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Evaluation of the effects of melatonin administration intraperitoneally on rats with acute pancreatitis induced by ductal ligation

Duktal ligasyon tekniğiyle oluşturulmuş akut pankreatitli sıçanlarda intraperitoneal melatonin enjeksiyonunun etkisinin değerlendirilmesi

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Background/aims: *The aim of this study was to address the protective effects of exogenous melatonin injections intraperitoneally on the histopathological changes in a model of obstructive acute pancreatitis.* ***Methods:*** *In this study, ductal ligation technique was used in 20 adult male Wistar Albino rats to develop a model of obstructive acute pancreatitis and beginning pancreatic atrophy. Melatonin 20 mg/kg solution was injected in one group of rats intraperitoneally for one week and results were compared with the control group regarding histopathological findings in the pancreatic tissue.* ***Results:*** *The apoptosis rate in control group rats was 30.5%, while it was 12.7% in the melatonin group. Pancreatic edema, hemorrhage and polymorphonuclear leukocyte infiltration decreased remarkably in the melatonin group compared to the control group.* ***Conclusions:*** *Injection of exogenous melatonin in rats with obstructive acute pancreatitis for seven days prevents acinar cell degeneration, apoptosis and polymorphonuclear leukocyte infiltration.*

Key words: Acinar cell degeneration, apoptosis, hemorrhage, melatonin treatment, obstructive acute pancreatitis, edema

Amaç: *Bu çalışma obstruktif akut pankreatit modelinde intraperitoneal olarak enjekte edilen ekzojen melatoninin histopatolojik değişiklikler üzerindeki koruyucu etkisini incelemek amacıyla gerçekleştirilmiştir.* ***Yöntem:*** *Bu çalışmada 20 erişkin Winstar Albino rat kullanılarak obstruktif akut pankreatit ve pankreatik atrofi modeli için pankreatikobilier kanal ligasyon tekniği uygulanmıştır. Bir grup rat'ta bir hafta süreyle intraperitoneal olarak 20 mg/kg melatonin solusyonu enjekte edildi ve pankreas dokusundaki histopatolojik değişiklikler yönünden kontrol grubu ratlarla karşılaştırıldı.* ***Bulgular:*** *Apoptozis görülme oranı kontrol grubunda %30.5 olduğu halde çalışma grubunda %12.7 bulunmuştur. Pankreatik ödem, kanama ve polimorf nükleer lökosit infiltrasyonu da kontrol grubuna gore melatonin grubunda daha az görülmüştür.* ***Sonuç:*** *Obstruktif akut pankreatitli ratlarda 7 gün süreyle ekzojen melatonin enjeksiyonu asiner hücre dejenerasyonu, apoptosis ve polimorf nükleer hücre infiltrasyonunu azaltmaktadır.*

Anahtar kelimeler: Asiner hücre dejenerasyonu, apoptozis, kanama, melatonin tedavisi, obstruktif akut pankreatit, ödem

INTRODUCTION

Acute pancreatitis has been defined as a disease that develops with perivascular infiltration and inflammation characterized by lipid necrosis, polymorphonuclear leukocyte (PMNL) infiltration, hemorrhage, acinar cell necrosis, and tissue edema in the pancreas. The pathogenesis of acute pancreatitis is complicated and unclear. Generally, it is believed that there is a close relationship between the modes of inflammatory reaction of the macrophages and acinar cell death. Acute pancreatitis still carries a high morbidity and mortality rate. It can be represented on a spectrum ranging from a self-limited course requiring only short hospitalization and medical treatment to a fulminant illness resulting in multiple organ failure with accompanying sepsis. The prognosis depends upon the degree of infected pancreatic necrosis and septic shock. There have been important changes in the management of acute pancreatitis over the last two decades. In patients with acute pancreati-

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tis without severe necrosis, the mortality rate of non-operative medical treatment has now been shown to be better than that of radical surgical procedures.

Melatonin is the main product of the pineal gland and is also secreted in the intestines. It is a hormone that affects the general immune system and cellular immunity both directly and indirectly. Melatonin is secreted diurnally. It can overcome all morphophysiological barriers in the human body and reach the intercellular area in all organs and cell nuclei easily due to its lipophilic and hydrophilic features (1).

Presently, it is accepted that melatonin shows an outstanding functional versatility, by exhibiting antioxidant, antiinflammatory and immunomodulatory properties. Inflammatory cytokines are produced in large amounts during acute pancreatitis. Melatonin downregulates production of inflammatory cytokines and protect animals from septic complications of acute pancreatitis. During the last decade, some valuable research in animal models has been published, emphasizing the importance of melatonin effects on acute pancreatitis (2-4).

The aim of this study was to address the protective effects of exogenous melatonin injections on the histopathological changes in acute pancreatitis.

MATERIALS AND METHODS

Animals

Twenty adult male Wistar Albino rats (average weight 200-250 g) were used in this study and were separated into two groups of 10. Ethical approval was obtained from the Animal Care Ethics Committee of Abant Izzet Baysal University. During the study, all the experimental animals were housed at standard room temperature (22°C) in a 12-h light/dark cycle and were fed with standard pellet rat food (210 kcal/100 g/day) and tap water.

In the rats in Group I (Control group), only experimental acute pancreatitis was conducted and they were not exposed to any medical applications. In Group II (Melatonin group), in order to eliminate the damages in the organisms caused by acute pancreatitis, melatonin (Sigma, St. Louis, MO, USA) 20 mg/kg/day solution was injected intraperitoneally every day over the period of one week between the hours of 18.00-20.00.

Surgical Procedures

Surgical ductal ligation was used in order to develop the obstructive acute pancreatitis model. This technique (ligation of the pancreatic duct) is not only a model of obstructive acute pancreatitis, but is also used as a model of pancreatic atrophy. All surgical procedures performed on the experimental animals were carried out on the same day under general anesthesia and standard sterile conditions. In all test subjects, a single intramuscular dose of 2.5 mg/kg Enrofloksasin (Baytril 10%, Bayer; Istanbul) injection was administrated as prophylactic antibiotic. For general anesthesia, 20 mg/kg ketamine HCl (Ketalar, Eczacibasi; Istanbul) intramuscular and 5 mg/kg xylazine HCl (Alphazin, Rompun Bayer) intramuscular were used.

Histopathological Evaluation

After re-anesthesia, laparotomy was performed in the test subjects at the end of the 7th day and pancreatic tissue samples were taken. The proximal part of the pancreas was used for histopathological evaluations. The samples taken were kept in 10% formaldehyde for 48 hours of fixation and then dehydrated using a graded series of ethyl alcohol, cleared in xylol and embedded in paraffin. The paraffin block was trimmed and 4 Ìm thick histological sections were taken. We used a system of visual evaluation of the histopathological changes induced to quantitatively describe acinar tissue damage. Samples were examined under light microscope after being stained with hematoxylin-eosin (H&E). Using the histological scoring described below, acinar cell degeneration, apoptosis, PMNL infiltration, edema, and hemorrhage in pancreatic tissue were recorded (5).

Acinar cellular degeneration scoring method: [0] points: no acinar cell degeneration in the pancreas, [1] point: acinar cell degeneration present in less than 15% of the cells in each section of the pancreas, [2] points: rate of acinar cell degeneration in the pancreas between 15 and 35%, and [3] points: rate of acinar cell degeneration in the pancreas >35%.

Apoptosis: In order to assess the apoptosis in acinar cells in the pancreas, taking 10 areas in each tissue section, the number of cells with clear apoptotic bodies was measured using a X40 objective, and the percentage of apoptotic acinar cells was recorded.

PMNL infiltration scoring method: [0] points: no leukocyte infiltration, [1] point: mild leukocyte infiltration in perivascular area, [2] points: moderate leukocyte infiltration in perivascular area, and [3] points: presence of acute and pervasive leukocyte infiltration in all areas.

Pancreatic edema scoring method: [0] points: no edema in the pancreas, [1] point: interlobular edema, [2] points: interlobular edema and mild intralobular edema, and [3] points: presence of both interlobular edema and acute intralobular edema.

Pancreatic hemorrhage scoring method: [0] points: no hemorrhage in the pancreas, [1] point: 1-2 hemorrhagic areas in each section in pancreas, [2] points: 3-5 hemorrhagic areas in each section in the pancreas, and [3] points: more than 5 hemorrhagic areas in each section in the pancreas.

Statistical Analysis

The statistical package for the Social Sciences (SPSS) for Windows, Version 11.0 (SPSS, Chicago, IL, USA) was used for data analysis. Kruskal-Wallis variance analysis and Mann- Whitney U tests were performed for statistical comparisons; a value of $p<0.05$ was accepted as statistically significant.

RESULTS

The microscopic appearance of normal pancreatic tissue of healthy rats that has not undergone any kind of operation is given in Figure 1. With the help of H&E and a X40 objective, it was observed that the structure of the lobules in the pancreatic sections was preserved quite well. The basal parts of the acinar cells were darker in color, while the parts on the nucleus were a relatively lighter color. Moreover, the acinar lumens and secretion ducts could be discerned clearly.

In the test subjects of the first group (control group) in which obstructive acute pancreatitis was formed through ductal ligation, serious edema in the pancreatic tissue and peripancreatic areas was observed macroscopically during the exploration done through relaparotomy (Figure 2). In the microscopic investigation of the pancreatic tissues of the rats in this group, it was observed that the lobules in the parenchyma were separated from each other, there was prevalent edema in the tissue, there were hemorrhagic areas in certain parts, and there was common PMNL infiltration in all parts (Figure 3). Edema in the pancreas was found to be serious in the lobules in certain rats, while it was serious between the lobules in others. The edema in the pancreatic tissue was quite serious either in or between the lobules in the test subjects. Likewise, PMNL infiltration was observed insularly between the lobules, whereas it was diffuse in the whole pancreatic tissue.

The apoptosis and acinar cell degeneration scores in the rats of the control group and the melatonin group are shown in Table 1. In some of the rats of the control group, low rates of acinar cell degeneration were observed. The acinar cell degeneration rate observed in this group was lower than 15%. On the other hand, no acinar cell degeneration was observed in the rats of the melatonin group. Comparing the test subjects for apoptosis rates, it was observed that the apoptosis rate in the control

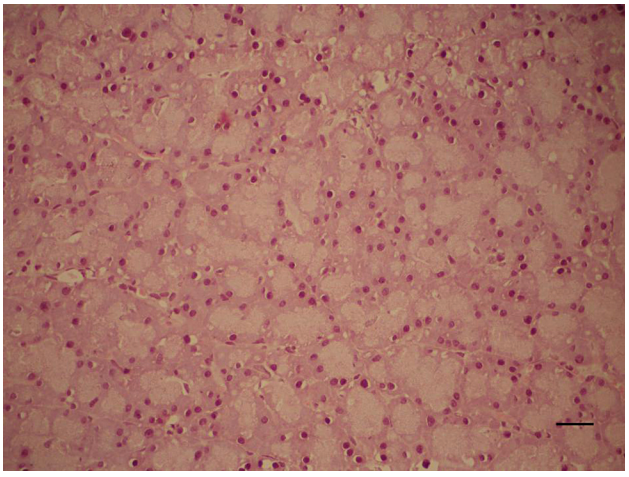

Figure 1. Light microscopic view of normal pancreatic tissue in rats (H&E x40).

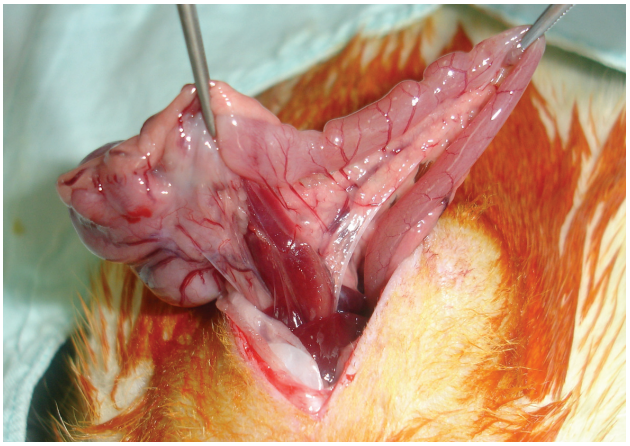

Figure 2. In test subjects following ductus ligation, certain findings regarding dilatation of choledochus and macroscopic edema in pancreatic tissue are seen.

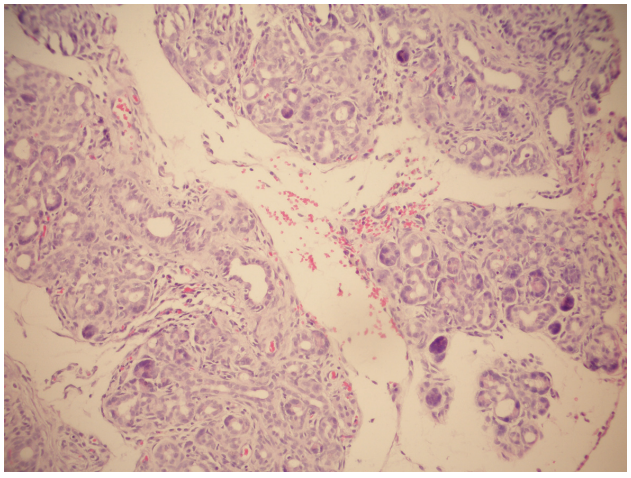

Figure 3. The microscopic view of the pancreas in the rats of the first group showing clear PMNL infiltration and edema, acinar dilatation in lobules, ductal transformations, and the hemorrhagic areas between the lobules (H&Ex20).

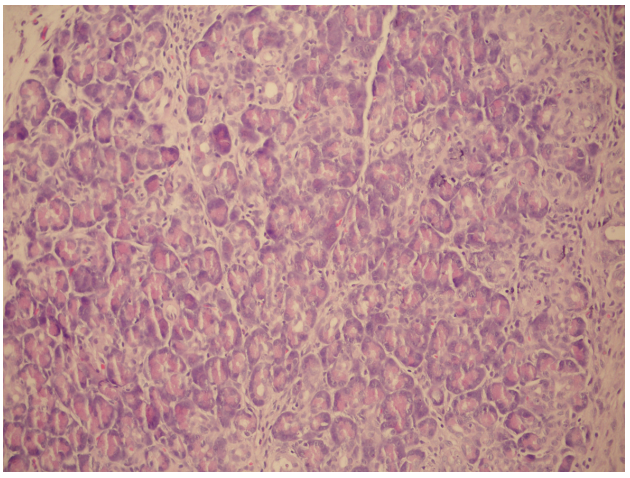

Figure 4. The view of the clear regression in pancreatitis in test subjects with exogenous melatonin treatment. A considerable increase was observed in the pancreatic connective tissue (H&Ex40).

group rats was 30.5%, while it was 12.7% in the melatonin group rats. The difference observed in the acinar cell degeneration and apoptosis rates of the two groups was statistically significant (t: 2.8, p=0.013 for acinar cell degeneration, t: 3.72, p=0.002 for apoptosis).

It was also observed that the acinar lumens in the control group rats were dilated to a great extent, that they had formed ductal transformations, and that there were hemorrhagic areas in some parts between the lobules. In the rats of this group, there was leukocyte infiltration dominated by neutrophils and their lumens were excessively dilated (Figure 3). In some test subjects, the acinar epithelium cells were flattened due to excessive dilatation in the lobules, which occurred because of edema and PMNL infiltration. Because of excessive edema in the pancreas, the islets of Langerhans were isolated and separated from the acini.

Nevertheless, it was observed that there were no structural defects in a considerable number of the test subjects, and that the normal view was maintained. In addition to these histological findings, a notable increase in the number of apoptotic bodies in the acinar cells was observed, especially in the subjects of the first group due to acute pancreatitis.

In the melatonin group, edema and PMNL infiltration were remarkably decreased when compared to the first group. Hemorrhagic areas were minimal and there was no acinar cell degeneration. However, ductal transformation also continued in this group. In the test subjects of this group, apoptotic bodies in the acinar cells were remarkably decreased when compared to the other group, and mitosis was not detected in the acinar cells (Figure 4). It was determined that the connective tissue between the lobules became more evident in certain parts and that the number of cells that had undergone apoptosis decreased.

The scores of PMNL infiltration, edema and hemorrhage in the pancreatic tissues of the control group (acute pancreatitis model) and the melatonin group (acute pancreatitis + melatonin) are shown in Table 1.

The PMNL infiltration score in the pancreatic tissue of the control group subjects was 2.44±0.88, while the score decreased to 1.22±0.83 in the melatonin group subjects, and the difference between the two groups was statistically significant. As seen in Table 1, a similar decrease was also observed in the edema and hemorrhage scores. However, the differences between the two groups concerning the edema and hemorrhage scores were not statistically significant. In the control group rats, the

Table 1. Scoring of PMNL infiltration, edema, hemorrhage, acinar cell degeneration and apoptosis in test subjects (Mean ± SD)

Histopathologic findings	Group I	Group II
Score for acinar cell degeneration	0.77±0.83	Not observed
Percentage for apoptosis	30.55±11.5	12.77±8.4
Score for PMNL infiltration	2.44±0.88	1.22±0.83
Score for edema	1.55±0.52	1.00±0.7
Score for hemorrhage	0.55±0.72	0.22±0.4

edema score was approximately 1.55±0.52, while the hemorrhage score was 0.55±0.72. In the second group, these scores were 1.0±0.7 and 0.22±0.4, respectively (t: 3.02, p=0.008 for PMNL infiltration, t: 1.89, p=0.07 for edema, t: 1.17, p=0.25 for hemorrhage).

DISCUSSION

Acute pancreatitis is a disease followed by interstitial edema at different stages, PMNL infiltration, hemorrhage, and acinar cell degeneration (6). The isolation of islet of Langerhans due to edema, dilatation and apoptotic bodies in the acini can be seen microscopically.

Acute pancreatitis develops with the effect of active lysosomal enzymes or the autoactivation of trypsinogen. The zymogens that are activated by trypsin lead to tissue damage (7). Melatonin is a hormone with a strong antioxidant effect and it is a free radical scavenger. It thusly protects the cells from oxidative damage and decreases tissue inflammation (8).

Many studies have been conducted that show the mechanism by which melatonin decreases apoptosis, and how apoptosis affects the physiopathology of acute pancreatitis. Kaiser et al. (9) stated that in acute pancreatitis, apoptosis develops as a consequence of obstruction of the pancreatic ducts, and that apoptosis causes atrophy in pancreatic tissue. It has also been asserted that apoptosis that develops in an acute pancreatitis model formed through ductal ligation is in fact a defense mechanism. It is also believed that apoptosis decreases enzyme secretion by causing atrophy in the pancreas, and thus it decreases the enzymatic activities of the pancreas (9, 10).

Fujimoto et al. (11) viewed the apoptosis in pancreas acinus cells microscopically at the 24th hour in their ischemia-reperfusion studies. In another ischemia-reperfusion study, protective and therapeutic applications of melatonin were tested. In that study, it was demonstrated that melatonin has a cytoprotective effect over oxidative stress and pancreatic tissue damage (12). In the current study, it was determined that in rats treated with melatonin, acinar cell degeneration could be prevented completely and apoptosis could be partly prevented (Table 1).

Apoptosis is a normal physiological occurrence, and this term describes the programmed and controlled cell death that occurs as a result of nucleus fraction characterized by DNA damage. Taking into consideration the findings in Table 1, the statistically significant difference between the control group and the melatonin group was interpreted as the prevention of apoptosis by exogenous melatonin in acute pancreatitis.

The cells in living organisms die because of two main mechanisms, apoptosis and necrosis, due to their different effects. Apoptosis is a form of programmed removal of superfluous, infected, transformed, or damaged cells that have lost their functions and are no longer needed by the organism by activation of an intrinsic suicide program (13). Necrosis is, however, a pathological cell death in which the cells die due to physical and chemical effects such as hypoxia, excessive temperature change and toxins. Apoptosis and necrotic cell death are irreplaceable. In contrast to necrosis, in apoptosis, no inflammation occurs in the tissue due to cell death (13, 14).

At the onset of apoptosis, nucleus chromatin degrades and the nucleus membrane fractions. Due to an increase in cytoplasmic proteins, shrinkage is observed in cells. Endoplasmic reticulum dilates and accretes with the cell membrane, forming crater-like structures on the cell surface. In this stage, the contraction of the cell membrane increases and the cell mangles. These small round structures surrounded with membrane are called “apoptotic bodies” (15). Phagocytosis of apoptotic bodies is speedily performed by the macrophages. As intracellular macromolecules cannot detach from the cell, apoptosis does not lead to any inflammatory reaction. Apoptotic reactions occur within the cell and do not damage the neighboring cells (15).

Injection of exogenous melatonin can cause the same effect as endogenous melatonin (16). While secreted mainly in the pineal gland, melatonin can also be secreted in the intestines and the retina in small amounts. Melatonin is found in different concentrations in certain tissues and systems in an organism. Konturek et al. (17, 18), in their immunoreactive studies conducted with ^{125}I-melatonin, determined that the amount of melatonin secreted in the pineal gland is 20 times more than that secreted in the intestines.

The pineal gland is located in the hypothalamus and works in a manner similar to a body clock along with the suprachiasmatic nucleus and secretion of the melatonin hormone. The secretion function of the pineal gland is maintained by two en-

dogenous substances, the indolamines and peptides. At night, a high amplitude of secretion is observed. Even though it has been found that melatonin is secreted in organs other than the pineal gland, such as the retina and intestines, the amount of melatonin secreted in these organs is minimal and has little effect on the general blood melatonin level (1).

In conclusion, we determined that there were differences in PMNL infiltration, acinar cell degeneration and apoptosis, but not in edema and hemorrhage, in the melatonin-treated group. We concluded in this study that injection of exogenous melatonin in rats with acute pancreatitis for 7 days prevented PMNL infiltration, acinar cell degeneration and apoptosis. Taking these findings into consideration, it was found that injection of exogenous melatonin in rats with acute pancreatitis has a protective effect on tissue damage. However, further studies are required concerning the use of melatonin in the treatment of acute pancreatitis.

REFERENCES

1. Arendt J. Melatonin and the pineal gland: influence on mammalian seasonal and circadian physiology. Rev Reprod 1998; 3: 13-22.
2. Gülben K, Ozdemir H, Berberoğlu U, et al. Melatonin modulates the severity of taurocholate-induced acute pancreatitis in the rat. Dig Dis Sci 2010; 55: 941-6.
3. Alhan E, Kalyoncu NI, Kural BV, Erçin C. Effects of melatonin on acute necrotizing pancreatitis in rats. Z Gastroenterol 2004; 42: 967-72.
4. Cöl C, Dinler K, Hasdemir AO, Bugdayci G. The effect of an intraperitoneal injection of melatonin on serum amylase levels in acute pancreatitis. JOP 2009; 10: 306-9.
5. Skandalakis LJ, Rowe JS, Gray SW, Skandalakis JE. Surgical embryology and anatomy of the pancreas. Surg Clin North Am 1993; 73: 661-97.
6. Sanfey H, Bulkley GB, Cameron JL. The pathogenesis of acute pancreatitis. The source and role of oxygen-derived free radicals in three different experimental models. Ann Surg 1985; 201: 633-9.
7. Banks PA, Freeman ML. Practice Parameters Committee of the American College of Gastroenterology. Practice guidelines in acute pancreatitis. Am J Gastroenterol 2006; 101: 2379-400.
8. Bulbuller N, Dogru O, Umac, et al. The effects of melatonin and pentoxiphylline on L-arginine induced acute pancreatitis. Ulus Travma Acil Cerrahi Derg 2005; 11: 108-14.
9. Kaiser AM, Saluja AK, Sengupta A, et al. Relationship between severity, necrosis, and apoptosis in five models of experimental acute pancreatitis. Am J Physiol 1995; 269: 1295-304.
10. Walker NI. Ultrastructure of the rat pancreas after experimental duct ligation. The role of apoptosis and intraepithelial macrophages in acinar cell deletion. Am J Pathol 1987; 126: 439-51.
11. Fujimoto K, Hosotani R, Wada M, et al. Ischemia-reperfusion injury on the pancreas in rats: identification of acinar cell apoptosis. J Surg Res 1997; 71: 127-36.
12. Muñoz-Casares FC, Padillo FJ, Briceño J, et al. Melatonin reduces apoptosis and necrosis induced by ischemia/reperfusion injury of the pancreas. J Pineal Res 2006; 40: 195-203.
13. Ozturk F. Apoptosis. J Inonu University Medical Faculty 2002; 9: 2.
14. Carson DA, Ribeiro JM. Apoptosis and disease. Lancet 1993; 341: 1251-4.
15. Buja LM, Eigenbrodt ML, Eigenbrodt EH. Apoptosis and necrosis. Basic types and mechanisms of cell death. Arch Pathol Lab Med 1993; 117: 1208-14.
16. Jaworek J, Nawrot-Porabka K, Leja-Szpak A, et al. Melatonin as modulator of pancreatic enzyme secretion and pancreatoprotector. J Physiol Pharmacol 2007; 6: 65-80.
17. Konturek SJ, Konturek PC, Brzozowski, et al. Role of melatonin in upper gastrointestinal tract. J Physiol Pharmacol 2007; 6: 23-52.
18. Konturek SJ, Konturek PC, Brzozowska I, et al. Localization and biological activities of melatonin in intact and diseased gastrointestinal tract (GIT). J Physiol Pharmacol 2007; 58: 381-405.