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Spexin Modulates the Glucose Homeostasis in Streptozotocin (STZ)-Induced Diabetes in Rat

Spexin, Sıçanlarda Streptozotosin (STZ) Kaynaklı Diyabette Glukoz Homeostazisini Düzenler

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Abstract

Background; Despite the various pharmacological agents and insulin treatment, still, there is lack of an effective and therapeutic treatment for diabetes. The present study investigated the possible therapeutic role of spexin (SPX) in glucose homeostasis both functionally and structurally in streptozotocin (STZ)-induced diabetic rats.

Methods; Male Wistar albino rats(n=28) were randomly divided into the control(n=4), diabetes mellitus (DM+saline;1mL/kg, n=6), DM+S10(SPX;10 µg/kg/Ml, n=6), DM+S30(SPX; 30 µg/kg/mL, n=6) and DM+S100 (SPX; 100 µg/kg/mL, n=6). Diabetes was induced by administering a single dose of STZ (35mg/kg, i.p. Blood and pancreatic tissue samples were taken for insulin level measurements by ELISA. Liver and muscle tissue samples were taken for glycogen measurement. Histopathological changes in liver, muscle and pancreas tissues were examined. Data were analyzed by ANOVA, and the Mann-Whitney-U test was used for multiple comparisons.

Results; STZ-induced diabetes increased blood and urine glucose levels in the DM group as compared to the control group and significantly decreased with 10 mg/kg dose of SPX treatment. Glucose loss rate (K value) in insulin sensitivity measurements was lowered by STZ-induced diabetes (8.030) vs the control group (9.973) witha weak negative correlation (R=-0.1786). Glycogen content in liver and muscle tissues has declined, and SPX limited the decrease in hepatic glycogen levels in the DM+S10 group. SPX treatment reduced the degeneration levels and tissue damage in all tissues.

Conclusions; Our findings indicated that SPX has a regulatory role in glucose homeostasis via insulin secretion, glycogen metabolism, and blood glucose regulation in a dose-dependent manner.

Keywords: Spexin, diabetes, glucose, insulin, glycogen.

Özet

Amaç: Çeşitli farmakolojik ajanlar ve insülin tedavisine rağmen halen diyabetin etkili ve tedavi edici bir tedavisi bulunamamıştır. Bu çalışma, streptozotosin (STZ) ile indüklenen diyabetik sıçanlarda spexinin (SPX) glukoz homeostazisindeki olası terapötik rolünü hem fonksiyonel hem de yapısal olarak araştırdı.

Gereç ve Yöntem: Erkek Wistar albino sıçanlar (n=28) rastgele olarak, kontrol (n=4), diyabet (DM+salin;1mL/kg, n=6), DM+S10(SPX;10 µg/kg/Ml, n=6), DM+S30(SPX; 30 µg/kg/mL, n=6) ve DM+S100 (SPX; 100 µg/kg/mL, n=6) gruplarına ayrıldı. Tek doz STZ (35mg/kg, i.p.) uygulanarak diyabet oluşturuldu. Kan ve pankreas dokusu örnekleri insülin düzeyinin ELISA ile ölçümü için alındı. Glikojen ölçümü için karaciğer ve kas dokusu örnekleri alındı. Karaciğer, kas ve pankreas dokularında histopatolojik değişiklikler incelendi. Veriler ANOVA ile analiz edilmiş ve çoklu karşılaştırmalarda Mann-Whitney-U testi kullanıldı.

Bulgular: STZ ile indükelenen diyabet, DM+salin grubunda kontrol grubuyla karşılaştırıldığında kan ve idrar glukoz düzeyleri arttı ve 10 mg/kg SPX tedavisi ile anlamlı düzeyde azaldı. İnsülin duyarlılığı ölçümlerinde hesaplanan glikoz kaybolma oranı (K değeri), zayıf bir negatif korelasyonla (R=-0,1786) kontrol grubuna (9,973) kıyasla STZ kaynaklı diyabet (8,030) ile azaldı. Karaciğer ve kas dokularındaki glikojen içeriği azaldı ve SPX, DM+S10 grubunda hepatik glikojen seviyelerindeki düşüşü sınırladı. SPX tedavisi tüm dokulardaki dejenerasyon düzeylerini ve doku hasarını azalttı.

Sonuç: Bulgularımız SPX'in insülin sekresyonu, glikojen metabolizması ve kan şekeri regülasyonu yoluyla glukoz homeostazisinde doza bağlı olarak düzenleyici bir role sahip olduğunu gösterdi.

Anahtar Kelimeler: Spexin, diyabet, glukoz, insülin, glikojen.

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INTRODUCTION

Diabetes does characterize by chronic hyperglycemia resulting from decreased circulating insulin concentration (insulin deficiency), the diminished response of peripheral tissues to insulin (insulin resistance), or both [1]. The development of diabetes is caused by several pathological processes, such as insulin deficiency resulting from the destruction of β cells in the pancreas or abnormalities arising from developing resistance to insulin action. Impairment of insulin effectiveness does cause by one or more of the pathways involved in the mechanism of action of the hormone insulin. Decreased insulin secretion or impaired cellular response to insulin underlies these disorders [2].

Spexin (SPX), known as neuropeptide Q, was first discovered in 2007 with Markov modelling [3]. SPX is expressed in many tissues in rats, including gastrointestinal tract, kidney, bladder, heart, uterus, lung, skeletal muscle, thymus, spleen, brain, hypothalamus, adenohypophysis, thyroid, adrenal, testis, and ovary [4]. Recent studies showed that SPX might improve glucose tolerance and insulin sensitivity, reduce HbAlc levels, and reduce the possibility of type 2 diabetes [5, 6]. Long-term SPX treatment has been found to improve liver function and increase glycogen storage in the liver in type-2 diabetic mice [7]. In addition, a recent study showed an association between SPX and increment in body weight which correlates positively with diabetes [8]. Researchers identified that SPX, galanin and kisspeptin genes are localized on the close ancestral chromosomes, and SPX is more related to galanin. According to Mirabeau et al. these peptides are natural ligands for GALR2/3 [3]. Galanin has a significant contribution by accelerating GLUT-4 to the plasma membrane of diverse insulin-sensitive cells, regulating glucose homeostasis, and reducing insulin resistance [9]. SPX increases glucose utilization by inhibiting galanin binding to GALR2. By these effects, SPX has been shown to regulate biological processes that occur in obesity, diabetes, and hepatic steatosis [10].

Well-established that diabetes is a global disease that significantly affects health and mortality [1] [2]. However, the influence of SPX on diabetes has remained unclear. According to recent studies, SPX may play a regulatory role in glucose metabolism. The experimental work presented here provides one of the first investigations into how SPX treatment impacts glucose homeostasis in diabetes in a dose-dependent manner. The present study lays the groundwork for future research to elucidate the tissue crosstalk of diabetes-associated hormones and newly discovered neuropeptides.

MATERIAL & METHODS

Animals

Male Wistar Albino rats (6-8 weeks old, 180-220 g) were supplied by the Trakya University Animal Center. All experimental procedures were applied according to Universal Declaration on Animal Welfare and approved by the Trakya University Animal Research and Ethics Committee, Edirne, Turkey (Approved date 27.12.2019, number:2019-12-03). Rats were housed in standard environmental conditions and had free access to food and water. The clinical signs constituted in all experimental procedures.

Rats (n=28) were randomly divided into the control (n=4), diabetes mellitus (DM, saline; 1mL/kg, i.p, n=6), DM+ SPX10 (Spexin; 10 µg/kg/mL, i.p, n=6), DM+SPX30 (Spexin; 30 µg/kg/mL, i.p, n=6) and DM+SPX100 (Spexin; 100 µg/kg/mL, i.p, n=6) groups. Spexin peptide was purchased from PolyPeptide (Cat.no: SC1547)). Diabetes induced with a single dose of streptozotocin (Santa Cruz Biotechnology, USA). (STZ 35 mg/kg, prepared in 0.1 M citrate buffer (pH 4.4, i.p.). Following 72 hours of STZ injection, blood glucose levels were measured from the tail vein of the rats after night fasting. Rats with a fasting plasma glucose level of 250 mg/dl or higher were considered diabetic. These values were acquired in all rats injected with STZ. Following the diagnosis of diabetes, saline or SPX was administered intraperitoneally at the above-mentioned doses once a day for five days. At the end of the study, rats were placed in a 24-hour metabolic cage, and water intake and urine output were monitored. Following metabolic measurements, rats were sacrificed under ketamine (75 mg/kg, i.p) and xylazine (10 mg/kg, i.p) anesthesia following an insulin sensitivity test. Blood samples were collected in a serum clot activator tube and centrifuged at 3000g (15 min at 4 °C). Pancreas, skeletal muscle (gastrocnemius), and liver tissues of each animal were removed and stored at −80 °C for glycogen and insulin measurements. The liver, gastrocnemius muscle, and pancreas tissue samples were fixed in formaldehyde for histopathological assessment (The experimental design showed in Figure 1).

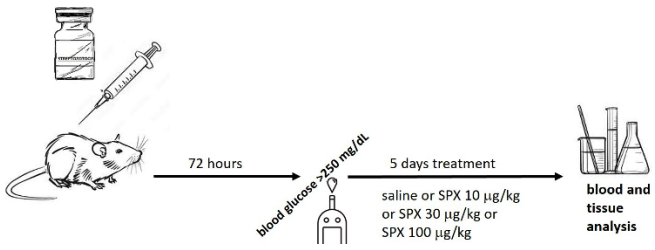


Figure 1. Graphical demonstration of the experiment

Insulin sensitivity test

The insulin sensitivity test was conducted according to Panigrahi et al. study [11]. The insulin sensitivity test stood carried out on the 5th day of the treatment. After 3 hours fasted periods the sensitivity test was performed under anaesthesia. Animals received human insulin (the dose of 0.1 U/kg) which has a short effect intravenously through the tail veins. Blood samples were collected from the tail veins at five times, 0 (just before insulin injection), 4, 8, 12, and 16 min of insulin

administration for glucose estimation. Insulin sensitivity showed glucose disappearance rate, which is calculated from the average slope of in the fitting curve by linear regression calculation. Then, multiplying the slope by −1, the -value (mg/dL/min) was calculated.

Blood samples

Blood samples were taken at the end of the experimental procedure, and glucose levels were measured by counter plus one (Ascensia Diabetes Care, Basel, Switzerland). In addition, insulin levels from the sacrificed rats at the end of experiments were measured by rat insulin ELISA kit (SUNRED 201-11-0708) according to the kit procedure.

Tissue glycogen content analyses

Tissue glycogen contents were measured in liver and gastrocnemius muscle according to Murat et al. [12]. Briefly, 1-1.5 g tissue was homogenized in 30% potassium hydroxide solution and then boiled at 100 °C for 30 minutes. Samples were then cooled in an iced cold solution with sodium sulfate. The glycogen was precipitated by adding ethanol, and the supernatant was discarded. The precipitate was brought into the solution by adding distilled water again. Glycogen content was determined spectrophotometrically (Shimadzu UV 1280) at a wavelength of 620 nm by adding the chemical anthrone reagent.

Histopathological analysis

Liver, gastrocnemius muscle, and pancreas tissues from rats in each group were gently dissected and immediately fixed in a 10% neutral buffered formalin solution for 72 hours. The samples were dehydrated in ascending (70%, 80%, 90%, 96%) ethanol series, cleared with xylene, and embedded in paraffin. The paraffin blocks were cut into 4-5 µm thick sections with a rotary microtome (Medite M530). Dewaxed cells were stained with Hematoxylin and Eosin (H&E) for histopathological evaluation, and for histochemical reaction, Periodic Acid Schiff (PAS) was used to demonstrate glycogen deposition. Morphological and histopathological changes in the liver, muscle, and pancreas tissues were examined using a computer-equipped (LasV 4.10 program), the camera attached photo-light microscope (Leica DM 2500, Germany), and micrographs were taken from sections. Pathological changes were assessed with vacuolization, intensely stained pyknotic nuclei, congested sinusoidal walls, lymphocyte cell infiltration, lipid droplet, and adipocyte accumulation. Semi-quantitative scoring criteria (0: no damage; 1: mild; 2: moderate; 3: severe) were used to detect tissue degeneration and PAS-positive cell distribution. The average scores were calculated for each group, and statistical analysis was performed.

Statistics

The results are expressed as the mean ± SD. To evaluate the level of statistical difference, One-way analysis of variance (ANOVA) and Mann-Whitney-U test were used for multiple comparisons (GraphPad Prism 6.0, San Diego, CA, USA). "p" values 0.05 have been accepted to be statistically significant.

RESULTS

Plasma glucose levels in the diabetic group (330 ± 66.28) were significantly higher than the control group (116,80 ± 8.73) at the beginning of SPX treatment ($p<0.05$) (Figure 2a). SPX at a dose of 10 ug/kg (290.1 ± 27.29) prevented the increase of plasma glucose level compared to the DM group (321.2±23.41) till the end of the experiment ($p<0.01$). At a dose of 100 ug/kg (454.8 ± 58.6), SPX has elevated plasma glucose levels significantly compared to DM and the control group ($p<0.01$). Urine glucose output was significantly elevated in the DM group (221.5 ± 32.09) as compared to the control group (250.9 ± 109.0, $p<0.05$). This elevation was determined in the DM+SPX10 group (285.3 ± 80.02) on the 1st day of treatment as compared to the DM group (333.6 ± 62.74, $p<0.05$). Within days, the urine glucose levels of the DM+SPX100 group gradually increased except on 4th day of the treatment (Figure 2b). The insulin sensitivity test measured glucose disappearance rates (K value). Glucose levels in the control group dropped within minutes, with a significant negative correlation (R=-0.8018) with insulin therapy. However, this strong negative correlation was not seen in the DM group (R= -0.1786). When comparing the K values, the control group was 9.973, while the DM group was 8.030. Treatments of SPX showed different effects in a dose-dependent manner. According to these data, the K value of the DM+SPX30 group was the most similar to the control group.

The weight changes showed a positive correlation with STZ injection which was R= 0.769 in the name of the control group as a sign of weight gain, while was R= - 0.405 for the DM group (Figure 2d) which means affects weight gain during the experimental days. Therefore, SPX treatments did not significantly affect weight changes on all the experimental days.

Plasma and pancreatic tissue insulin levels were examined (Table 1). We did not find any significant difference between the DM and the control group. However, there was a substantial increase in plasma insulin levels in the DM+S10 group compared to the DM group ($p<0.05$). In addition, liver and muscle tissue glycogen contents were measured, and STZ injection caused a significant decrease in a large amount of glycogen from both tissues compared to the control group ($p<0.05$). Liver glycogen content was not improved by SPX treatment in any doses. However, muscle glycogen content recoveries at 100 ug doses of SPX compared to the saline-treated DM group ($p<0.01$).

Light microscopic evaluation of the H&E stained liver tissue

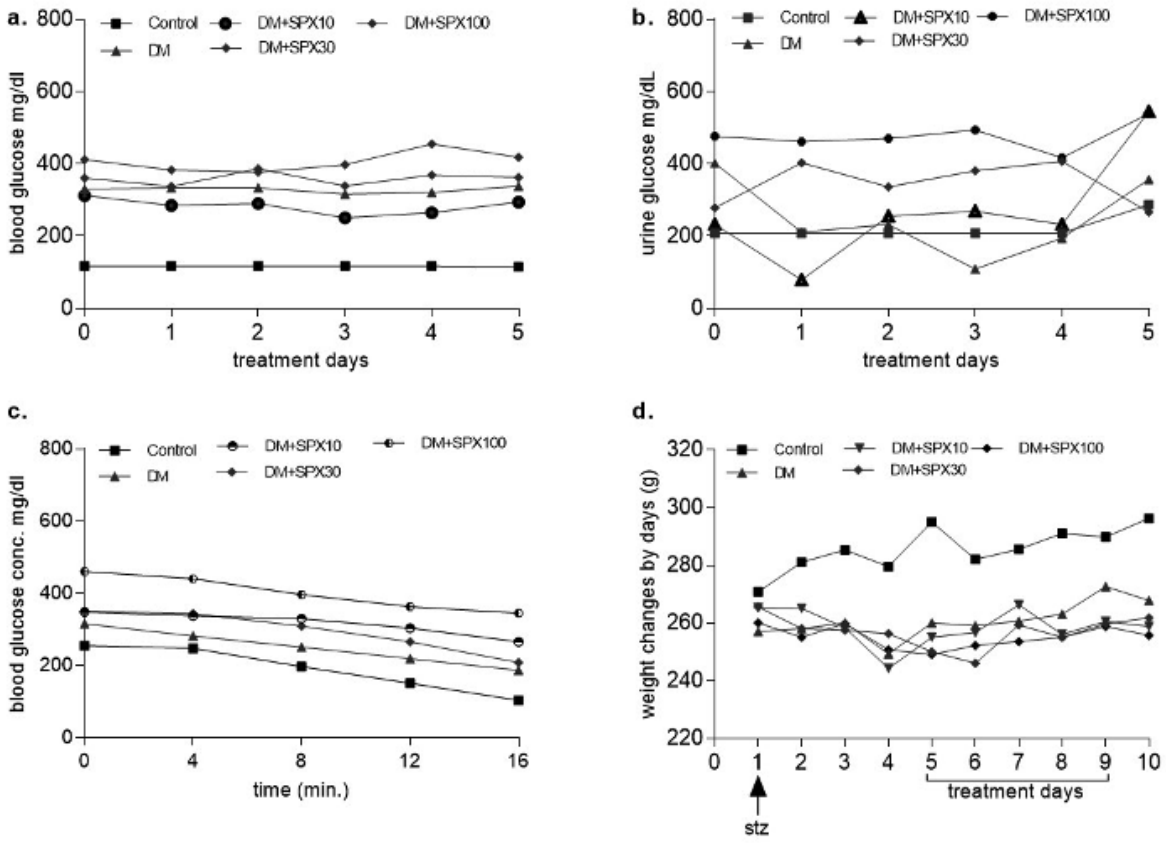
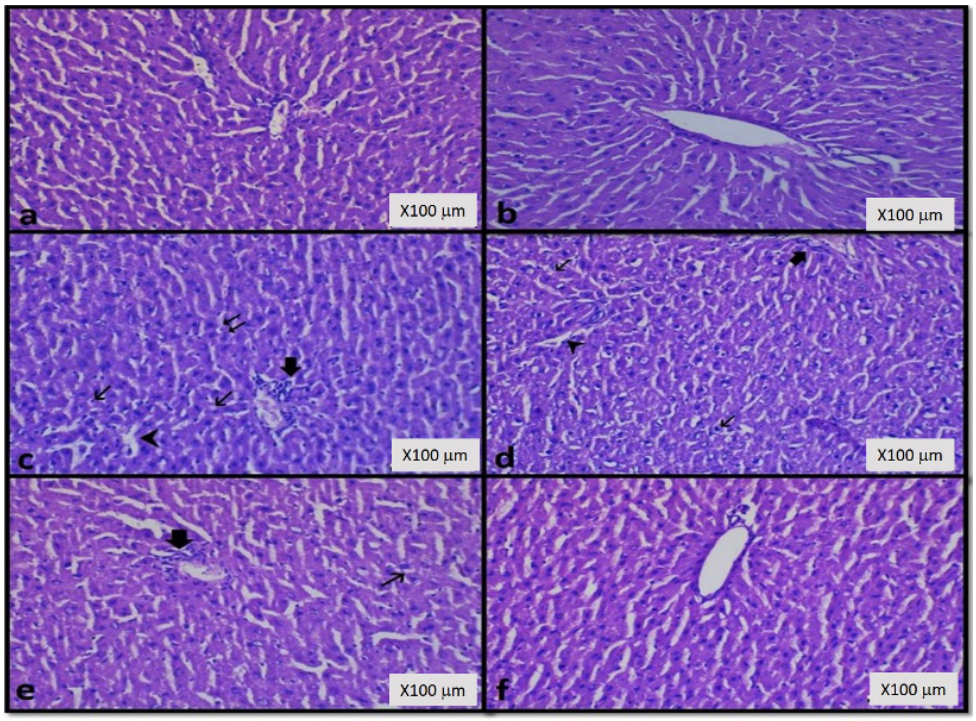


Figure 2. The figure shows plasma glucose levels (a), urine glucose levels (b), plasma glucose disappearance slope with insulin (c), and weight changes by days (d) in experimental groups.

Table 1. The table shows plasma insulin levels, pancreatic tissue insulin content, liver glycogen content, and skeletal muscle glycogen content in experimental groups. (*p<0.05 vs control group. +p<0.05 vs the DM group).

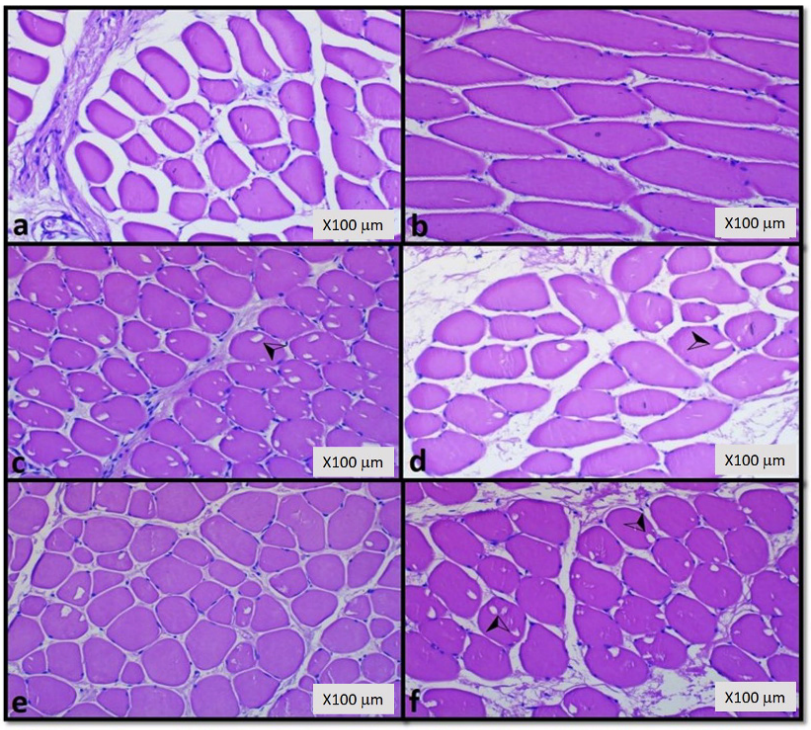
Table 1.	Control	DM	DM+SPX10	DM+SPX30	DM+SPX100
Plasma insulin IU/mL	6.21±0.67	6.28±0.49	8.41±1.5^{+}	7.12±0.81	9.02±3.28
Pancreatic insulin IU/mg tissue	9.90±3.40	17.29±7.35	6.04±1.86	6.18±1.78	7.21±2.12
Liver glycogen (mg/100 mg tissue)	8218±4051	2905±1040*	2991±1839	2923±1285	2705±847.1
Muscle glycogen (mg/100 mg tissue)	1413±122.6	324 ±162.2*	528 ±254.2	454±299.4	953±103.4^{++}

of the control group rats revealed hepatocyte cords radiating from the central vein, polygonal hepatocytes with eosinophilic cytoplasm, central round nucleus, some binucleated cells, hepatic sinusoids between cords, and portal triads (Figure 3a-b). Skeletal muscle tissue investigation of the control group revealed a regular fascicle structure with perimysium, endomysium, skeletal muscle fibers, and their peripherally located nuclei (Figure 4a,b). Pancreas tissue of the same group showed intact Langerhans Islets and exocrine area with serous acini and ducts (Figure 5a-b). However, lymphocytic infiltration around the central vein, congested liver sinusoids, some hepatocytes with pyknotic nuclei, and diffuse vacuolar changes in hepatocytes were observed in the liver tissue of the Diabetes (DM) group (Figure 4c). Distinct and abundant lipid droplets were detected in skeletal muscle fibers of all Diabetes group animals (Figure 4c). In addition, enlarged Langerhans Islets and vacuolization in serous acini were seen in the pancreas tissue of the same group (Figure 5c). The degenerative changes seen in the diabetic group were also detected in the liver, muscle, and pancreatic tissues of the DM+SPX10



Groups	Control	DM	DM+SPX10	DM+SPX30	DM+SPX100
Vacuolization	0.33±0.51	2.66±0.51*	2.66±0.51	2.33±0.51	2.33±0.51
Pyknotic nuclei	0.33±0.51	2.66±0.51*	2.66±0.51	2.33±0.51	2.68±0.51
Congested sinusoids	0.33±0.51	1.5±0.55*	1.5±0.54	1.33±0.52	1.33±0.52
Lymphocyte cell infiltration	0	2.67±0.52**	2.66±0.51	2.33±0.52	2.33±0.51

Figure 3. Photomicrographs and histological scores showing liver tissue of the control (a,b), diabetes mellitus (c), DM+SPX10 (d), DM+SPX30 (e), and DM+SPX100 (f) groups. Lymphocytic infiltration around the central vein (⬇), congested sinusoids (⮜), pyknotic nucleus (⇆), and vacuolar changes (↙) in hepatocytes are seen (Hematoxylin and eosin, X100 µm)(*p<0.05, **p<0.01 vs the control group).



Groups	Control	DM	DM+SPX10	DM+SPX30	DM+SPX100
Vacuolization	0.33±0.52	0.16±0.4	0.16±0.4	0	0
Pyknotic nuclei	0.16±0.4	2.66±0.52**	2.66±0.52	2.33±0.52	2.68±0.52

Figure 4. Photomicrographs and histological scores showing skeletal muscle tissue of the control (a,b), diabetes mellitus (c), DM+SPX10 (d), DM+SPX30 (e), and DM+SPX100 (f) groups. Arrowheads (⮟) represent lipid droplets in skeletal muscle fiber sarcoplasm (Hematoxylin and eosin, X100 µm). (**p<0.01 vs the control group).

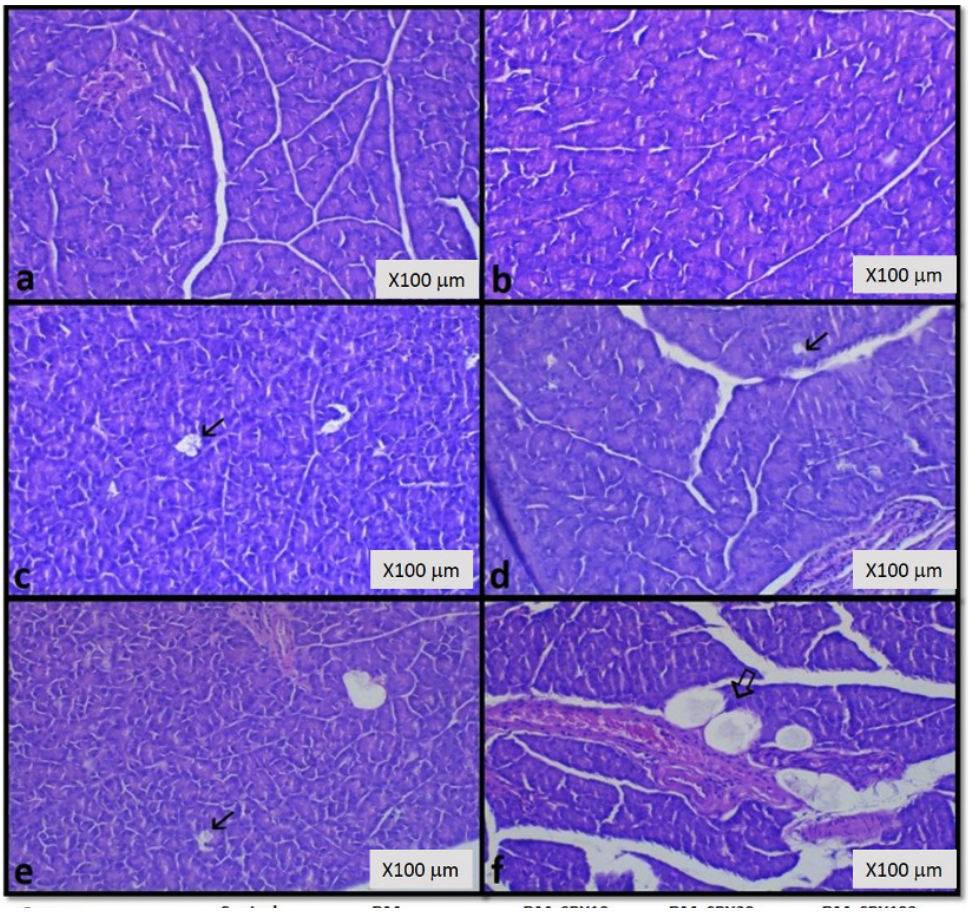


Figure 5. Photomicrographs and histological scores showing pancreatic tissue of the control (a,b), diabetes mellitus (c), DM+SPX10 (d), DM+SPX30 (e), and DM+SPX100 (f) groups. Vacuolization in serous acini (↙) and interlobular adipocytes (⇨) are clearly seen (Hematoxylin and eosin, X100 µm) (*p<0.05 vs the control group).

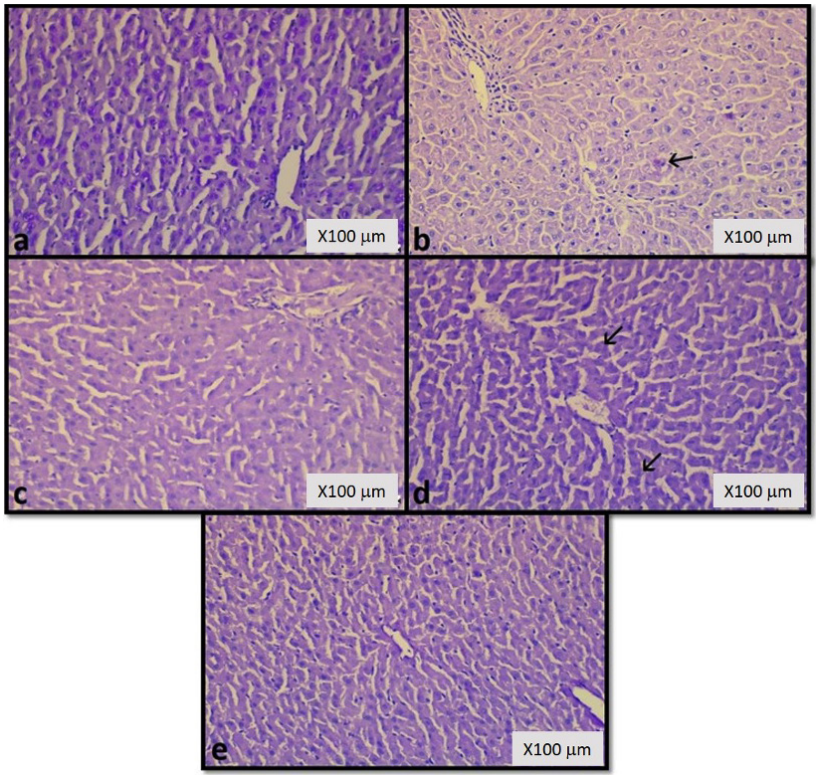


Figure 6. Photomicrographs demonstrating PAS histochemistry in liver tissue of the control (a), diabetes mellitus (b), DM+SPX10 (c), DM+SPX30 (d), and DM+SPX100 (e) groups. Arrows (↑) represent PAS-positive cells (Periodic Acid-Schiff, X100 µm).

group (Figures 3d, 4d, 5d), but they slightly declined in the DM+SPX30 group (Figures 3e,4e,5e). Although the liver and pancreatic tissues of the DM+SPX100 group have significantly resembled the DM+SPX30 group, interlobular adipocyte formation was observed in pancreatic tissue (Figure 5f), and an increased number of lipid droplets were still detected in the skeletal muscle (Figure 4f). Light microscopic evaluation of the PAS histochemistry revealed a strong positive reaction in hepatocyte cytoplasms of the control group (Figure 6a). In contrast, the reaction was weak and uneven in DM (Figure 6b) and DM+SPX10 (Figure 6c) treated groups. A moderate response was observed in the DM+S30 group (Figure 6d), and decreased weak reaction was detected in the DM+SPX100 group (Figure 6e). Besides the histological appearance when we compared the histological score between the groups, while STZ showed degenerative changes in DM groups, we did not observe significant changes by spexin treatment. As mentioned by the histological score in the DM group hepatic tissue showed degenerative changes with vacuolization, pyknotic nuclei, congested sinusoids, and lymphocyte cell infiltration (Figure 3.). Meanwhile, muscle tissue pyknotic nuclei levels were considerably higher in the DM group as compared to the control group (Figure 4, $p<0.05$). The pancreatic tissue histological score showed high levels of vacuolization in the DM group (Figure 5, $p<0.05$).

DISCUSSION

DM is a common condition which is needed to be a detailed understanding of its pathophysiology by identifying key agents that are effective in metabolic changes. It is important to elucidate these mechanisms in order to find possible effective treatments. Recently defined substances that enable tissue crosstalk to gain importance. Spexin, a peptide hormone, has gained considerable attention for its role in regulating glucose levels, energy homeostasis, and metabolism.

To maintain normal physiological functions, the human body must control plasma glucose levels within a narrow range of 4–6 mM [13]. Along with the continuous carbohydrate taken to the body with meals throughout the day, plasma glucose homeostasis is maintained using glycogen sources. Miscellaneous hormones affect the cellular use of glucose and control plasma glucose levels though there are two most significant hormones; insulin and glucagon. Glucagon delivers glucose from hepatic glycogen between meals and sleeps [14]. Insulin has opposite effects on plasma glucose and lowers plasma glucose for using cellular uptake during meals [15]. This study showed increased plasma glucose levels by STZ, and SPX showed its beneficial effect in the 10 ug/mL dose on plasma glucose and plasma insulin levels. Recent studies indicated that SPX levels decreased in patients with Type I and Type II diabetes or obesity. These results consider that SPX may influence glycemic control in diabetes and metabolic diseases [9, 16, 17]. Our result suggests that SPX endeavors decrease plasma glucose levels by increasing the plasma insulin level. The slight decrease in insulin content in pancreatic tissue, actually not statistically significant, in groups treated with SPX confirms our hypothesis partially.

Cellular insulin resistance due to impaired insulin sensitivity is observed in diabetes, mainly developing with a metabolic disease background in type-2 diabetes[18]. In this study, we observed insulin sensitivity development by STZ treatment, and SPX helps to recover from its effects on plasma glucose on a 10 ug/kg dose. These results suggest that SPX may have therapeutic effects on plasma glucose levels in an insulin-dependent manner. Sassek et al. study also supports our results, which is that SPX treatment reduced insulin secretion from cultured pancreatic islet cells when fronted with high glucose [19]. The SPX at 30 mg/mL and 100 mg/mL is beyond the beneficial effects, showing elevated plasma glucose levels. We think these doses are in supraphysiological quantities, so we predict it has such an effect. Sherman et al. previously showed that at supraphysiological doses, spexin lost its effect on thermogenic and weight compared to standard doses [20]. Urine glucose output also increased in DM groups, and the spexin at 10 mg/kg dose limited this increase on the first day of treatment. However, high-dose spexin treatment increased urine glucose output, such as plasma glucose levels. We had difficulty explaining fluctuations in urinary glucose, but we attributed this to changes in urine output.

Glycogenolysis is also crucial for fasting periods and is promoted by insulin [13]. The muscle and liver have buffer activity for plasma glucose changes after meals by storing the osmotically active metabolite in an inert form, such as glycogen [21]. However, glycogen storage is impaired and is one of the typical pathological causes seen in diabetes. As shown in this study, muscle and liver glycogen content was decreased by STZ treatment. Two main steps in glucose metabolism are essential here; the transport of glucose to tissue and the conversion of glucose to glycogen by glycogen synthase [22]. Interfering with glycogen metabolism with various agents, such as reducing glycogen breakdown with glycogen phosphorylase inhibitors, has beneficial effects on plasma glucose levels in diabetes [23]. Other interventions on glycogen metabolism, like inhibition of glycogen synthase kinase-3, have also shown beneficial effects on plasma glucose control [22, 24]. Muscle glycogen synthase is not stimulated in type-2 diabetes under euglycemic hyperinsulinemia [25] but the studies are conducted under the ambient hyperglycemia of type 2 diabetes, the defect in muscle glucose uptake is less apparent [25, 26]. The study about diurnal glycogen uptake in type-2 DM, find out that with a lack of insulin stimulation glycogen synthesis liver has a normal capacity for store of glycogen. This is probably the difference in the rate-limiting of cellular uptake of glucose via GLUT4

[21]. Suggesting these results with our study, SPX recovered the glycogen content of the muscle with the 100 ug/kg dose. Also, the liver glycogen content decreased by STZ treatment but we couldn't see recovery with spexin treatment. There is a lack of studies about muscle glycogen storage in diabetes. Further studies should be carried on to clarify the effects of glucose transporters on muscle glycogen synthesis in diabetes.

It has been known for years that diabetes causes focal sclerosis, inflammation, fat accumulation, and arteriosclerosis in the pancreas [27]. In addition, studies showed that patients with type 1 diabetes have a smaller pancreas than those with healthy and type 2 diabetes patients [28]. And it has been demonstrated that the exocrine pancreas is smaller in patients with both type 1 and type 2 diabetes and is more tending to fibrosis, fatty degeneration, infiltration of inflammatory cells, and atherosclerosis [29, 30]. The histopathological findings of the present study are supported by the biochemical results that STZ-induced diabetes has significant degenerative effects on the liver and muscle tissue besides the pancreas. Furthermore, for the first time in the literature, we indicated the therapeutic impact of SPX treatment on cellular damage in DM.

CONCLUSION

The present study set out to explore the potential influence of SPX in DM. The findings of our study complement those of earlier studies that point out plasma SPX levels were low in diabetic patient groups, making us think that SPX may play a crucial role in modulating glucose homeostasis. This paper contributes to recent concerns about the effects of SPX on insulin secretion. We have revealed the regulatory role of SPX on plasma glucose levels and pancreatic and hepatic cellular damage, glycogen metabolism, and insulin secretion. Moreover, we demonstrated the effectiveness of SPX tested at different doses. Further studies need to indicate the in vitro and in vivo effects of SPX by the increased number of subjects, especially by working on intracellular mechanisms with specialized cell lines.

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