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Investigation of Immunohistochemical Localization of Oxytocin Receptor in Diabetic and Non-Diabetic Mouse Heart

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Abstract

The aim of this study was to investigate the immunohistochemical localization of oxytocin receptor (OTR) in diabetic and non-diabetic mouse heart tissue. Eighteen male Balb-c adult (8-12 week) mice were used in the study. Animals were divided into three groups; control, sham and diabetes. The diabetes group was given STZ by intraperitoneally (i.p) injections and diabetes was induced. Sham group was again treated with sodium citrate solution by i.p. The animals in the control group did not receive any treatment. After 30 days of STZ application, mice were cervical dislocated under ether anesthesia and their heart tissues were removed. Each heart tissue was vertically divided into two parts and routine histological procedures were applied and then tissues were blocked in paraffin and sections were taken. For histological examination, Haematoxylin&Eosin (H&E), Crossman's Triple staining and Periodic Acid Schiff (PAS) were applied to the sections. Immunoreactivity of OTR was determined by Avidin-Biotin-Peroxidase Complex (ABC) method. At the end of the study period; the body weight of the groups, blood glucose level, tissue weights and immunohistochemical localization of OTR in heart tissue samples and histological structure of tissue were compared. When weights of heart tissue were compared between the groups, there was no statistically significant difference between the groups ($p>0.05$). As a result of histological examinations, it was found that there was more degeneration in the cells in the myocardium of the heart in the diabetes group compared to the other groups. Immunohistochemical examinations showed that OTR showed similar immunoreactivity in sham and control groups. In the diabetic group, the immunoreactivity of OTR was similar in endothelial and capillary areas, but less in cell membrane, cytoplasm and purkinje cells. In conclusion, the results of this study showed that there is a significant relationship between the OTR, diabetes and heart tissue. As a result, it is thought that diabetes may have an effect on the cardiovascular system through the OTR ($p<0.05$) .

Key Words: Diabet, heart, ımmunohistochemistry, oxytocin, oxytocin receptor

Diabetik ve Non-Diabetik Fare Kalbinde Oksitosin Reseptörünün İmmunohistokimyasal Lokalizasyonunun İncelenmesi

Öz

Bu çalışmada, diyabetik ve non-diyabetik fare kalp dokusunda oksitosin reseptörünün (OTR) immunohistokimyasal lokalizasyonunun incelenmesi amaçlanmıştır. Çalışmada 18 adet Balb-c cinsi ergin (8-12 haftalık) erkek fare; kontrol, sham ve diyabet grubu olarak belirlenmiştir. Diyabet grubu intraperitoneal (i.p.) enjeksiyonla streptozotosin (STZ) verilerek oluşturulmuştur. Sham grubuna ise i.p. yolla sodyum sitrat çözeltisi uygulanmıştır. Kontrol grubundaki hayvanlara ise herhangi bir uygulama yapılmamıştır. STZ uygulandıktan 30 gün sonra farelere eter anestezi altında servikal dislokasyon yapılarak kalp dokuları alınmıştır. Alınan her bir kalp dokusu düşey olarak ikiye bölünerek rutin histolojik işlemlerden geçirilerek parafinde bloklanıp kesitler alınmıştır. Alınan kesitler histolojik olarak incenlenmek üzere Hematoksilen Eosin (H&E), Crossman'ın üçlü boyaması ve Periyodik Asit Schiff (PAS) yapıldı. Oksitosin reseptörünün (OTR) immunoreaktivitesi Avidin-Biotin-Peroksidaz kompleks (ABC) metodu uygulanarak belirlenmiştir. Müdahale sonrasında, grupların canlı ağırlıkları, kan glikoz seviyesi, doku ağırlıkları ve kalp doku örneklerinde oksitosin reseptörünün immunohistokimyasal lokalizasyonu ve dokunun histolojik yapısı karşılaştırılmıştır. Kalp dokusunun ağırlıkları gruplar arasında karşılaştırıldığında istatistiksel düzeyde anlamlı bir fark yoktur ($p>0.05$). Histolojik incelemeler sonucunda diyabet grubunda diğer gruplara kıyasla kalbin miyokard bölgesindeki hücrelerde daha fazla dejenerasyon olduğu tespit edildi. İmmunohistokimyasal incelemeler sonucunda, sham ve kontrol grupları arasında OTR'nin immunoreaktivite seviyesi açısından istatistiksel olarak anlamlı bir fark yoktur. Diyabet grubunda diğer gruplara kıyasla OTR'nin immunoreaktivite seviyesi hücre membranı, sitoplazma ve purkinje hücrelerinde istatistiksel olarak anlamlı düzeyde daha az iken endotel ve kapiller alanlarda fark saptanmamıştır. Bu çalışma sonuçları oksitosin reseptörü, diyabet ve kalp dokusu arasında önemli bir ilişkinin olduğunu göstermiştir. Sonuç olarak, diyabetin oksitosin reseptörü vasıtasıyla kardiovasküler sistem üzerinde etkili olabileceği düşünülmektedir ($p<0.05$).

Anahtar Kelimeler: Diyabet, immünohistokimya, kalp, oksitosin, oksitosin reseptörü

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease triggered by hyperglycemia caused by lack insulin secretion in pancreas beta cells or impaired insulin action. Since DM affects diabetic cardiomyopathy, hypertension, autonomic neuropathy and endothelial dysfunction, it can increase cardiovascular mortality (1-3).

Oxytocin (OT) is a peptide hormone consisting of 9 amino acids, and effective on glucose metabolism (4) so it is also effective glycemic control, and causes to increase related hormones (5). OT and OTR in the heart have cardioprotective effects directly or by stimulating nitric oxide (NO) of atrial natriuretic peptide (6-8). It is also known to trigger parasympathetic impulses to decrease systemic blood pressure (9). For this reason, it has been stated that oxytocin prevents the development of hypertension by causing a decrease in arterial pressure (10).

OT has come to the fore with its anorexigenic effect. In addition to a decrease in food intake and concomitant weight loss, getting better in oral glucose tolerance test and insulin sensitivity can be observed following OT administration (11). It is also known that OT level increases in hypoglycaemia (12). OT application increases the secretion of OTR (13), insulin and glucagon in pancreas (14, 15), and causes a significant increase in insulin levels in rabbits (16) and increase plasma glucose and insulin in dogs (17).

While the negative effects of diabetes on cardiovascular system attract intense scientific attention, the possible beneficial effects of the OT on the cardiovascular system are not yet known. From this point of view, we aimed to investigate the effect of diabetes on OTR in heart tissue by using immunohistochemical technique.

MATERIAL AND METHODS

Experimental Animals

Approval for this study was obtained from Kafkas University Experimental Animals Local Ethics Committee (Date: 25 May 2016, Number: 042). The experimental animals used in this study were obtained from Atatürk University Medical Experimental Application and Research Center. Eighteen male Balb-c adult (8-12 week) mice were used, and they were randomly divided into three groups and mice in each group kept in one cage. The animals were housed at constant conditions in terms of temperature and humidity on a 12-hour dark-light cycle. The samples were fed with pellet in steel containers on cages and tap water in drinkers with steel balls. The cages, containers and drinkers were cleaned daily. The animals were allowed to adapt to the environment without any application for two weeks after obtaining them. After these two weeks for adaptation, we named groups as sham, control and diabetic each of which had 6 mice. The diabetic group received once at dose of 100 mg/kg streptozotocin (STZ) (S0130-1G, Sigma-Aldrich, USA), via intraperitoneally (i.p.) (18). STZ was dissolved in 0.1 M Citrate buffer (pH:4.5). The sham group was treated only once via i.p. with sodium citrate solution in which STZ was dissolved. The control group did not receive any experimental treatment. The day of STZ injections was accepted as day 0, and the experimental period lasted for 30 days. After 12 hours fast, the blood glucose was measured, and body weight was scaled by a precision digital balance (Precisa-XB220A) on 0^{th} and 30^{th} of the study.

Determination of Blood Glucose Level

Blood glucose level of all groups was measured with glucometer (Rocho Accucheck Go) by taking blood drop from the tail vein. All animals left fasted for 12-hour before the blood glucose level was measured. Animals in the diabetic group were fasted for 12-hour to determine 72 hours later blood glucose level. It was considered as diabetic if blood glucose level equal or above 200 mg/dL (19). On the 30^{th} day of the study, before the tissues were collected, the animals were again fasted for 12 hours and their blood glucose level were measured for the last time.

Taking Tissue Samples

Before the tissues were taken, the animals were fasted for 12 hours to measure their glucose level and to scale their weight. On the 30^{th} day after STZ administration, cervical dislocation under ether anaesthesia was performed and all animals were euthanized. Then, the heart tissues of the subjects were removed and weighted. The heart tissues divided into two parts to be examined histologically and immunohistochemically.

Histological and Immunohistochemical Procedures

Taken the heart tissues were fixed in 10% formol to be examined histologically and immunohistochemically. Then, the tissues were passed through graded alcohols, methyl benzoate and benzyl series, and at the end they were blocked in paraffin. The 5 µm thick sections were taken from these paraffin blocks to the slides which were coated with chrome alum gelatine. H&E, Crossman's Triple staining and PAS staining techniques were applied to examine the heart tissue (20). PAS was used to determine glycogen in cardiac muscle cells and elastic fiber in great vessels wall and valves. The preparations were examined under a light microscope (Olympus Bx43, Japan) for evaluation, and the necessary parts were photographed with the Olympus DP21 camera.

Some of the sections (5 µm thickness) were used for routine histological examination, and some of them used to examine the immunohistochemical localization of the OTR in heart tissue. Avidin-Biotin-Peroxdase Comples (ABC) was used to detect the OTR in heart tissue (21). After deparafinization and dehydration processes, sections were washed in phosphate buffered saline (PBS) (0.1 M, pH;7.2). Endogenous peroxidase activity in tissues was blocked by incubating them with 3% H2O2 prepared in PBS for 20 min. After washing with PBS, sections were kept in a microwave oven for 12 min to reveal the antigens. They were washed with PBS again, and then incubated in serum (%10) which was compatible with seconder antibody. Sections were washed with PBS, and incubated with anti-OTR antibody (abcam:ab217212, Cambridge, UK) at a dilution of 1:250 for 1 hour at room temperature. Then, tissues were washed

with PBS and incubated with biotinylated secondary antibody which is specific to species in which the primary antibody was produced. After washed with PBS, sections were incubated with streptavidin peroxidase for 30 min at room temperature. After washed with PBS again, Diamibobenzidine (DAB) (Thermo TA-125-HD, USA) was used for chromogen application. Finally, haematoxylin was applied to the sections as a counter staining, and the sections were covered with a coverslip with the help of entellan. The prepared slides were examined under the light microscope and photographed.

The degree of immunoreaction was determined as 0 (no reaction), +1 (weak reaction), +2 (moderate reaction) and +3 (strong reaction). In order to make clear whether the immunoreactivitiy was specific or not, the primary antibody step was skipped and all other processes were applied in the negative control slides.

Statistical Analysis

SPPS version 16.0 was used for statistical analysis (SPSS 2007). One-way ANOVA was used for multiple comparisons between groups. Bonferroni test was used for homogenous groups and Tamhane tests were applied for non-homogeneous groups. Paired sample t-test was used for pairwise comparisons. Statistical significant was determined as $p<0.05$.

RESULTS

Body Weight Results

The body weights of the animals scaled on the 0^{th} and 30^{th} days were evaluated statistically. In our study, body weight of each group was statistically compared within itself in order to determine whether there is a difference between 0^{th} and 30^{th} days. There was a statistically significant decrease in diabetic group between body weights on 0^{th} and 30^{th} ($p<0.05$). On the other hand, there was an increase in the control group between body weights on 0^{th} and 30^{th} ($p<0.05$). There was an increase but not statistically significant in body weight of sham group between those days ($p>0.05$), (Table 1).

Table 1. Comparison of body weight of each group by days

Group/Day	N	Body weight mean (g) ± SD	p-value
Diabetic 0^{th} day	6	37.67±3.27	0.010*
Diabetic 30^{th} day	6	31.00±3.90	
Sham 0^{th} day	6	36.17±4.36	0.217
Sham 30^{th} day	6	39.00±2.97	
Control 0^{th} day	6	35.00± 4.34	0.026*
Control 30^{th} day	6	41.00 ±3.52	

*p<0.05, there was significant difference. (SD: standard deviation). Each group was evaluated separately.

Heart Weight Results

The heart tissue was taken on the 30^{th} of the study and were weighted as soon as they were taken. As a result of the statistical evaluation in our study, there was not a statistically difference between groups in terms of heart weight (Table 2).

Table 2. Comparison of heart weights between groups

Group	N	Heart weight mean (g)±SD	F
Diabetic	6	1.62±0.29	
Sham	6	1.76 ±0.12	2.428
Control	6	1.88±0.17	

(SD: standard deviation, F: F value)

Blood Glucose Level

The blood glucose levels were measured on 0^{th} and 30^{th} days. When the results were compared between groups, it was found that only the blood glucose level of diabetic group on 30^{th} day was statistically significant higher than that of diabetic group on 0^{th} day and other groups on 0^{th} and 30^{th} days (Table 3).

Table 3. Comparison of blood glucose level on 0^{th} and 30^{th} days between groups

Group/Day	N	Blood glucose (mg/dL) ± SD	Min.	Max.	F
Diabetic 0th day	6	81.50^{a}±10.05	71	95	
Sham 0th day	6	91.00^{a}±13.89	72	108	
Control 0th day	6	86.33^{a}±6.62	78	95	88.75*
Diabetic 30th day	6	265.50^{b}±40.49	218	318	
Sham 30th day	6	87.67^{a}±9.09	76	102	
Control 30th day	6	93.50±8.76	83	106	

Different superscript letters indicate a statistically significant difference. *p<0.05 (SD: Standard deviation)

Histological Results

The tissues stained with H&E, Crossman's Triple and PAS were histologically examined. We saw that heart valves and great vessels wall were most prominently stained with PAS in all group (Figure 1C-D). Heart muscle cells close to the pericardium in the diabetic group had weaker staining than those of control and sham groups when we examined slides prepared with Triple staining (Figure 1E-F). In the control and sham groups, there was not a change in terms of the histological structure part of heart such as endothelium, Purkinje cells and cardiac muscle cell. In diabetic group, it was seen that there was mild degeneration of the heart muscle cells in some myocardial regions (Figure 1).

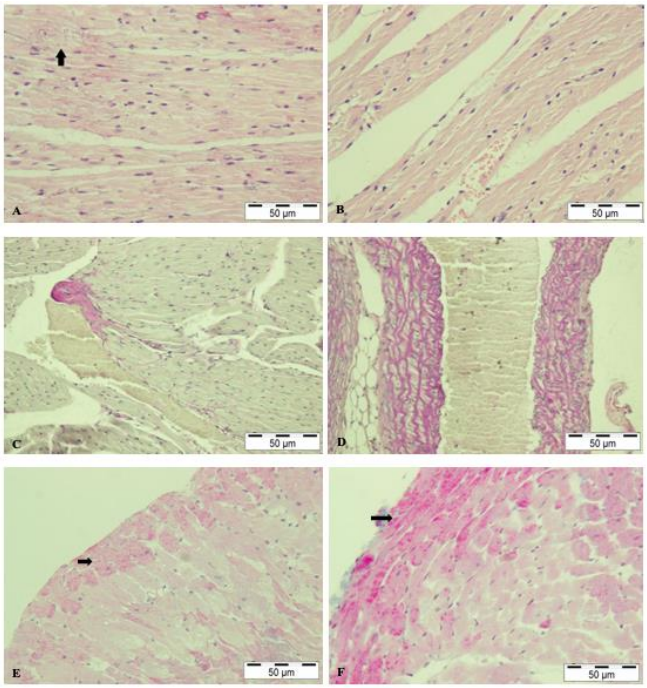

Figure 1. A. Focal mild degeneration of myocyte (black arrow) in diabetic group. H&E. Bar: 50µm. B. The histological view of myocytes of control group. H&E. Bar: 50µm. C. The histological view of heart tissue and valve tissue in diabetic group. PAS. Bar: 50µm. D. The histological view of great vessel walls in control group PAS. Bar: 50µm. E. Muscle cells nearby pericardium (black arrow) in diabetic group. Triple. Bar: 50µm. F. Muscle cells nearby pericardium (black arrow) in control group. Triple. Bar: 50µm.

Immunohistochemical Results

When the localization of OTR in heart tissues were examined immunohistochemically, it was observed in the myocyte membrane, heart valves, capillaries and endothelial layer in all groups (Figure 2). The immunoreactivity was observed in the cytoplasm of a few myocytes in some regions (Figure 2). It was observed that the OTR immunoreactivity was stronger in control and sham groups than diabetic group (Figure 2) (Table 4).

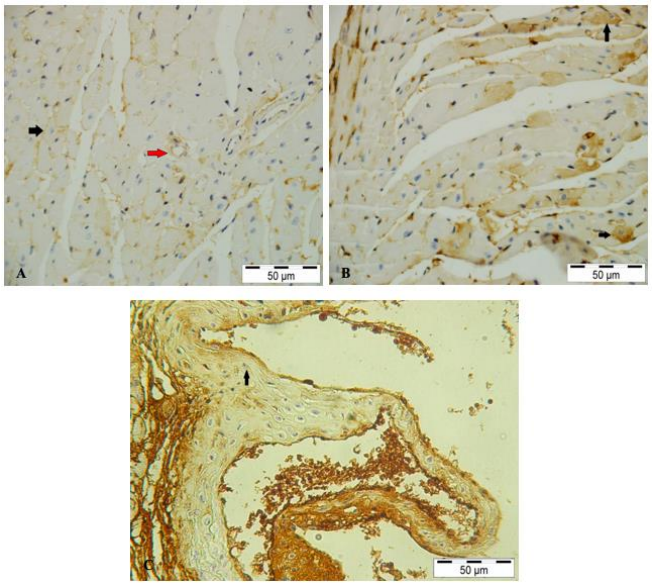

Figure 2. A. Oxytocin receptor immunoreactivity in mouse heart tissue of diabeteic group. Red arrow: immunoreactivity in endothelial cells. Black arrowhead: immunoreactivity in cell membrane. Bar: 50µm B. Oxytocin receptor immunoreactivity in mouse heart tissu of control group. Immunoreaction in some cytoplasm of myocyte. Arrow: Immunoreaction surround of nukleus. Bar:50µm. C. Oxytocin immunoreactivity in valve of mouse heart in sham group (arrow). Bar:50µm.

Table 4. Scores of OTR immunoreactivity

Part of heart	N	Control group	Sham group	Diabetic group
Cell membrane	6	+++	+++	++
Cytoplasm	6	++	++	+
Nucleus	6	++	++	+
Endothelium	6	+++	+++	+++
Purkinje cell	6	++	++	+
Small vessel	6	+++	++	+++

DISCUSSION AND CONCLUSION

In our study, experimental diabetes was induced in mice by applying STZ. In addition to examine immunohistochemical localization of OTR, the body and heart weight of mice and their fasting blood glucose levels were evaluated.

In studies, different STZ doses have been used to induce diabetes in mice such as 100 mg/kg (18), 150 mg/kg (22) and 200 mg/kg (23). We induced diabetes in mice of diabetic group using STZ at dose of 100 mg/kg via i.p. injection.

The fasting mice blood glucose level, which is consider sign of diabetes, differs from one study to another study. Yeğin and Mert (2013) confirmed mice as diabetic if their blood glucose level was above 210 mg/dL (24), Demir and Yılmaz (2013) accepted mice as diabetic if blood glucose level was between 140-200 mg/dL (25), and Kanitkar and Bhonde (2004) verified mice as diabetic if it was 200 mg/dL. In our study, mice with ≥ 200 mg/dL fasting blood glucose level established as diabetic (19).

The relationship between body weight and diabetes was evaluated by many studies. Grover et al. (2001) reported that diabetes did not cause body weight loos in mice with experimental diabetes by applying STZ at 150 mg/kg dose (22). Koroğlu et al. (2014) stated that diabetes created body weight loos in rats which were applied STZ at 65 mg/kg dose and kept for 28 days (26). Shin et al. (2014) determined that diabetes caused body weight loos in mice with experimental diabetes which they created with using STZ (23). We found that body weight decreased statistically significant in diabetic group during the 30 days. We thought that this result became because of glucose metabolism deficit during diabetes.

Harackova and Murphy (1988) reported that there were not significant changes in ventricular muscle cells of rats between diabetic and control groups (27). Contrary to this study, Cai et al. (1989) stated that myocardia layer was thinner in diabetic male rat heart (28). Cai et al. (1989) and Sun et al. (1993) expressed that there was damage to myofibrils in the heart muscle cell (28,29). Çetin et al. (2013) mentioned that pycnotic nuclei and eosinophilic cytoplasm were observed in myocyte of heart in the experimental diabetic group (30). They also stated that vacuoles with miscellaneous dimensions were seen in cytoplasm of those cells. We determined that degeneration of myocytes in some region and irregularity in some intercalated discs in diabetic mice heart.

When we reviewed the literature, we could not encounter any study about OTR immunoreactivity in diabetic

mouse heart. Jankowski et al. (2004) announced that they showed OTR in endothelial cells of rat hearts at the 21st day of embryonic development and at the 1st postnatal day in their study related to development ontogeny. Wigger et al. (2004) reported that OTR was shown in the heart muscles, and in vessel wall and endothelial cells (31). Merz et al. (2020) showed OTR immunoreactivity in heart tissue (32). We saw OTR in membrane of myocytes, cytoplasm of some cells, valve of heart and small capillaries. We also determined that the OTR immunoreactivity in the diabetic mice heart was weaker than that of others. We considered that there is a suppressing effect of hyperglycaemia on OTR expression as Liu et al. (33) previously mentioned.

In conclusion, we found that diabetes caused degeneration in some part of heart, and decreased OTR immunoreactivity in heart tissue. Because of this results, we considered that there is a clear relationship between OTR, diabetes and heart tissue triad. In conclusion, we thought that diabetes might have an effect on the cardiovascular system through OTR.

CONFLICTS OF INTEREST

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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REFERENCES

1. Çavuşoğlu T, Çiftçi ÖD, Çağıltay E, et al. (2017). Diyabetik Kardiyomiyopati Sıçan Modelinde Oksitosin Etkilerinin Histolojik ve Biyokimyasal Olarak Incelenmesi. Dicle Tıp Dergisi. 44(2): 135-143.
2. Işık S, Delibaşı T, Berker D, Aydın Y, Güler S. (2009) Kalp Hastalıklarında Diyabet Yönetimi. Anadolu Kardiyoloji Dergisi. 9: 238-247.
3. Özel CB, Arıkan H, Dağdelen S, et al. (2021). Investigation of Cardiovascular Disease Risk Factors Knowledge and Physical Activity Levels in Patients with Type 2 Diabetes. Journal of Exercise Therapy and Rehabilitation. 8(1): 99-105.
4. Du Vigneaud V. (1956). Trail of Sulfur Research: From Insulin to Oxytocin. Science. 123: 967-974.
5. Bjorkstrand E, Eriksson M, Uvna Moberg K. (1996). Evidence of Peripheral and a Central Effect of Oxytocin on Pancreatic Hormone Release in Rats. Neuroendocrinology. 63: 377-383,
6. Wang P, Wang SC, Liu X. (2022). Neural Functions of Hypothalamic Oxytocin and its Regulation. American Society for Neurochemistry. 14: 1-33.
7. Paquin J, Danalache BA, Jankowski M, Mccann SM, Gutkowska J. (2004). Oxytocin Induces Differentiation of P19 Embryonic Stem Cells to Cardiomyocytes. Proc Natl Acad Sci. 99: 9550-9555.
8. Jankowski M, Danalache B, Wang D, et al. (2004). Oxytocin in Cardiac Ontogeny. Proc. Natl. Acad. Sci. USA 101: 13074-13079.
9. Houshmand F, Faghihi M, Zahediasl S. (2009). Biphasic Protective Effect of Oxytocin on Cardiac Ischemia/Reperfusion Injury in Anaesthetized Rats. Peptides. 30(12): 2301-2308.
10. Ondrejcakova M, Ravingerova T, Bakos J, Pancza D, Jezova D. (2009). Oxytocin Exerts Protective Effects on in Vitro Myocardial Injury Induced by Ischemia and Reperfusion. Can J Physiol Pharmacol. 87: 137-142.
11. Balazova L, Krskova K, Suskp M, Sısovsky V, Hlavacova N, Olszanecki R. (2016). Metabolic Effects of Subchronic Peripheral Oxytocin Administration in Lean and Obese Zucker Rats. J Physiol Pharmacol. 67:4531-4541.
12. Gutkowska J, Jankowski M, Lambert C, Mukaddam-Daher S, Zingg HH, Mccann SM. (1997): Oxytocin Releases Atrial Natriuretic Peptide by Combining with Oxytocin Receptors in the Heart. Proc Natl Acad Sci. 94: 11704-11709.
13. Biggs LM, Hammock EAD. (2022). Oxytocin via Oxytocin Receptor Excites Neurons in the Endopriform Nucleus of Juvenile Mice. Scientific Reports. 12(1): 11401.
14. Mineo H, Ito M, Muto H, Kamita H. (1997). Effect of Oxytocin Arginine-Vasopressin and Lysine-Vasopressin on Insulin and Glucagon Secretion in Sheep. Res in Veterinary Science. 62: 105-110.
15. Wallin LA, Fawcet CP, Rosenfeld CR. (1996). Oxytocin Stimulates Glucagon and Insulin Section in Fetal and Neonatal Sheep. Endoc.125: 2289-2296.
16. Knudizon J. (1981). Acute Effect of Oxytocin and Vasopressin on Plasma Levels of Glucagon, Insulin and Glucose in Rabbits, Horm Metab Res. 15:103-106.
17. Aitszuler N, Hamshire J. (1981). Oxytocin Infusion Increases Plasma Insulin and Glucagon Levels and Glucose Production and Uptake in the Dog. Diabetes. 30: 112-114.
18. Bingöl SA, Kocamış H. (2010). The Gene Expression Profile by RT-PCR and Immunohistochemical Expression Pattern of Catalase in the Kidney Tissue of Both Healthy and Diabetic Mice. Kafkas Univ Vet Fak Derg. 16 (5): 825-834.
19. Shaukat A, Hussain G, Irfan S, Ijaz MU, Anwar H. (2022). Therapeutic Potential of MgO and MnO Nanoparticles within the Context of Thyroid Profile and Pancreatic Histology in a Diabetic Rat Model. Dose-Response. 20(3): 1-11.
20. Luna LG. (1968). Manual of Histologic Staining Methods of Armed Forces Institute of Pathology. 3th ed., pp. 222-226. Mc GrawHill Book Comp, New York.
21. Hsu SM, Raine L, Fanger H. (1981). A Comparative Study of the Peroxidase-Antiperoxidase Method and an Avidin-Biotin Complex Method for Studying Polypeptide Hormones with Radioimmunoassay Antibodies. American Journal of Clinical Pathology. 75(5): 734-738.
22. Grover JK, Vats V, Rathi SS, Dawar R. (2001). Traditional Indian Anti-Diabetic Plants Attenuate Progression of Renal Damage in Streptozotocin Induced Diabetic Mice. J Ethnopharmacol. 76(3): 233-238.
23. Wada T, Furuichi K, Sakai N, et al. (2000). Upregulation of Monocyte Chemoattractant Protein-1 in Tubulointerstitial Lesions of Human Diabetic Nephropathy. Kidney Int. 58(4): 1492-1499.
24. Yeğin SÇ, Mert N. (2013). Deneysel Olarak Diyabet Oluşturulmuş Sıçanlarda HbA1c, MDA, GSH-Px ve SOD Miktarlarının Tayini. YYU Veteriner Fakültesi Dergisi. 24: 51-54.
25. Demir E, Yılmaz Ö. (2013). Streptozotosin Ile Tip-2 Diyabet Oluşturulan Sıçanlarda Çam Yağının Antihiperglisemik ve Bazı Biyokimyasal Parametrelere Etkisi. Marmara Fen Bilimleri Dergisi. 25(3): 140- 156.

26. Koroglu P, Senturk GE, Yucel D, Ozakpinar OB, Uras F, Arbak S. (2014) The Effect of Exogenous Oxytocin on Streptozotocin (STZ)-Induced Diabetic Adult Rat Testes. Peptides. 63: 47-54.
27. Harackova M, Murphy MG. (1998). Effects of Chronic Diabetes Mellitus on the Electrical and Contractile Activities, 45Ca2+ Transport, Fatty Acid Profiles and Ultrastructure of Isolated Rat Ventricular Myocytes. Pflugers Arc. 411: 564-572.
28. Cai F. (1989). Studies of Enzyme Histochemistry and Ultrastructure of the Myocardium in Rats with Streptozotocin Induced Diabetes. Zhonghua Yixue Za Zahi. 69(5): 276-278.
29. Sun S, Dawuti A, Gong Difei. (2022). Puerarin-V Improve Mitochondrial Respiration and Cardiac Function in a Rat Model of Diabetic Cardiomyopathy via Inhibiting Pyroptosis Pathway through P2X7 Receptors. Int J Mol Sci. 23(21): 13015.
30. Çetin A, Vardı N, Orman D. (2013). Deneysel Diyabetin Sıçan Kalp Dokusunda Meydana Getirdiği Histolojik Değişiklikler Üzerine Aminoguanidinin İyileştirici Etkileri. İnönü Üniversitesi Sağlık Hizmetleri Meslek Yüksekokulu Dergisi. 4(1): 1–11.
31. Wigger DC, Gröger N, Lesse A. (2020). Maternal Separation Induces Long-Term Alterations in the Cardiac Oxytocin Receptor and Cystathionine *γ*-Lyase Expression in Mice. Oxidative Medicine and Cellular Longevity. 2020: 1-10.
32. Merz T, Denoix N, Wigger D. (2020). The Role of Glucocorticoid Receptor and Oxytocin Receptor in the Septic Heart in a Clinically Relevant, Resuscitated Porcine Model with Underlying Atherosclerosis. Frontiers in Endocrinology. 11: 1-8.
33. Liu J, Liang Y, Jiang X, et al. (2021). Maternal Diabetes-Induced Suppression of Oxytocin Receptor Contributes to Social Deficits in Offspring. Frontiers in Neuroscience. 15: 634781.

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