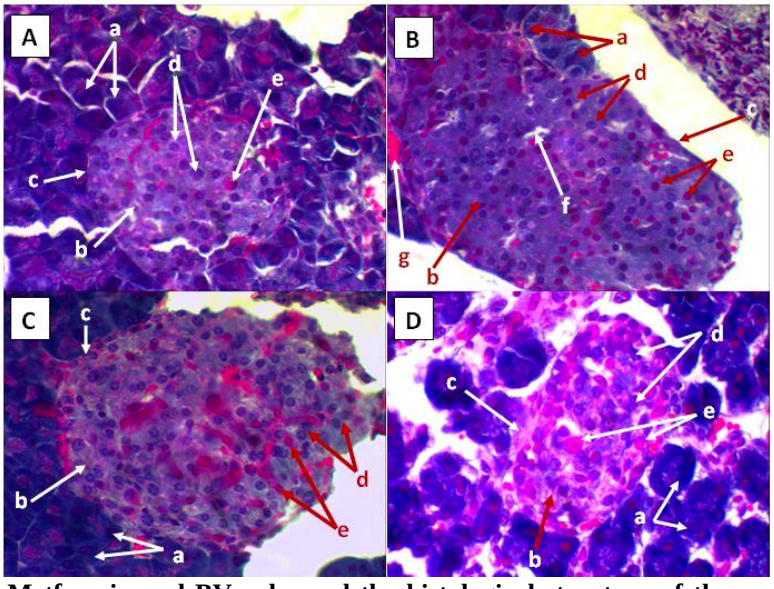

Figure 6. Metformin and BV enhanced the histological structure of the pancreas in mice.

A. Control mice. a. acinar cells. b. islet of langerhans. c. connective tissue capsule. d. beta cells. e. alpha cells

B. Diabetic mice. a. acinar cells. b. islet of langerhans. c. delicate capsular tissue. d. beta cell. e. alpha cells. f. islet vacuolization. g. blood vessel bleeding.

C. Metformin treatment. a. acinar cells. b. islet of langerhans. c. capsular connective tissue. d. alpha cells. e. beta cells.

D. BV treatment. a. endocrine acinus. b. islet of langerhans. c. capsular connective tissue. d. alpha cells. e. beta cells. Gomori stain, 40X.

4. Conclusion

Clearly, both metformin and BV showed potent antidiabetic action through regulating glucose and insulin levels in diabetic mice. This potential impact is interesting as regulating these markers are important to manage diabetic condition and its consequences. Furthermore, both agents showed ability to ameliorate the alteration histological structure of the pancreas restored almost all deterioration which could be through elimination free radicals and inflammation. Based on these results, BV could be potent therapeutic antidiabetic agent that might prevent worsen the diabetic condition.

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