# EFFECT OF SPEXIN TREATMENT ON CARDIOMETABOLIC CHANGES IN OBESE TYPE 2 DIABETIC RATS

By

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## ABSTRACT

**Background:** Spexin is a peptide hormone that was expressed in brain regions and peripheral tissues of many species including human and rat. Several studies investigated its blood level changes with obesity and type 2 diabetes, but few studies assessed its effect on cardiometabolic, histological and morphometric changes in diabetes with some controversies in their results.

**Objective:** To explore the effect of spexin treatment on cardiometabolic, histological and morphometric changes of pancreas and heart tissue in obese type 2 diabetic rats, and the possible mechanisms involved.

**Materials and Methods:** Thirty adult male albino rats were divided randomly into 5 equal groups; control (fed ordinary laboratory chow along this 8 weeks' study), diabetic [fed high fat diet along this 8 weeks' study, but at the start of the 4th week, overnight fasted rats were injected with a single streptozotocin injection intraperitoneally (40 mg/kg body weight, dissolved in 0.01M citrate buffer, pH 4.5) to induce diabetes], diabetic metformin-treated [rats were managed as in diabetic group and in addition to that, metformin was given orally (300 mg/kg/day) by oral gavage to rats for 4 weeks from the start of 5th week to the end of 8th week], diabetic vildagliptin treated [rats were manipulated as in diabetic group and in addition to that, vildagliptin was given orally (10 mg/kg/day) using oral gavage to rats for 4 weeks from the start of 5th week to the end of 8th week], and diabetic spexin treated [rats were managed as in diabetic group and in addition to that, spexin dissolved in normal saline was injected intraperitoneally (35 μg/kg/day) for 4 weeks from the start of 5th week to the end of 8th week] groups. For histological, immunohistochemical and morphometric examinations, fresh heart and pancreatic specimens were collected from rats that were sacrificed.

**Results:** In diabetic group, a significant increase was detected in final body mass index, serum glucose, serum insulin, homeostasis model assessment-insulin resistance, serum total cholesterol, serum triglycerides, serum low density lipoprotein, atherogenic index, serum dipeptidyl peptidase-IV, serum tumor necrosis factor alpha serum interleukin-1 beta, serum malondialdehyde, serum lactate dehydrogenase, serum creatine kinase-myoglobin binding and mean arterial blood pressure, with a significant decrease in homeostasis model assessment-beta cell function serum high density lipoprotein, serum superoxide dismutase and serum spexin in comparison to that in the control group. With spexin treatment (as well as with the administration of standard drugs metformin and vildagliptin), all these changes were reversed significantly in comparison with those in the diabetic group. Diabetes induced histopathological changes in cardiac muscle and pancreatic structure which were ameliorated by treatment with spexin as well as with the standard anti-diabetic drugs (metformin and vildagliptin). The morphometric analysis confirmed the histopathological results, as a statistically significant difference was detected in area percent of collagen deposition, area percent of insulin immune-reaction in pancreas and cardiac muscle Bax expression between

both diabetic group and all other groups, with no statistically significant difference between diabetic metformin treated, diabetic vildagliptin treated and diabetic spexin treated groups.

**Conclusion:** Spexin ameliorated diabetes induced deleterious cardiometabolic, histopathological and morphometric disturbances. The anti-obesity, dipeptidyl peptidase-IV inhibitory, hypoglycemic, hypolipidemic, antioxidant, anti-inflammatory and cardio-protective properties of spexin may contribute to its useful effects.

**Key words:** Spexin, Cardiometabolic changes, Obese type 2 diabetic rats, Metformin, Vildagliptin.

# INTRODUCTION

Spexin is a peptide hormone that was intensely expressed in brain regions and peripheral tissues of human and rat, including the hypothalamus, cerebral cortex, pons, retina, esophagus, pancreas, stomach, heart, kidney, thyroid, liver, ovary, testis, adrenal glands and adipose tissue *(Gu et al. 2015* and *Liu et al. 2019).*

Diabetes mellitus is a metabolic disorder that is caused by either lack of secretion of insulin or decreased sensitivity to insulin by the tissues *(American Diabetes, 2010).* It is one of the major health problems due to its micro and macrovascular hazards *(Omar and Aboulkhair, 2017).* Also, beta cells of islets of Langerhans and hence insulin secretion is markedly affected by diabetes mellitus *(Nurdiana et al., 2017). Yu et al. (2012)* declared that one of the most dangerous complications of diabetes is the diabetic cardiomyopathy.

*Kolodziejski et al. (2018a)* observed that circulating spexin levels were low in type 2 diabetes mellitus (T2DM) patients and inversely related to blood glucose and lipids. Also, *Al-Daghri et al. (2018b)* noted that spexin levels were significantly lower in obese children but without associations with markers of insulin resistance. Moreover, *Ge et al. (2016)* reported that spexin treatment in mice suppressed hepatic lipids and long-chain fatty acid uptake, contributing to loss in body weight.

Glucagon-like peptide-1 plays a climacteric role in secretion and signaling of insulin, and is degraded by dipeptidyl peptidase-IV (DPP-IV) *(Silva Júnior et al., 2019). Lamers et al. (2011)* noticed a rise in DPP-IV release in obese persons who had a higher risk of developing insulin resistance and diabetes *(Almass et al., 2016).* Also, *Kirino et al. (2009)* reported that plasma DPP‑IV activity increased in rats after streptozotocin (STZ) injection and positively correlated with blood glucose levels. The DPP‑IV inhibitor vildagliptin is an anti‑hyperglycemic drug that may benefit patients with diabetes by regulating blood glucose levels, increasing weight loss and providing cardiovascular advantages with fewer associated adverse events *(Ji et al., 2016).*

Metformin, an insulin sensitizer, is used for the treatment of T2DM as it decreased the elevated blood glucose level, reduced fat mass, decreased hepatic glucose production by suppressing gluconeogenesis and activating peripheral glucose utilization in muscle, intestine and liver *(Mithieux et al., 2006).* Some studies were published regarding the association between chronic hyperglycemia and cardiovascular complications in T2DM, including systemic hypertension and atherosclerosis *(Boudina & Abel, 2010*;

*Rutter & Nesto, 2011*; *Giorgino et al., 2013* and *Wright et al., 2019).*

Few studies were conducted to investigate the association between spexin and cardiometabolic changes with obesity complicated with diabetes and their results showed some controversies *(Al-Daghri et al., 2018a* and *Kumar et al., 2018).*

Thus, this study was planned to explore the effect of spexin treatment on cardiometabolic changes (including also the histopathological and morphometric changes in heart and pancreas) in obese type 2 diabetic rats and the possible mechanisms involved.

## PATIENTS AND METHODS

In Physiology Department, Zagazig Faculty of Medicine, this study was carried out during the period between August 2019 and November 2019. Thirty adult male albino rats of a local strain weighing 140–190 g, obtained from Animal House, Faculty of Medicine Zagazig University, housed in steel wire cages (50x30x20 cm), 3 rats/cage, in a light- and temperature-controlled room on normal light–dark cycle and fed a standard pellet lab chow with ad libitum access to tap water.

The experimental protocol in this study was conducted according to the data guiding the use of research animals and was approved by the Institutional Animal Care and Use Committee (IACUC), Zagazig University.

**Experimental design:** After acclimation period of 2 weeks, rats were divided randomly into 5 equal groups; control, diabetic, diabetic metformin treated, diabetic vildagliptin treated and diabetic spexin treated groups. In control group, rats were fed ordinary diet consisted of mixed rat laboratory chow (25.8 % protein, 62.8 % carbohydrate and 11.4 % fat) obtained from Faculty of Zagazig Agriculture and administered normal saline orally 1 ml/day using oral gavage for 8 weeks (study period).

At the start of the 4th week, 0.01M citrate buffer, pH 4.5 was injected intraperitoneally (i.p.) to mimic the STZ injections. In diabetic group, high fat diet (HFD), formed of protein (16.45%), carbohydrate (25.6%) and fat (58.0%) in the form of cotton seed oil added to the laboratory chow diet *(Cha et al., 2000)*, was fed to rats for 8 weeks. At the start of the 4th week, overnight fasted rats were injected with a single STZ injection (40 mg/kg body weight, i.p., dissolved in 0.01M citrate buffer, pH 4.5) to induce diabetes *(Suman et al., 2016).*

The animals were allowed to drink 5% glucose solution overnight to overcome drug induced hypoglycemia. Blood glucose was estimated 7 days after STZ injection and rats with blood glucose (> 200 mg/dl) were considered diabetic *(Rajesh et al., 2017)*. In diabetic metformin treated group, rats were managed as in diabetic group and in addition to that, metformin (obtained as Cidophage 500 mg tabs, from CID, Egypt) was given orally (300 mg/kg/day) *(Oliveira et al., 2014)* by oral gavage to rats for 4 weeks from the start of 5th week to the end of 8th week.

**Table (1): Experimental design**

| Parameter \ Group | Control | Diabetic | Diabetic metformin treated | Diabetic vildagliptin treated | Diabetic spexin treated |
|---|---|---|---|---|---|
| **Duration (weeks)** | 8 | 8 | 8 | 8 | 8 |
| **Number of rats** | 6 | 6 | 6 | 6 | 6 |
| **Ordinary diet along the study** | ✓ | | | | |
| **High fat diet along the study** | | ✓ | ✓ | ✓ | ✓ |
| **STZ i.p. at the start of the 4th week** | | ✓ | ✓ | ✓ | ✓ |
| **Metformin orally from the start of the 5th to the end of the 8th week of the study** | | | ✓ | | |
| **Vildagliptin orally from the start of the 5th to the end of the 8th week of the study** | | | | ✓ | |
| **Spexin i.p. from the start of the 5th to the end of the 8th week of the study** | | | | | ✓ |

**STZ, streptozotocin; i.p., intraperitoneal**

In diabetic vildagliptin treated group, rats were manipulated as in diabetic group and in addition to that, vildagliptin (DPP-IV inhibitor obtained as Gliptus 50 mg tabs, from EVA Pharma, Egypt) was introduced orally (10 mg/kg/day) *(Rajesh et al., 2017)* using oral gavage to rats for 4 weeks from the start of 5th week to the end of 8th week. In diabetic spexin treated group, rats were managed as in diabetic group. In addition, spexin (purchased from Phoenix Pharmaceuticals; Belmont, CA, USA) was dissolved in normal saline and injected i.p. (35 μg/kg/day) *(Sassek et al., 2018)* for 4 weeks from the start of 5th week to the end of 8th week.

Final body mass index (BMI) (g/cm2) was assessed in different studied groups by dividing final body weight (g) on Length2 (cm2) where rat length was notified by estimating the distance between the anus and the nose *(Novelli et al., 2007)*.

At the end of the experiment, the mean arterial blood pressure (MABP) was measured by the tail-cuff device (NARCO, Biosystem, Inc., Huston, Texas) after the rats have been warmed in a metabolic chamber for 30 min to be maintained at approximately 30oC. The mean of the three consecutive measurements of MABP was considered as the pressure value for each rat *(Sakamaki et al., 1987)*.

After measuring MABP, blood samples were collected from the tail vein *(Suman et al., 2016)* under ether anesthesia, left for 30 min at room temperature to clot and then centrifuged at 3000 rpm for 15 min and serum was separated and stored at -80oC for estimation of biochemical parameters.

In the Biochemistry Laboratory, Zagazig Faculty of Medicine, serum was collected and used to estimate levels of spexin using commercial kits (catalog No. DEIA10757; Creative Diagnostics, USA).

Also, serum levels of glucose, insulin, total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), tumor necrosis factor alpha (TNFα, interleukin 1 beta (IL-1β, superoxide dismutase (SOD), malondialdehyde (MDA), lactate dehydrogenase (LDH), creatine kinase-myoglobin binding (CK-MB) and dipeptidyl peptidase-IV (DPP-IV) by kits (purchased from Sigma-Aldrich, USA) with catalog No. CBA086, RAB0904, MAK043, MAK266, MAK045, RAB0479, RAB0277, 19160, MAK085, MAK066, MAK116 and MAK088, respectively.

Both insulin resistance and β-cell function were calculated by homeostasis model assessment (HOMA):

Insulin resistance (HOMA-IR) = (Fasting glucose in mg/dl x Fasting insulin in µIU/ml)/405 *(Sun et al., 2007)*.

β- Cell function (HOMA-□) = (360 x Fasting insulin in µIU/ml)/(Fasting glucose in mg/dl-63) *(Hermans et al., 1999)*.

Atherogenic index was calculated from the following formula: Atherogenic index (AI) = (TC-HDL)/ HDL *(Kayamori and Igarashi, 1994)*.

LDL was estimated using the following formula: LDL (mg/dl) = [TC] – [(HDL) + (TG / 5)] *(Friedewald et al., 1972)*.

**Histopathological studies:** After the animals were sacrificed at the end of the experiment, the heart and pancreas were fixed immediately in 10% buffered neutral formalin solution and embedded in paraffin to prepare paraffin sections of 5 µm thickness that were deparaffinized, rehydrated and stained with the following stains:

1. Hematoxylin and eosin stain: to visualize the microscopic architecture of the tissues under light microscope *(Kiernan, 1999)*.

2. Mallory trichrome stain: to detect distribution of collagen fibres in pancreatic tissue *(Bancroft & Gamble, 2008)*.

3. Immunohistochemical localization of insulin: Sections from pancreatic specimens were selected for immune-histochemical study using the anti-insulin antibody; (Thermo Fisher Scientific, Fremont, CA, USA) that was prepared to detect the immune-reaction of insulin. Hydrogen peroxide block was used for 15 minutes to decrease the background staining of endogenous peroxidase, then washing by phosphate buffer, later on incubation in the ultra V block for 5 minutes at room temperature. After that, sections were reared in the anti-insulin antibody after being diluted (1:200) for 30 minutes at room temperature. Then, light microscope was used to determine the immunohistochemical localization of insulin *(Abdelrahim, 2013)*.

4. Immunohistochemical measurement of Bax expression and distribution: prepared cardiac muscle paraffin sections were treated according to the instructions of the staining kit, previously were treated with 3% H2O2 to neutralize endogenous peroxidase, then by serum blocking and heat-induced antigen recovery techniques. The specific primary antibody was added, and then sections were nursed overnight at 4oC. Later, the secondary antibody was added with 30 min

incubation period, then staining by DAB and hematoxylin counterstaining and observed under a microscope *(Li et al., 2018).*

**Morphometric study:** The morphometric study was performed in Image Analyzing Unit of Human Anatomy and Embryology Department, Faculty of Medicine, Zagazig University, Egypt, using the computerized image analyzer (the Image J software plugin) to measure the followings:

1. Mean area percent of collagen in Mallory's trichrome-stained pancreatic specimens.

2. Mean area percent of insulin immune-expression in islets of Langerhans in insulin immunostained sections *(Abunasef et al., 2014* and *Omar & Aboulkhair, 2017).*

3. Bax immunostained cardiac muscle area. Automatically by calibration, the image analyzer converts the measurement units (pixels) into micrometers. For each specimen, ten readings from non-overlapping fields were recorded, and the mean was calculated. The measured data were investigated at 400× magnification by the use of the interactive measurement menu.

**Statistical Analysis:**

The results were expressed as mean ± standard deviation (SD). For statistical significance, one-way analysis of variance (ANOVA) and Tukey HSD for Post hoc multiple comparisons were used to compare means. The software, IBM Statistical Package for Social Sciences (SPSS) Version 26 Software for Windows (SPSS, Inc., Chicago, IL, USA), was used for that purpose.

Also, Graph Pad Prism (Version 8 Software for Windows) was used to analyze the Pearson's correlation coefficient between serum levels of spexin and some studied parameters within the diabetic group. Significance was considered with P value ≤ 0.05.

## RESULTS

**Cardiometabolic changes in diabetic group (Table-2):** There was a significant increase in final BMI, serum glucose, serum insulin, HOMA-IR, serum TC, serum TG, serum LDL, atherogenic index, serum DPP-IV, serum TNFα, serum IL-1β, serum MDA, serum LDH, serum CK-MB and MABP, with a significant decrease in HOMA-β, serum HDL, serum SOD and serum spexin in comparison to that in the control group.

**Cardiometabolic changes in diabetic metformin treated group (Table-2):** A significant decrease was found in final BMI, serum glucose, serum insulin, HOMA-IR, serum TC, serum TG, serum LDL, atherogenic index, serum DPP-IV, serum TNFα, serum IL-1β, serum MDA, serum LDH, serum CK-MB and MABP, with a significant increase in HOMA-β, serum HDL, serum SOD and serum spexin in comparison to that in the diabetic group.

**Cardiometabolic changes in diabetic vildagliptin treated group (Table-2):** There was a significant decrease in final BMI, serum glucose, serum insulin, HOMA-IR, serum TC, serum TG, serum

LDL, atherogenic index, serum DPP-IV, serum TNFα, serum IL-1β, serum MDA, serum LDH, serum CK-MB and MABP, with a significant increase in HOMA-β, serum HDL, serum SOD and serum spexin in comparison to that in the diabetic group.

**Cardiometabolic changes in diabetic spexin treated group (Table-2):** A significant decrease was noticed in final BMI, serum glucose, serum insulin, HOMA-IR, serum TC, serum TG, serum LDL, atherogenic index, serum DPP-IV, serum TNFα, serum IL-1β, serum MDA, serum LDH, serum CK-MB and MABP,

with a significant increase in HOMA-β, serum HDL, serum SOD and serum spexin in comparison to that in the diabetic group.

**Correlation between serum spexin level and some studied parameters within the diabetic group (Table-3):** Spexin serum level was negatively correlated with each of BMI, serum glucose, HOMA-IR, serum TC, serum DPP-IV, atherogenic index, serum TNFα, serum LDH and serum CK-MB, but it was positively associated with both HOMA-β and serum SOD.

**Table (2): Biochemical changes in different studied groups** (number of rats in each group= 6)

| Groups / Parameters | Control | Diabetic | Diabetic metformin treated | Diabetic vildagliptin treated | Diabetic spexin treated |
|---|---|---|---|---|---|
| Final BMI (g/cm²) | 0.57±0.04 | 0.82±0.02ᵃ | 0.66±0.03ᵃ&ᵇ | 0.69±0.05ᵃ&ᵇ | 0.71±0.03ᵃ&ᵇ |
| Serum glucose (mg/dl) | 80.17±1.33 | 363.83±6.68ᵃ | 166±6.16ᵃ&ᵇ | 162.17±6.08ᵃ&ᵇ | 169.5±8.92ᵃ&ᵇ |
| Serum insulin (μIU/ml) | 8.58±0.5 | 30.12±2.89ᵃ | 15.37±1.14ᵃ&ᵇ | 17.33±1.14ᵃ&ᵇ | 15.99±0.64ᵃ&ᵇ |
| HOMA-IR | 1.7±0.12 | 27.09±2.98ᵃ | 6.3±0.53ᵃ&ᵇ | 6.94±0.56ᵃ&ᵇ | 6.69±0.35ᵃ&ᵇ |
| HOMA-β | 180.5±11.1 | 36.01±2.91ᵃ | 53.88±4.98ᵃ&ᵇ | 63.08±4.98ᵃ&ᵇ | 54.43±5.98ᵃ&ᵇ |
| Serum TC (mg/dl) | 70±5.1 | 287.67±9.73ᵃ | 116±4.15ᵃ&ᵇ | 107.83±3.49ᵃ&ᵇ | 107.83±6.34ᵃ&ᵇ |
| Serum TG (mg/dl) | 77±6.1 | 315.33±10.76ᵃ | 138.83±7.19ᵃ&ᵇ | 135.17±7.17ᵃ&ᵇ | 133±7.4ᵃ&ᵇ |
| Serum HDL (mg/dl) | 38.67±4.55 | 20±2.28ᵃ | 30.67±2.42ᵃ&ᵇ | 30±2.61ᵃ&ᵇ | 28.33±3.33ᵃ&ᵇ |
| Serum LDL (mg/dl) | 15.93±1.38 | 204.6±10.62ᵃ | 57.57±3.17ᵃ&ᵇ | 50.8±5.53ᵃ&ᵇ | 52.9±4.29ᵃ&ᵇ |
| Atherogenic index | 0.82±0.09 | 13.56±1.84ᵃ | 2.8±0.28ᵃ&ᵇ | 2.62±0.36ᵃ&ᵇ | 2.84±0.33ᵃ&ᵇ |
| Serum DPP-IV (microunit/ml) | 4.9±0.15 | 41.53±1.57ᵃ | 27.4±1.75ᵃ&ᵇ | 13.64±1.02ᵃ,ᵇ,ᶜ | 19.37±0.59ᵃ,ᵇ,ᶜ&ᵈ |
| Serum TNFα (pg/ml) | 23.65±0.73 | 93.59±1.74ᵃ | 41.5±0.62ᵃ&ᵇ | 45.13±1.12ᵃ,ᵇ&ᶜ | 42.92±1.26ᵃ,ᵇ&ᵈ |
| Serum IL-1β (pg/ml) | 36.36±0.6 | 60.28±0.66ᵃ | 47.31±0.51ᵃ&ᵇ | 48.86±1.01ᵃ,ᵇ&ᶜ | 50.53±1.34ᵃ,ᵇ,ᶜ&ᵈ |
| Serum SOD (Unit/ml) | 354.99±4.8 | 169.75±1.35ᵃ | 296.57±4.11ᵃ&ᵇ | 287.19±2.95ᵃ,ᵇ&ᶜ | 298.63±5.77ᵃ,ᵇ&ᵈ |
| Serum MDA (nmol/ml) | 11.36±0.32 | 22.56±0.43ᵃ | 15.5±0.41ᵃ&ᵇ | 14.84±0.2ᵃ,ᵇ&ᶜ | 15.26±0.3ᵃ&ᵇ |
| Serum spexin (ng/ml) | 1.78±0.06 | 0.73±0.05ᵃ | 1.24±0.04ᵃ&ᵇ | 1.18±0.02ᵃ&ᵇ | 1.79±0.03ᵇ,ᶜ&ᵈ |
| Serum LDH (U/L) | 256.83±7.74 | 522.15±5.65ᵃ | 418±4.24ᵃ&ᵇ | 392.1±6.84ᵃ,ᵇ&ᶜ | 398.63±7.17ᵃ,ᵇ&ᶜ |
| Serum CK-MB (U/L) | 133.12±5.43 | 264.12±3.98ᵃ | 200.63±3.6ᵃ&ᵇ | 189.35±2.71ᵃ,ᵇ&ᶜ | 193.97±2.24ᵃ,ᵇ&ᶜ |
| MABP (mmHg) | 117.5±2.74 | 138.83±2.64ᵃ | 118.33±2.16ᵇ | 119.33±2.16ᵇ | 118.5±1.87ᵇ |

Data were expressed as mean±SD. ᵃP<0.05 when compared with control group. ᵇP<0.05 when compared with diabetic group. ᶜP<0.05 when compared with diabetic metformin treated group. ᵈP<0.05 when compared with diabetic

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vildagliptin treated group. BMI, body mass index; HOMA-IR, homeostasis model assessment insulin resistance; HOMA-β, homeostasis model assessment β-cell function; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein; LDL, low density lipoprotein; DPP-IV, Dipeptidyl peptidase-IV; TNFα, tumor necrosis factor alpha; IL-1β, interleukin-1 beta; SOD, superoxide dismutase; MDA, malondialdehyde; LDH, lactate dehydrogenase; CK-MB, creatine kinase-myoglobin binding; MABP, mean arterial blood pressure.

**Table (3): Pearson's correlation coefficient (r) between serum spexin level and some studied parameters within the diabetic group** (number of rats= 6)

| Parameters / Correlations | | Final BMI | Serum Glucose | HOMA-IR | HOMA-β | Serum TC | Serum DPP-IV | Serum SOD | Atherogenic index | Serum TNFα | Serum LDH | Serum CK-MB |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Serum spexin | r | -0.859 | -0.96 | -0.947 | 0.997 | -0.965 | -0.824 | 0.866 | -0.976 | -0.913 | -0.914 | -0.93 |
| | P Value | <0.05 | <0.01 | <0.001 | <0.001 | <0.01 | <0.05 | <0.05 | <0.001 | <0.05 | <0.05 | <0.01 |

BMI, body mass index; HOMA-IR, homeostasis model assessment insulin resistance; HOMA-β, homeostasis model assessment β-cell function; TC, total cholesterol; DPP-IV, Dipeptidyl peptidase-IV; TNFα, tumor necrosis factor alpha; SOD, superoxide dismutase; LDH, lactate dehydrogenase; CK-MB, creatine kinase-myoglobin binding.

### Histopathological results:

**Hematoxylin and eosin (H&E) stained pancreatic sections:** The control group showed normal histological structure, with presence of lobules, formed of adherent acini. The acini are formed of pyramidal shaped cells with apical acidophilic cytoplasm and basal basophilic nuclei. The islets of Langerhans appeared as pale regular oval areas with blood capillaries present in between the cellular groups in addition to big number of β- cells with distinctive round nuclei (Fig.1A).

But diabetic group showed pathological variations of both exocrine and endocrine pancreatic components, where the acini were irregularly organized, widely separated with presence of inflammatory cell infiltrations. The acinar cells appeared swollen with cytoplasmic vacuoles and dark stained nuclei, dilated ducts with presence of congested blood vessels. Islets of Langerhans appeared disturbed with irregular outline, and even lost, with vacuolations in between the cells of islet (Fig.1B&1B*). Diabetic metformin treated rats showed regeneration of the islet cells with nearly regular outline and slight normal appearance of most cells (Fig.1C).

Diabetic vildagliptin treated rats showed approximately normal organization and restoration of the islets' size, also the exocrine part showed decrease in vacuoles and trophic changes (Fig.1D). In diabetic spexin treated rats, a picture nearly similar to that of control group, with restoration of the borders between the exocrine and the endocrine pancreatic parts (Fig.1E).

**Mallory's trichrome-stained pancreatic sections:** The control group showed delicate collagen fibers in-between the pancreatic acini, around the blood vessels and the interlobular duct (Fig.2A). In diabetic group, there was an apparent increase in collagen fibers around the dilated ducts, congested blood vessels and within the islet (Fig.2B&2B*), while in metformin treated diabetic group (Fig.2C&2C*) and vildagliptin treated diabetic group (Fig.2D) there was apparent decrease in amount of collagen fibers around the apparently dilated ducts. Spexin treated

diabetic group showed minimal amount of collagen fibers around the interlobar ducts and in-between acini (Fig.2E &2E*).

**Anti-insulin immunostained pancreatic sections:** Positive cytoplasmic staining of the islets of Langerhans in control group was detected in all cells especially in the center and appeared dense brown in color (Fig.3A). In diabetic group there was marked decrease in immune-reaction with presence of vacuolations (Fig.3B). In diabetic metformin treated, diabetic vildagliptin treated and diabetic spexin treated groups, apparent increase in immune-reaction of the cells was observed as compared with the diabetic group (Fig. 3C, 3D&3E).
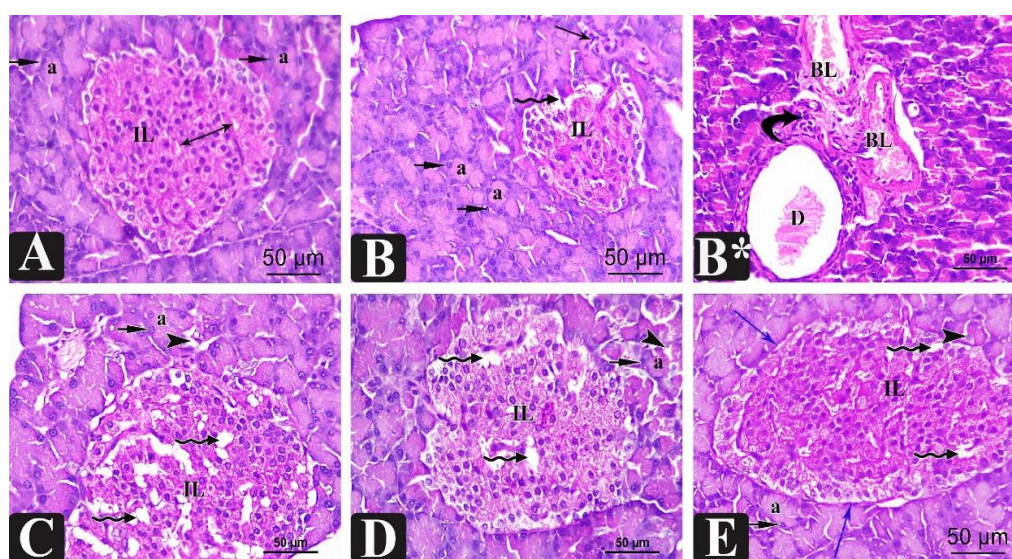


**Fig. (1): Photomicrographs of pancreatic sections. A.** Control group displaying normal pancreatic histological structure with exocrine and endocrine parts. Tightly packed acini (**a**) arranged into small lobules, with acidophilic cytoplasm and basal basophilic nuclei (arrow). The islet cells (IL) are seen intermingled with the acinar cells and are lightly stained with capillaries in between (double headed arrow). **B.&B*.** Diabetic group revealed abnormal shape of both the exocrine and endocrine pancreatic parts. The acinar cells are puffy with small vacuoles (arrow head) and some with dark stained nuclei (long arrow). Apparently dilated duct (D) and congested dilated blood vessels (BL) with inflammatory infiltration (curved arrow). The islet cells (IL) are disturbed with dark stained nuclei and multiple vacuoles (zigzag arrow)**.** Interlobular ducts were slightly dilated and lined with flattened epithelium (red arrow). **C.** Diabetic rats treated with metformin showing slight alteration of the pancreatic structure, the acini showed cytoplasmic vacuolations (arrow head). **D.** Diabetic rats treated with vildagliptin showing approximately normal organization of islets of Langerhans (IL) with decreased vacuoles (zigzag arrow)**,** trophic changes of the exocrine part were less severe with basophilic nuclei (arrow) of the acini (a) and decreased vacuoles (arrow head). **E.** Diabetic rats treated with spexin showed apparent restoration of histological structure of the islets (IL), decreased the vacuolations within the islets of Langerhans (zigzag arrow) and in the acinar cells (arrow head), restoration of the borders between the exocrine and the endocrine pancreatic parts (blue arrows). (H&E X400)

**Hematoxylin and eosin stained cardiac sections***:* Cardiac muscle in control group showed normal histological pattern with regular arrangement of muscle fibers and normal cardiac vasculature with clear eosinophilic cytoplasm and flattened centrally located ellipsoid nuclei. The fibers were connected by intercalated discs. Flat dark nuclei of the fibroblasts in the endomysium were detected (Fig.4A).

In the diabetic group the muscle fibers were irregularly arranged and the striations were lost with highly widened endomysium. Degeneration of the

cardiac myocytes with fragmentations of some and presence of inflammatory cell infiltration were noted. The nuclei looked faintly stained and others are pyknotic or karyolytic (Fig.4B&4B*). In diabetic metformin treated, diabetic vildagliptin treated and diabetic spexin treated groups there was an apparent restoration of the normal structure of the cardiac muscle fibers with slight widening between the muscle fibers (Fig.4C, 4D&4E).

Immunohistochemical staining of the cardiac muscle*:* Bax expression technique displayed an apparent increase of the brown color in diabetic group as compared to the other groups (Fig.5B), while in diabetic metformin treated, diabetic vildagliptin treated and diabetic spexin treated groups, there was an apparent decrease in Bax expression as compared to the diabetic group (Fig. 5C, 5D&5E).

**Morphometric results** (Table-4):

Mean area percent of collagen in pancreatic tissue, presented a statistically significant increase in diabetic, diabetic metformin treated, diabetic vildagliptin treated and diabetic spexin treated groups versus to the control group, with a statistically significant decrease in diabetic metformin treated, diabetic vildagliptin treated and diabetic spexin treated groups as compared to the diabetic

group. But no statistically significant difference was detected between diabetic metformin treated, diabetic vildagliptin treated and diabetic spexin treated groups.

Mean area percent of insulin immune-reactivity in diabetic, diabetic metformin treated, diabetic vildagliptin treated and diabetic spexin treated groups showed a significant decrease in mean area percent of insulin as compared to the control group, while in diabetic metformin treated, diabetic vildagliptin treated and diabetic spexin treated groups it revealed a significant increase as compared to diabetic group. However, no statistically significant difference was detected between diabetic metformin treated, diabetic vildagliptin treated and diabetic spexin treated groups.

Bax expression showed a significant increase in diabetic, diabetic metformin treated, diabetic vildagliptin treated and diabetic spexin treated groups as compared control group. Also, diabetic metformin treated, diabetic vildagliptin treated and diabetic spexin treated groups were presented with a significant decrease in Bax expression as compared to diabetic group. In addition, no statistically significant difference was detected between diabetic metformin treated, diabetic vildagliptin treated and diabetic spexin treated groups.
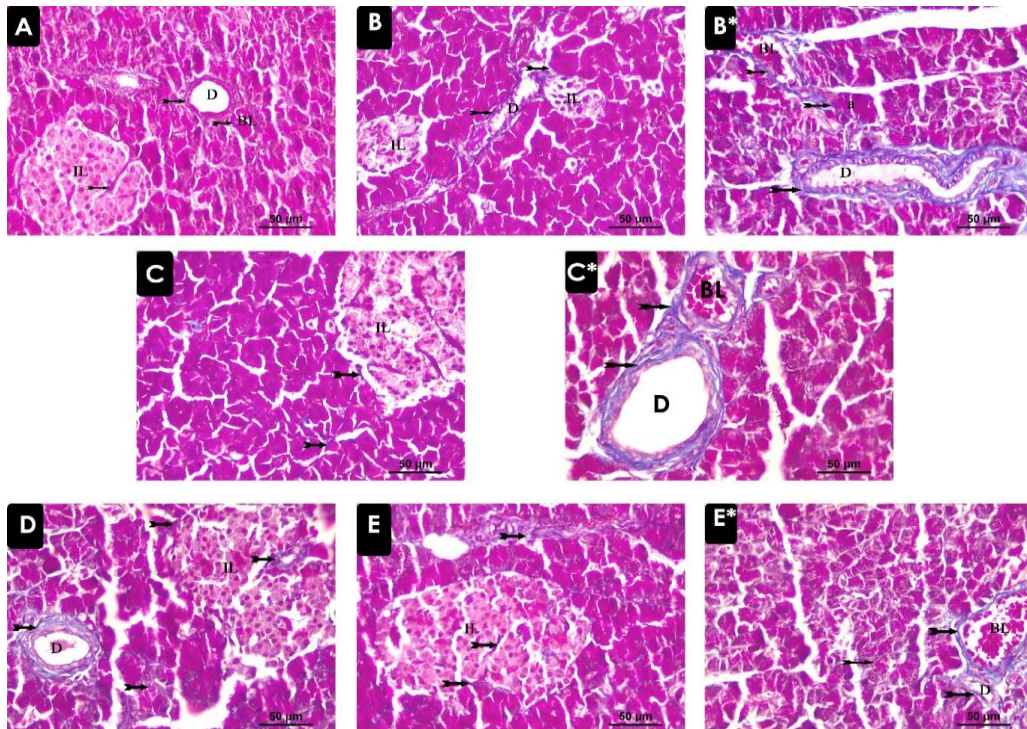
**Fig. (2): Mallory's trichrome stained pancreatic sections. A.** control group showed delicate collagen fibers in-between the pancreatic acini (tailed arrow), around the blood vessels (BL) and the interlobular duct (D). **B.&B\*.** diabetic group showed an apparent increase in collagen fibers around the dilated ducts (D), congested blood vessels (BL) and within the islet (IL) (tailed arrow). **C.&C\*.** metformin treated diabetic group and **D.** vildagliptin treated diabetic group showed apparent decrease in amount of collagen fibers. **E.&E\*** Spexin treated diabetic group showed minimal amount of collagen fibers around the interlobar ducts (D), in-between acini (tailed arrow). (Mallory's Trichrome X400).
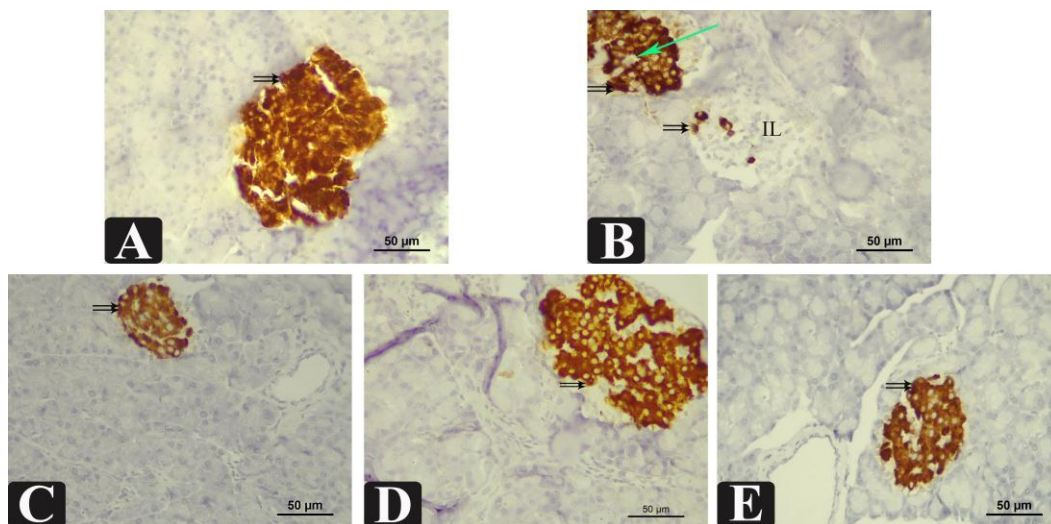


**Fig. (3): Photomicrographs of pancreatic specimens immune-stained with (Anti-insulin Immunostaining X400). A**. control group showed strong positive immunoreaction (dark brown color) (double arrow) mainly in the center of the islets (B-cells). **B**. diabetic group displaying noticeable decrease in the immunoreaction with irregular outline of the islets and apparent decrease in the area of islets with presence of vacuoles (green arrow). **C.** metformin treated diabetic group and **D.** vildagliptin treated diabetic group showed apparent increase in area of islets as compared with diabetic group. **E.** spexin treated diabetic group showed an obvious increase in beta-cells immunoreaction as compared with diabetic group.
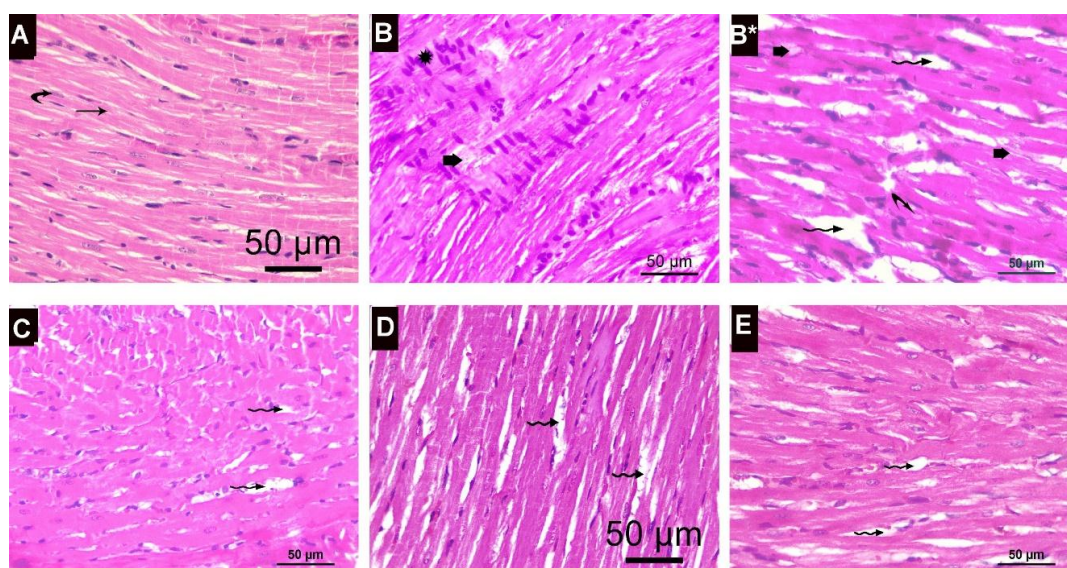
**Fig. (4): Photomicrograph displaying section of cardiac muscle. A**. control group cardiac muscle fibers were regular (arrow) with flattened nucleus (curved arrow). **B.&B\*.** diabetic group cardiac muscle fibers were irregular, widely separated (zigzag arrow) and showed myolysis (thick arrow), with presence of inflammatory cell infiltrate (\*), the nucleus showed karyolysis (curved arrow). **C.** metformin treated diabetic, **D.** vildagliptin treated diabetic group and **E.** spexin treated diabetic group showed apparent improvement of the histological changes found in the diabetic group with decreased separation between cardiac muscle fibers (zigzag arrow). **(H&E X400)**

# DISCUSSION

To declare the effect of spexin treatment on cardiometabolic changes occurred in type 2 diabetes mellitus and the possible mechanisms involved, a rat model of high fat diet- and a low dose streptozotocin induced diabetes was used in this study. The results of this study confirmed occurrence of cardiometabolic changes in obese type 2 diabetic rats which were ameliorated with treatment by spexin as well as by the standard drugs (metformin and vildagliptin).
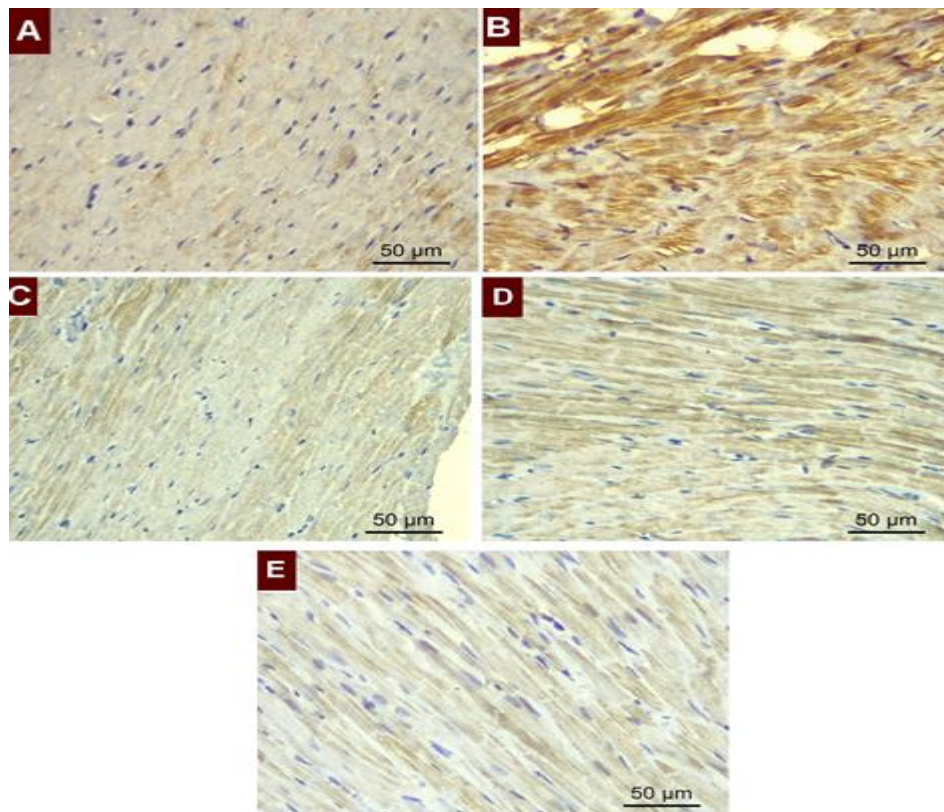
**Fig. (5): Photomicrographs of cardiac specimens immune-stained with Bax expression (X400).** A. control group showed minimal Bax expression B. diabetic group showed enhanced Bax expression C. diabetic rats treated with metformin, D. diabetic rats treated with vildagliptin group and E. diabetic rats treated with spexin showed apparent decrease in Bax expression as compared to the diabetic group.

**Table (4): The mean area percent (Area %) of (collagen in pancreatic tissue, insulin immune-reactivity and Bax expression) in the different groups. Data were expressed as mean±SD.** Number of rats in each group= 6.

| Groups / Variables | Control | Diabetic | Diabetic metformin treated | Diabetic vildagliptin treated | Diabetic spexin treated |
|---|---|---|---|---|---|
| **Area % of collagen** | 15.66 ±1.71 | **39.21 ±1.83[a]** [a]P<0.001 | **20.56±1.84[a&b]** [a]P<0.01 [b]P<0.001 | **22.94±2.4[a&b]** [ab]P<0.001 | **20.7±2.23[a&b]** [a]P<0.01 [b]P<0.001 |
| **Area % of insulin immune-reactivity** | 13.26 ± 1.08 | **2.03 ± 0.42[a]** [a]P<0.001 | **9.20 ± 1.16[a&b]** [a,b]P<0.001 | **10.03 ± 1.56[a&b]** [a,b]P<0.001 | **10.18 ± 1.23[a&b]** [a,b]P<0.001 |
| **Area % Bax expression** | 23.33 ± 1.08 | **68.24 ± 5.4[a]** [a]P<0.001 | **39.60 ± 1.7[a&b]** [a,b]P<0.001 | **41.28 ± 2.24[a&b]** [a,b]P<0.001 | **37.68.9±1.95[a&b]** [a,b]P<0.001 |

[a]P when compared with control group. [b]P when compared with diabetic group.

In the diabetic group, significant cardiometabolic changes were noticed, in comparison with the control group, in the form of disturbed, glucose homeostasis (hyperglycemia, hyperinsulinemia, insulin resistance and decreased beta cell function), lipid profile (increased serum level of TC, TG and LDL and increased

atherogenic index, with decreased serum level of HDL) and cardiac function (increased serum level of both LDH and CK-MB with increased MABP).

These results were supported by *Rajesh et al. (2017)* who confirmed presence of hyperglycemia, insulin resistance and decreased pancreatic beta cell function in obese rats. Also, *Al-Daghri et al. (2018a)* stated that elevation in serum CK-MB, a cardiac enzyme, reflected the extent of cardiac damage. The current changes could be explained according to the results of this study by presence of obesity (evidenced by the significant increase in final BMI) which was considered as one of the risk factors of insulin resistance as discussed by *Almass et al. (2016)* and *Kolodziejski et al. (2018a)*. Also, other mechanisms that could explain cardiometabolic disturbances in obese diabetic rats as declared by the present study included the significant increase in serum levels of proinflammatory cytokines (TNFα and IL-1β), increased oxidative stress (decreased serum level of SOD with increased serum level of MDA), and increased activity of DPP-IV which degraded glucagon-like peptide-1 that affected glucose homeostasis as stated by *Silva Júnior et al. (2019)*.

These results were in agreement with *Wright et al. (2019)* who stated that oxidative stress was associated with overproduction of reactive oxygen species and played a vital role in the development of complications of diabetes. Also, *Lamers et al. (2011)* reported a rise in DPP-IV release in obese individuals who had an increased risk of having insulin resistance and diabetes. In addition, *Kirino et al. (2009)* found an increase in plasma DPP‑IV activity in rats after streptozotocin injection. Moreover, the current study declared that the decrease in serum spexin level in obese type 2 diabetic rats could be considered as another mechanism that could explain the cardiometabolic changes occurred with type 2 diabetes as spexin serum level was decreased significantly and was negatively associated with each of final BMI, serum glucose, HOMA-IR, serum TC, serum DPP-IV, atherogenic index, serum TNFα, serum LDH and serum CK-MB, but it was positively associated with both HOMA-β and serum SOD.

This was supported by *Walewski et al. (2014), Kolodziejski et al. (2018a) and Al-Daghri et al. (2018b)* who explored a decrease in serum spexin in patients with metabolic syndrome and in obese adults. Also, *Walewski et al. (2010)* declared that the gene encoding spexin (Cg12orf39) was the most downregulated gene in fat tissue in humans with obesity. In addition, *Gu et al. (2015)* reported an inverse correlation between serum levels of both spexin and glucose in adults with type 2 diabetes mellitus.

In addition, *Lin et al. (2018)* found a negative correlation between serum levels of both spexin and total cholesterol. Moreover, *Kumar et al. (2016)* stated that in obese children, spexin was negatively correlated with body weight. Thus, to confirm the role of spexin in the pathogenesis of cardiometabolic changes found with diabetes, it was evaluated for efficacy in treatment of type 2 diabetes in comparison with the standard drugs (metformin and vildagliptin).

The previously mentioned cardiometabolic disturbances of type 2

diabetes mellitus explained the histopathological and inflammatory changes in the diabetic cardiac muscle detected in this study. The muscle fibers were irregularly arranged with loss of striations and widened endomysium, in hematoxylin and eosin stained sections of the cardiac muscle of diabetic group. In addition, degenerative changes of the cardiac myocytes and fragmentations of some with presence of inflammatory cell infiltrate were also detected. The nuclei looked faintly stained while, others were pyknotic or karyolytic. These findings are similar to those of *Thent et al. (2012), Abdelhafez & El-Wahsh (2019),* and *El-shaer & Nofal (2019)*.

The Bax protein, one of the proapoptotic members, is situated in the cytoplasm. Its structure and position are changed under the effect of injury, leading to formation of heterodimer that affects the mitochondrial membrane causing stimulation of apoptosis signaling pathway. Apoptosis consumes more energy, that in turn enhance more apoptosis producing a vicious circle *(Zhao et al., 2001).* Morphometric statistical analysis of Bax expression in this study revealed a significant increase in diabetic group as compared to the control one which was supported by the results of *Yu et al. (2012)*.

The current study showed histopathological changes in pancreatic tissue of diabetic group that support the biochemical and physiological results, as in both exocrine and endocrine components the acini were irregularly organized, widely separated with presence of inflammatory cell infiltrations. The

acinar cells appeared swollen with cytoplasmic vacuoles and dark stained nuclei. Islets of Langerhans appeared disturbed with irregular outline, and even lost. These results were similar to those of *El-Esawy et al. (2016), Nurdiana et al. (2017)* and *Almalki et al. (2019)*.

Morphometric analysis of the area percent of collagen and area percent of immune-stained beta-cells of islets of Langerhans showed significant increase in diabetic group as compared to the control one.

The results of the current study confirmed that, spexin (as the standard drugs metformin and vildagliptin) utilization in the treatment of obese type 2 diabetic rats significantly ameliorated the accompanied cardiometabolic disturbances through its, anti-diabetic effects (spexin treatment decreased serum levels of both glucose and insulin, decreased insulin resistance as noticed by the decrease in HOMA-IR, and increased beta cell function as evidenced by the increase in HOMA-β), anti-obesity effect (spexin treatment decreased the final BMI) and hypolipidemic effects (spexin treatment improved lipid profile as it decreased the elevated serum level of TC, TG and LDL, and also decreased the atherogenic index, but it increased serum level of HDL).

These results were supported by *Al-Daghri et al. (2019)* who reported that spexin was involved in weight regulation with a potential role for therapy of obesity. Also, *Walewski et al. (2014), Ge et al. (2016)* and *Lin et al. (2018)* stated that the long-term injection of spexin, in obese rats, reduced caloric intake, body weight and serum cholesterol level. In

addition, *Ge et al. (2016)* found that spexin reduced long chain fatty acids uptake by adipocytes and improved both glucose tolerance and insulin resistance in obese type 2 diabetic mice.

Moreover, *Kolodziejski et al. (2018b)* who declared that spexin stimulated uptake of glucose in human adipocytes. In diabetic metformin treated and diabetic vildagliptin treated groups apparent improvement in histopathological changes was noticed. As regard to the diabetic spexin treated group obvious amelioration in the histological structure of the cardiac muscle and a picture nearly similar to the normal was noticed this is similar to the results of *Yu et al. (2012)* who proved the protective effect of curcumin in diabetic cardiomyopathy, by improving the oxidative stress and the metabolic disorders.

Also, there was a significant decrease in Bax expression in diabetic metformin treated, diabetic vildagliptin treated and diabetic spexin treated groups as compared to the diabetic group which ensured the protective effect of the **standard drugs** (metformin and vildagliptin) in addition to spexin, in ameliorating the diabetic cardiac effects, this was in agreement with the results of *El-shaer and Nofal (2019)* who proved the role of the anti-diabetic effect of the medicinal plant (chamomile) in improving the cardiac muscle histological structure.

In addition, the present study reported that spexin may exert its effects through,

affecting DPP-IV pathway (spexin treatment decreased serum level of DPP-IV and thus it could decrease the degradation of glucagon-like peptide-1), its anti-inflammatory effects (spexin treatment decreased the elevated serum level of both TNFα and IL-1β), its antioxidant effects (spexin treatment increased serum level of SOD but decreased serum level of MDA) and its cardio-protective effects (spexin treatment decreased serum level of both LDH and CK-MB, and decreased MABP). All these effects of spexin (as the standard drugs metformin and vildagliptin) treatment were statistically significant in comparison with the diabetic group. These results were supported by *Sassek et al. (2018)* who stated that spexin regulated insulin secretion and pancreatic beta cell viability and proliferation.

Also, histopathological findings in diabetic treated groups with either spexin or standard drugs (metformin and vildagliptin) ensured marked improvement in changes in pancreatic tissue when compared with diabetic group. In metformin treated diabetic rats, regeneration of the islet cells with nearly regular outline and slight normal appearance of most cells was observed which was in accordance with results of *El-Soud et al. (2016)* who compared the effect of metformin versus honey on beta-cells of pancreas. Also, vildagliptin treated diabetic rats showed approximately normal organization and restoration of the islets' size, also the exocrine part showed decrease in vacuoles and trophic changes. These results were similar to those of *Hassan et al. (2019)* who proved the protective effect of

Egyptian honey bee compared with vildagliptin in diabetic rat.

In spexin treated diabetic rats a picture nearly similar to that of control group, with restoration of the borders between the exocrine and the endocrine pancreatic portions. Morphometric analysis of the area percent of collagen and area percent of immune-stained beta-cells of islets of Langerhans in diabetic metformin treated, diabetic vildagliptin treated and diabetic spexin treated groups showed a significant decrease as compared with the diabetic group. These results were in line with *Abunasef et al. (2014)* who proved the protective effect of caffeine on the histochemical and histological alterations of beta-cells in diabetic rats. In addition, *Domouky et al. (2017)* proved a significant increase of collagen deposition in pancreas of diabetic rats versus mesenchymal stem cell and insulin producing cell treated rats. Also, *Lin et al. (2018)* confirmed that spexin had antioxidant activity as it significantly reduced MDA serum level which is a marker of lipid peroxidation in different organs including the heart. In addition, *Liu et al. (2019)* reported that spexin was expressed in the heart and that it had a protective effect on cardiomyocytes from hypoxia-induced metabolic dysfunction. Moreover, *Toll et al. (2012)* found that spexin modulated arterial blood pressure regulation.

Limitations of this study included that it was achieved on rats and the results obtained may be different from human. Also, small numbers of rats were used. Moreover, absence of healthy control groups treated with metformin, vildagliptin or spexin for comparisons.

Furthermore, changes in serum level of glucagon-like peptide-1 were not assessed. In addition, electrocardiogram changes in different groups were not recorded. Thus, further studies should be conducted to confirm the current results.

## CONCLUSION

Spexin ameliorated diabetes induced deleterious cardiometabolic, histopathological and morphometric disturbances. The anti-obesity, DPP-IV inhibitory, hypoglycemic, hypolipidemic, antioxidant, anti-inflammatory and cardio-protective properties of spexin may contribute to its useful effects. These findings give an insight into pathophysiology of diabetes induced cardiometabolic disturbances and render spexin as a potential therapeutic target.

## ACKNOWLEDGEMENT

## CONFLICT OF INTEREST

Nothing.

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# تأثير العلاج باستخدام الإسبيكسن على التغيرات فى القلب والأيض في الجرذان السمينة المصابة بداء السكرى من النوع الثانى

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**خلفيـــة البحــث:** يوجــد هرمــون الإسبيكسـن فــي العديــد مــن مناطــق المــخ، وكـذلك فــى العديــد مــن الأنســجة الطرفيــة فــى كثيــر مــن الفصائــل بمــا فــي ذلــك الإنســان والجــرذان. ولقــد بحثـت العديــد مــن الدراسـات فــي تغيــرات مســتوى هرمــون الإسبيكسـن فــى الــدم مــع الســمنة ومــرض الســكري مــن النــوع الثــانى، ولكــن القليــل مــن الدراسـات قيمـت تـأثيره علــى التغيــرات القلبيــة، وكـذلك تغيــرات عمليــة الأيــض فــي مــرض الســكري مــع وجود بعض التباين في نتائج تلك الدراسات.

**الهــدف مـن البحــث:** استكشــاف تــأثير العــلاج بمــادة الإسبيكسـن علــى التغيــرات البنكرياسـية والقلبيــة وتغيــرات عمليــة الايــض فــي الجــرذان المصابــة بالسـمنة المفرطــة والتــى تعــانى مــن مــرض الســكري مــن النــوع الثــانى والآليــات المحتملــة لطريقــة عمــل هذا الهرمون.

**مــواد وطــرق البحــث:** تــم تقسـيم ثلاثيــن مــن ذكــور الجــرذان البيضــاء البــالغين بشـكل عشوائي إلى ٥ مجموعات متساوية:

**المجموعـــة الأولـــي (الضــابطة):** تغــذت علــى الغــذاء المعتــاد لحيوانــات التجــارب طوال مدة هذه الدراسة (٨ أسابيع).

**المجموعـــة الثانيـــة (المصابة بمــرض السـكري):** تغــذت علــى غــذاء عــالي الــدهون طــوال مــدة هــذه الدراســة (٨أســابيع)، ولكــن فــي بدايــة الأسـبوع الرابــع مــن الدراســة، تــم حقــن الجــرذان بعــد الصيــام (١٢ سـاعة) بحقنــة واحــدة مــن الإستربتوزوتوسـين داخـل الغشــاء البريتـونى بجرعــة (٤٠ ملــغ / كيلــوغرام مـن

وزن الجســم، مــذاب فــي ٠,٠١ مــول مــن محلــول الســترات المتعــادل، الــرقم الهيدروجيني ٤,٥) لإحداث مرض السكري من النوع الثانى.

**المجموعـــة الثالثـــة (المصـــابة بمـــرض الســكري والمعالجـــة بـــالميتفورمين):** تمـــت معاملـــة الجـــرذان فـــى هـــذه المجموعـــة كمـــا فـــى المجموعـــة الثانيـــة، وبالإضـــافة إلـــى ذلــك، أعطيــت الجـــرذان الميتفـــورمين عـــن طريــق الفــم (٣٠٠ مجـــم / كجـــم / يـــوم) لمـــدة ٤ أســابيع مـــن بدايــة الأسبوع الخـــامس وحتـــى نهايــة الأسبوع الثامن.

**المجموعـــة الرابعـــة (المصـــابة بمـــرض الســكري والمعالجـــة بالفيلـــداجليبتن):** تمـــت معاملـــة الجـــرذان فـــى هـــذه المجموعـــة كمـــا فـــى المجموعـــة الثانيـــة، وبالإضـــافة إلـــى ذلــك، أعطيت الجـــرذان الفيلـــداجليبتن عـــن طريـــق الفـــم (١٠ مجـــم / كجـــم / يـــوم) لمـــدة ٤ أســابيع مـــن بدايـــة الأسبوع الخـــامس وحتـــى نهايـــة الأسبوع الثامن.

**المجموعـــة الخامســـة (المصـــابة بمـــرض الســـكري والمعالجـــة بالإسبيكســـن):** تمـــت معاملـــة الجـــرذان فـــى هـــذه المجموعـــة كمـــا فـــى المجموعـــة الثانيـــة، وبالإضـــافة إلـــى ذلــك، تـــم حقـــن الإسبيكســـن المـــذاب فـــي محلـــول ملحـــي متعـــادل داخـــل الغشـــاء البريتـــونى للجـــرذان بجرعـــة (٣٥ ميكروغـــرام / كجـــم / يـــوم) لمـــدة ٤ أســـابيع مـــن بدايــة الأسبوع الخـــامس وحتـــى نهايـــة الأسبوع الثـــامن. للفحص النســيجي، المنـــاعي، والفحوصـــات المورفومتريـــة، تـــم جمـــع عينـــات جديـــدة مـــن القلب والبنكرياس من الفئران التي تم التضحية بها.

**النتـــائج:** فـــي المجموعـــة المصـــابة بمـــرض الســكري، حـــدثت زيــادة ذات دلالـــة إحصائية فـــي كـــل مـــن (مؤشـــر كتلـــة الجســـم النهائي و الجلوكـــوز و الأنســولين و تقييم نمـــوذج التـــوازن للأنســـولين و الكولســترول الكلـــي و الـــدهون الثلاثيـــة و الـــدهون منخفضـــة الكثافـــة و مؤشـــر تصـــلب الشـــرايين و داى ببتيـــدايل ببتيـــداز الرابـــع و عامـــل نخـــر الـــورم ألفـــا و انترلـــوكين - ١ بيتـــا و مالونديالديهايـــد و هيـــدروجين اللاكتـــات و ملزمـــة الكرياتين كينـــاز-ميوغلـــوبين ومتوسـط ضـــغط الـدم الشـرياني ، بينمـا كـان هنـاك انخفاضـــا ذا دلالـــة إحصائيـــة فـــي تقيـيم نمـــوذج التـــوازن لوظيفـــة خلايـــا بيتـــا و الـــدهون عاليـــة الكثافـــة و ديســـميوتاز الفـــائق الاكســـجين بالمقارنـــة مـع المجموعـــة الضـــابطة. مـع العــلاج بالإسبيكسن (وكـذلك مــع اسـتخدام الأدويـة القياسـية لمـرض السـكرى

الميتفـورمين والفيلـداجليبتن)، تـم عكـس كـل هـذه التغييـرات بشـكل كبيـر بالمقارنـة مـع تلـك الموجـودة فـي المجموعـة المصـابة بمـرض السـكري. كمـا أكـد التحليـل المورفـومتري نتـائج التحلـي حيـث كشـف عـن وجـود فـروق ذات دلالـة إحصـائية فـي منطقـة ترسـب الكـولاجين، ورد الفعـل المنـاعي للأنسـولين فـي البنكريـاس وتعبيـر بـاكس العضـلي القلبـي بـين كـل مـن المجموعـة السـكرية وجميـع المجموعـات الأخـرى، مـع عـدم وجـود فـروق ذات دلالـة إحصـائية بـين المجموعـات المصـابة بالسكرى المعالجة بالميتفورمين أو بالفيلداجليبتن أو بالإسبيكسن.

**الاسـتنتاج:** أدى عـلاج الجـرذان السـمينة المصـابة بـداء السـكرى مـن النـوع الثـانى بالإسبيكسـن الـى تحسـين التغيـرات المصـاحبة فـى القلـب والأيـض والتغيـرات النسـيجية فـي نسـيج البنكريـاس والقلـب. وتعتبـر خصـائص الإسبيكسـن كمضـاد للسـمنة ومثـبط لإنـزيم الـداى ببتيـدايل ببتيـديز وخـافض لمسـتوى كـلا مـن الجلوكـوز والـدهون بالـدم، وكمضـاد للأكسـدة، ومضـاد للإلتهابـات وأيضـا كعامـل حمايـة للقلـب سـببا فـى تأثيراته المفيدة.