ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Determination of Ghrelin Immunoreactivity in the Pancreas Tissue of Rats Supplied with Melatonin

Melatonin Uygulanan Ratların Pankreas Dokusunda Ghrelin İmmünoreaktivitesinin Belirlenmesi

Ayse Aydogan[1], Engin Yalmanci[2], Seher Koc Saltan[3], Sahin Aslan[3]

[1]Department of Histology and Embryology, Faculty of Veterinary Medicine, Necmettin Erbakan University, Konya;
[2]Prof. Dr. Fahrettin Kirzioglu Science and Art Center; 3Department of Histology and Embryology, Faculty of Veterinary Medicine, Kafkas University, Kars, Türkiye

ABSTRACT

Aim: *This study aimed to determine the histological effect of melatonin administration on the pancreas and the localization of ghrelin, secreted from the pancreas, using immunohistochemical techniques.*

Material and Method: *The study utilized 30 male Sprague Dawley rats aged 10–12 weeks. Melatonin was dissolved in ethanol and injected intraperitoneally into the experimental group at 10 mg/kg for 21 days, diluted with a physiological saline solution. After the experimental period, pancreatic tissues were collected from the rats. These tissue samples were fixed in 10% formalin for histological and immunohistochemical examinations and underwent routine histological procedures. The tissues were then treated with a ghrelin antibody (diluted to 1/50) to determine the immunohistochemical localization of ghrelin.*

Results: *Upon examination of the general histological structure of the pancreatic tissue, no differences were observed between the groups. Ghrelin immunoreactivity was detected in the islets of Langerhans and the pars initialis epithelium of the pancreas in both the sham and control groups but not in the melatonin group.*

Conclusion: *Melatonin administration did not induce any histological disorders in the pancreas. However, it was found to decrease the ghrelin levels secreted from the pancreas.*

Key words: *ghrelin; immunohistochemistry; melatonin; pancreas*

ÖZET

Amaç: *Melatonin uygulamasının, pankreas üzerindeki histolojik etkisi ve pankreastan sekresyonu sağlanan ghrelinin, immünohistokimyasal olarak lokalizasyonunun belirlenmesi amaçlanmıştır.*

Materyal ve Metot: *Çalışmada 10–12 haftalık 30 adet erkek Spraque Dawley türü rat kullanıldı. Melatonin, etanolde çözdürülüp serum fizyolojik su ile sulandırılarak 10 mg/kg dozda ve 21 gün boyunca intraperitoneal yolla deneme grubuna enjekte edildi. Deneysel uygulamanın sonunda ratların pankreas dokuları alındı. Histolojik ve immünohistokimyasal incelemelerde kullanılmak üzere pankreas doku örnekleri %10'luk formalde tespit edilerek rutin histolojik işlemlerden geçirildi. Ghrelinin immünohistokimyasal lokalizasyonunu belirlemek amacıyla, dokulara ghrelin antikoru (1/50 dilüsyonunda) uygulandı.*

Bulgular: *Pankreas dokusunun genel histolojik yapısı incelendiğinde, gruplar arasında bir farklılık olmadığı tespit edildi. Sham ve kontrol gruplarında ghrelin immünoreaktivitesi pankreasın, Langerhans adacıklarında ve pars inisiyalis epitelinde görüldü. Melatonin grubunda ise immünoreaktivite görülmedi.*

Sonuç: *Melatonin uygulamasının, pankreasta herhangi bir histolojik bozukluğa yol açmadığı görüldü. Melatonin uygulamasının pankreastan salgılanan ghrelin seviyesini düşürdüğü tespit edildi.*

Anahtar kelimeler: *ghrelin, immünohistokimya, melatonin, pankreas*

Introduction

Melatonin is a hormone synthesized and secreted from the pineal gland located between the cerebral hemispheres, bone marrow cells, lens, ovary, gastrointestinal system, and bile in the brain of mammals[1,2]. Melatonin does not show a homogeneous distribution in the gastrointestinal tract and is present in different concentrations and parts. Gastrointestinal tract melatonin is entirely related to serotonin concentration, which supports the idea that melatonin production in the gastrointestinal tract is independent of the pineal gland. It is known that the gastrointestinal tract melatonin level is not affected by pinealectomy[3,4]. Literature on the pancreas indicates that melatonin has a protective effect on this organ[5].

İletişim/Contact: *Ayse Aydogan, Department of Histology and Embryology, Faculty of Veterinary Medicine, Necmettin Erbakan University, Konya, Türkiye* • ***Tel:*** *0536 675 79 84* • ***E-mail:*** *aydoganayse12@gmail.com* • ***Geliş/Received:*** *12.07.2023* • ***Kabul/Accepted:*** *16.02.2024*

ORCID: *Ayşe Aydoğan: 0000-0001-7317-6486 • Engin Yalmancı: 0000-0001-9416-3158 • Seher Koç Saltan: 0000-0003-3795-5145 • Şahin Aslan: 0000-0002-4565-9832*

Kafkas J Med Sci 2024; 14(1):48–53
doi: 10.5505/kjms.2024.98957

It is known that ghrelin, which can secrete growth hormone from the stomach of rats, was detected in 1999[6]. It is known that ghrelin is derived from the word 'ghre', which means 'to grow' in Proto-Indo-European languages[7]. The ghrelin is a hormone found in mammals and other vertebrate species such as chickens, frogs, and fish, and a delicious peptide hormone consisting of 28 amino acids[7,8]. The ghrelin has two different molecular forms. These are acyl ghrelin of 28 amino acids (modified form) and des-acyl ghrelin of 27 amino acids (unmodified form)[9,10]. Acyl ghrelin forms one-fifth of the ghrelin immunoreactivity in rat stomach[11]. In the gastrointestinal system, ghrelin has stimulating effects on the motility of the digestive tract during satiety and hunger[10].

Ghrelin is primarily produced in the stomach, particularly in the densest areas[12,7]. Additionally, it is produced in various other locations within the body, including the hypothalamus, brain, pituitary gland, adipose tissue, heart, lungs, and pancreas[7,13,14].

The literature has documented that the pancreas is an organ that produces ghrelin. It is known that the pancreatic cells responsible for ghrelin secretion are α and β cells[15,16]. In the postnatal period, the pancreatic ghrelin-expressing cells are high in number (around 10% of all endocrine cells)[17]. The ghrelin mRNA and protein are known to be found in the pancreas. These proteins are expressed in α[16] and β[15] cells of the pancreas. Several studies show that ghrelin upregulates insulin production in the pancreas[18,19]. It is also known to reduce plasma glucose levels in humans[20].

The study aims to determine the histological effect of melatonin administration on the pancreas and the localization of ghrelin, of which secretion is provided from the pancreas immunohistochemically.

Materials and Methods

Ethical approval of the study was obtained from the Animal Experiments Local Ethics Committee of Kafkas University (Date: 25.05.2022, Decision No: 107 and Research Code: KAU-HADYEK/2022-107). All stages of the study were conducted in the Department of Histology and Embryology Laboratory, Faculty of Veterinary Medicine, Kafkas University.

Experimental Animal Material: The experimental animals used in the study were obtained from the Erzurum Veterinary Control Institute. Thirty male *Spraque Dawley* rats, which were 10–12 weeks old, were used in the study. During the experiment, the subjects were fed rat chow as *adlibitum*. After a 15-day adaptation period, the subjects were divided into three groups: the experimental group, the sham group, and the control group. The subjects were housed in standard cages with a 12-hour light-dark cycle and a room temperature of 22±2°C.

Experimental Groups: The study included three experimental groups (control, sham, and melatonin). Melatonin (Sigma-M5250), stored at -20°C, was brought to the laboratory under cold chain conditions. Melatonin, dissolved in ethanol and diluted with physiological saline water at a dose of 10 mg/kg, was administered to the experimental group intraperitoneally for 21 days (in the evening hours). The sham group received injections of the same volume of ethanol and saline water as the experimental group for 21 days. No administration was made to the control group. A total of 30 rats were used, with 10 subjects in each group.

Removal of Pancreatic Tissues: At the end of the 21-day experimental period, the subjects were euthanized under anesthesia, and their pancreatic tissues were removed. The pancreatic tissue samples were fixed in 10% formalin for histological and immunohistochemical examinations.

Histological Studies: 5 μm thick sections were taken from the paraffin-blocked tissues on the slides coated with chrome alum gelatin. Crossman's Triple Staining was performed on tissue sections to examine the histological structure of the pancreas.

Immunohistochemical Studies: 5 μm thick sections were taken from the blocked tissues to examine the immunohistochemical localization of ghrelin in the pancreatic tissue. Deparaffinization and dehydration processes were applied to the prepared sections. It was washed in phosphate-buffered saline (PBS) and incubated for 15 minutes in 3% H_2O_2 prepared in 0.1 M PBS. After the sections were rewashed with PBS, 600 watts of heat were applied to the citrate buffer in a microwave oven for 10 minutes. It was rewashed with PBS and incubated for 10 minutes in UV serum (10%), suitable for the secondary antibody to prevent non-specific bindings. After washing with PBS again, the anti-ghrelin antibody (Phonex H-031-31, at 1:50 dilution ratio) was administered to the sections and incubated for 24 hours at room temperature. At the end of the period, the biotinylated secondary antibody was administered to the tissues that were rewashed with

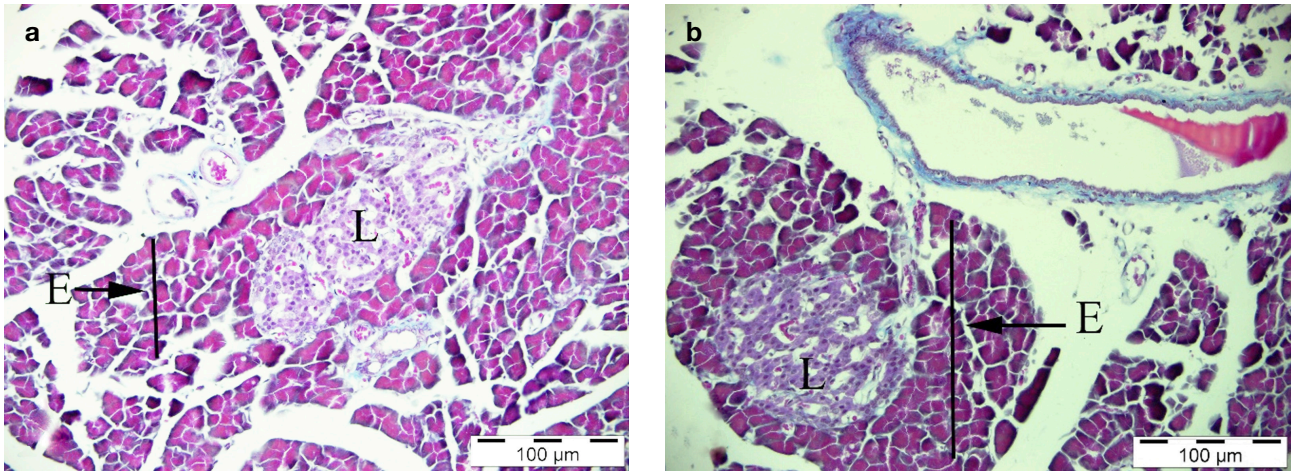

Figure 1. *a, b. Pancreas image with melatonin application (a). Pancreas image of the control group (b) (E: Exocrin pancreas, L: Islets of Langerhans, Triple staining).*

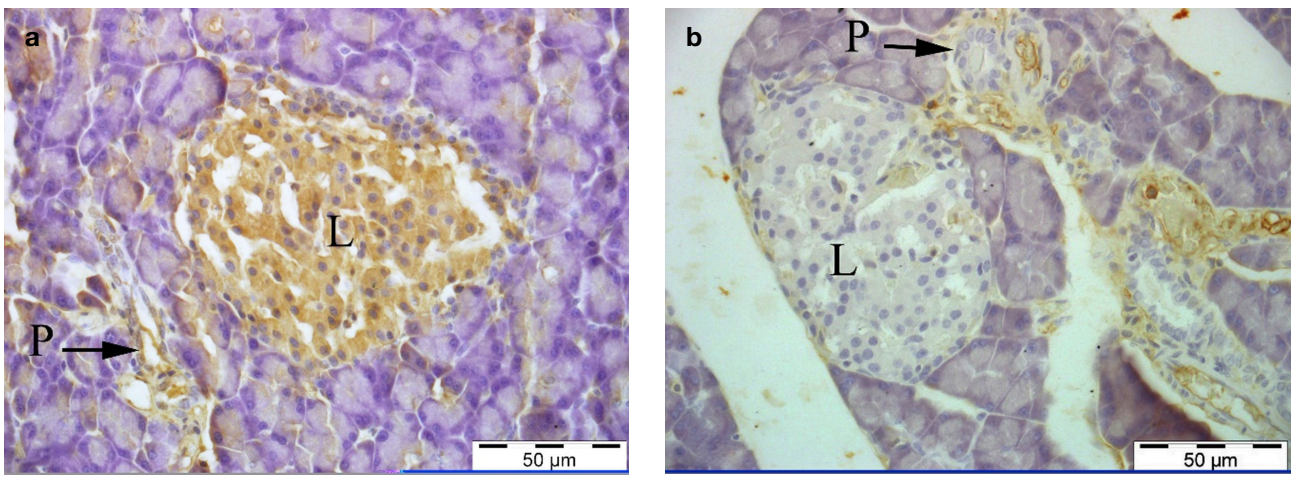

Figure 2. *a, b. Ghrelin immunoreactivity of the control group (a). Ghrelin immunoreactivity of the Melatonin group (b) (L: Islets of Langerhans, P: Pars initialis).*

PBS and left at room temperature for 30 minutes. After the repeated washing process, the sections were incubated for 30 minutes by administering streptavidin peroxidase. After washing with PBS again, chromogen was administered diaminobenzidine (DAB (Thermo TA-125-HD)). After administering chromogen solution to the sections, the reaction was stopped with distilled water in a controlled manner according to the formation of immunoreactivity. As a result of the dehydration and clearing processes by administering hematoxylin as a counter dye to the prepared tissues, the sections were closed with a coverslip with the help of entellan.

The prepared slides were examined under a light microscope and photographed. The density grades of cells with immunoreactivity were determined.

All steps were administered similarly, except for administering the ghrelin antibody to the negative control to determine the specificity of tissue ghrelin immunoreactivity.

Results

Histological Findings: The tissue samples from all three groups (melatonin, sham and control) were treated with triple staining to examine the pancreatic tissue's general histological structure. According to the evaluations, it was determined that the administration of melatonin did not cause any damage to the tissues, and the melatonin group and sham group pancreatic tissues were similar to the control group. It was observed that the structures in the endocrine and exocrine parts of the pancreas were normal in all groups (Fig. 1).

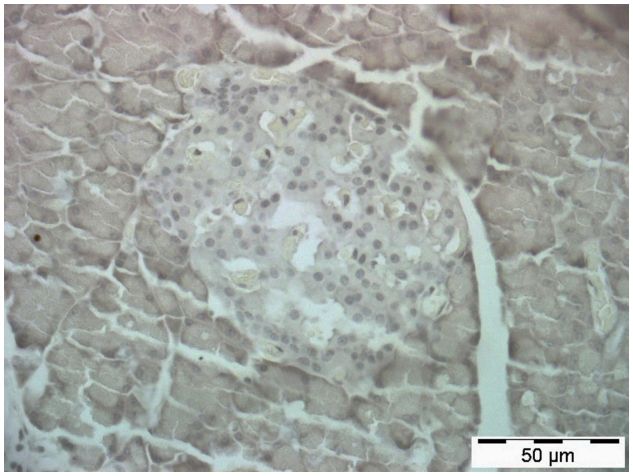

Figure 3. *Negative control of the ghrelin immunoreactivity in the pancreatic tissue.*

Immunohistochemical Findings: In our study, the ghrelin expression was examined immunohistochemically in the experimental, sham, and control groups. According to the evaluations, the immune reaction was observed in the islets of Langerhans, connective tissue areas, and pars initialis epithelium of the pancreatic sections in the control group. In the melatonin group, the ghrelin immunoreactivity was positive in the islets of Langerhans, connective tissue areas, and pars initialis epithelium of the pancreas. However, the immunoreactivity in the melatonin group was found to be weaker than in the control group. The immunohistochemical findings show that the melatonin administration suppresses the ghrelin expression (Fig. 2). It was observed that there was no immunoreaction in the negative staining of ghrelin (Fig. 3).

Discussion

It was determined that melatonin increased the activities or gene expressions of some antioxidant enzymes such as superoxide dismutase, glutathione reductase, and glutathione peroxidase and suppressed oxidative stress in this way[2]. In the oxidative damage induced by paraquat, melatonin was reported to reduce the level of lipid peroxidation product in rats[21]. According to the study of Akçay[22] (2017), melatonin administration prevents damage by increasing the antioxidant level in the gastrointestinal tract. It has a protective effect on the digestive tract canal. Yuzüak[23] (2014) reported that melatonin is a potent radical scavenger; it has an antidamage impact on the pancreas and thus protects the pancreatic tissue. The study of Javorek et al.[24] (2012) stated that the melatonin administration made to the rats exposed to acute pancreatitis and pineal gland removal by pinealectomy significantly reduced the lesions formed in the pancreatic tissue.

In our study, it was determined that the administration of melatonin did not cause any histological disorder in the pancreatic tissue of the rats, and the pancreas images obtained in all groups were in accordance (Ross and Pawlina 2011)[25].

Bianchini et al.[26] (2012) reported that ghrelin immunoreactivity was found in the duodenal epithelium in the sleeve gastrectomy they made in Wistar rats. In a study conducted by Akbalik[27] (2019), the ghrelin immunoreactivity in the gastrointestinal system of Partridge (Alectoris chukar) was in the epithelial layer of the digestive tract. In many studies conducted in rats, it was reported that there was a strong ghrelin immunoreaction in the alveolar and duct epithelial cells of the mammary tissue[28,29]. A study conducted by Tanaka et al.[30] (2005) reported that the ghrelin immunoreactivity was intense in the lamina muscularis layer of the human stomach.

The immunoreactivity of ghrelin in pancreatic tissue was evaluated in many organisms. Some of these studies are in humans, rats[15], mice, fish, birds[31], chickens[32], geese[33], and frogs[34]. According to the results of immunohistochemistry performed by Raghay et al.[35] (2013) in rat pancreas, it was reported that ghrelin immunoreactivity was found in islet cells in the center of the pancreas. According to the immunohistochemical study conducted by Wang et al.[31] (2017) in the pancreas of African ostrich chicks (Struthio camelus), they stated that the immunoreactivity of ghrelin was positive in both pancreatic islets and acinar cell regions. It was determined that the reaction was most intense in the islets of Langerhans and the areas close to it. Wang et al.[31] (2017) stated that the ghrelin immunoreactivity was also found in the ducts of the exocrine pancreas. In a study conducted on rats, Sönmez[36] (2017) reported that melatonin had a suppressive effect on the level of ghrelin in the serum.

Our study evaluated the ghrelin immunoreactivity in the pancreatic tissue of rats treated with tonin. It was determined that the findings obtained were under the studies carried out. In the control group, the immune reaction was detected in the islets of Langerhans, connective tissue areas, and pars initialis epithelium of the pancreatic sections. In the melatonin group, it was determined that the ghrelin immunoreactivity was positive in the islets of Langerhans, connective tissue areas,

and pars initialis epithelium of the pancreas. Still, the immunoreactivity in the melatonin group was weaker than in the control group. Our findings were observed to be parallel with Sönmez's[36] (2017) data, which showed that the melatonin administration suppressed the ghrelin expression.

Melatonin administration suppresses oxidative stress by increasing antioxidant enzyme activities and gene expressions. It is also known that increasing the antioxidant level prevents damage to various tissues of the organism. It was observed that melatonin administration did not cause histological deterioration in the pancreatic tissue. It was determined that the ghrelin immunoreactivity was suppressed. The ghrelin triggers insulin release in the pancreas. It is also known to lower the plasma glucose level. Therefore, we think more detailed studies should be done on this subject.

Conflict of Interest

The authors declare no conflict of interest regarding the publication of this manuscript.

References

1. Liebmann PM, Wölfler A, Felsner P, Hofer D, Schauenstein K. Melatonin and the immune system. İnt. Arch. Allergy. İmmunol. 1997;112:203–211.
2. Reiter RJ, Tan D, Osuna C, Gitto E. Actions of melatonin in the reduction of oxidative stress. J. Biomed Sci. 2000;7:444–458.
3. Konturek SJ, Konturek PC, Brzozowska I, Pawlik M, Sliwowski Z, Czesnikiewicz-Guzik M, et al. Localization and biological activities of melatonin in intact and diseased gastrointestinal tract (GIT). J Physiol Pharmacol. 2007;58:381–405.
4. Bubenik GA. Localization, physiological significance and possible clinical implication of gastrointestinal melatonin. Biol Signals Recept. 2001;10:350–366.
5. Ebelt H, Peschke D, Bromme HJ, Morke W, Blume R, Peschke E. Influence of melatonin on free radicalinduced changes in rat pancreatic beta-cells in vitro. J Pineal Res. 2000;28:65–72.
6. Kojima M, Hosada H, Date Y. Ghrelin is a growth hormone releasing acylated peptide from stomach. Nature. 1999;402:656–660.
7. Kukol A. Ghrelin Vitamins and Hormon, The Structure of Ghrelin. 2008;77–79.
8. Kojima M, Kangawa K. Ghrelin: structure and function. Physiol. 2005;85:495–522.
9. Ariyasu H, Takaya K, Tagami T, Ogawa Y, Hosoda K, Akamizu T, et al. Stomach is a major source of circulating ghrelin, and feding state determines plasma ghrelin-like immunoreactivity levels in humans. J Clin Endocrinol Metab. 2001;86:4753–4758.
10. Fujimiya M, Asakawa A, Ataka K, Chen CY, Kato I, Inui A. Ghrelin, des-acyl ghrelin, and obestatin: regulatory roles on the gastrointestinal motility. Int J Pept. 2010;
11. Hosoda H, Kojima M, Matsuo H, Kangawa K. Purification and characterization of rat desGln14-Ghrelin, a second endogenous ligand for the growth hormone secretagogue receptor. J Biol Chem. 2000;275:21995–2000.
12. Sakata I, Nakamura K, Yamazaki M, Matsubara M, Hayashi Y, Kangawa K, et al. Ghrelin-producing cells exist as two types of cells, closed- and opened-type cells, in the rat gastrointestinal tract. Peptides. 2002;23:531–536.
13. Cowley M, Smith R, Diano, Tschop M, Pronchuk N, Stasburger C, et al. The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. Neuron. 2003;37:649–661.
14. Korbonits M, Bustin SA, Kojima M, Jordan S, Adams EF, Lowe DG, et al. The expression of the growth hormone secretagogue receptor ligand ghrelin in normal and abnormal human pituitary and other neuroendocrine tumors. J Clin Endocrinol Metab. 2001;86:881–887.
15. Date Y, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, et al. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. Endocrinology. 2000;141:4255–4261.
16. Volante M, Allia E, Gugliotta P, Funaro A, Broglio F, Deghenghi R, et al. Expression of ghrelin and of the GH secretagogue receptor by pancreatic islet cells and related endocrine tumors. J Clin Endocrinol Metab. 2002;87:1300–1308.
17. Chanoine JP, Wong AC. Ghrelin gene expression is markedly higher in fetal pancreas compared with fetal stomach: effect of maternal fasting. Endocrinology. 2004;145:3813–3820.
18. Adeghate E, Ponery AS. Ghrelin stimulates insulin secretion from the pancreas of normal and diabetic rats. Journal of neuroendocrinology. 2002;14(7):555–560.
19. Lee HM, Wang G, Englander EW, Kojima M, Greeley GH. Ghrelin, a new gastrointestinal endocrine peptide that stimulates insulin secretion: enteric distribution, ontogeny, influence of endocrine, and dietary manipulations. Endocrinology. 2002;143(1):185–190.
20. Broglio F, Arvat E, Benso A, Gottero C, Muccioli G, Papotti M, et al. Ghrelin, a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans. The Journal of Clinical Endocrinology & Metabolism. 2000;86(10):5083–5083.
21. Melchiorri D, Reiter RJ, Attia AM. Potent protective effect of melatonin on in vivo paraquat-induced oxidative damage in rats, Life Sci. 1994;56:83–89.
22. Akçay GB. Stretozotosin İle Diyabet Oluşturulan Sıçanlarda Bağırsak Dokusunda Gelişen Oksidan Hasara Karşı Melatonin Tedavisinin Olası Koruyucu Etkilerinin İncelenmesi, Marmara Üni., Sağlık Bilimleri Enstitüsü, Farmakoloji Anabilim Dalı, İstanbul, 2017.

23. Yüzüak H. Melatoninin streptozotosin kaynaklı deneysel diyabet üzerine olası koruyucu etkisi. Klinik ve Deneysel Araştırmalar Dergisi. 2014;5(4):592–598.
24. Jaworek J, Szklarczyk J, Jaworek AK, Nawrot-Porąbka K, Leja-Szpak A, Bonior J, et al. Protective Effect of Melatonin on Acute Pancreatitis. International Journal of Inflammation. 2012;1–8.
25. Ross H, Wojciech Pawlina. Histology A Text and Atlas, Histoloji. ISBN 978–0–7817–7200–6, 2011.
26. Bianchini M, Fantazzinni TR, Vieira DSC, Teive AM, Costa A. Quantitative immunohistochemical analysis of duodenal ghrelin cells after sleeve gastrectomy in Wistar rats, Acta Cirurgica Brasileira. 2012;27(9):535 599.
27. Akbalık ME. Keklik (Alectoris chukar) Bağırsağında Ghrelin, Leptin ve Obestatin Dağılımı. Dicle Üniv Vet Fak Derg. 2019;12(2):117–121.
28. Grönberg, M, Tsolakis AV, Magnusson, L, Janson ET, Saras J. İnsan Dokularında Obestatin ve Ghrelin Dağılımı. Histokimya ve Sitokimya Dergisi. 2008;56(9);793–801.
29. Zhang W, Zhang Z, Chen J, Tong D. Ghrelin is Expressed in the Pregnant Mammary Glands of Dairy Goats and Promotes the Cell Proliferation of Mammary Epithelial Cells. Gen Comp Endocrinol. 2018;260:115–124.
30. Tanaka-Shintani M, Watanabe M. Distribution of ghrelin-immunoreactive cells in human gastric mucosa: comparison with that of parietal cells. J Gastroenterol. 2005;40:345–349.
31. Wang J, Li P, Zhang XT. Distribution and developmental changes of ghrelin-immunopositive cells in the pancreas of African ostrich chicks (Struthio camelus) . Poultry science. 2017;96(9):3445–3451. PMID:28595319.
32. Wei FM, Li YL, Ye Y, Zhang YJ, Jiang QY. Localization and development of ghrelin-immunopositive cells in periphery organs of broiler chickens. Acta Vet. Zootech. Sin. 2010;41:341–346.
33. Fang FG, Li MQ, Tao Y, Jiang SD, Li FB. Immunohistochemical localization of ghrelin in the liver and pancreas of adult Wanxi white goose. Chin. J. Anat. 2008;31:28–30.
34. Suzuki H, Yamamoto T. Ghrelin-and growth hormone secretagogue receptor- immunoreactive cells in Xenopus pancreas. Regul. Pept. 2011;169:64–69.
35. Raghay K, Gallego R, Scoazec JY, Garcia-Caballero T, Morel G. Different ghrelin localisation in adult human and rat endocrine pancreas. Cell Tissue Res. 2013;352:487–494.
36. Sönmez S. Sıçanlarda Pinealektomi ve Melatonin Uygulamasının Serum Melatonin, Nesfatin-1 ve Ghrelin Düzeylerine Etkisi. Selçuk Üni. Sağlık Bilimleri Enstitüsü, Yüksek Lisans Tezi, Konya, 2017.