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EFFECTS OF IN VIVO / IN VITRO MELATONIN APPLICATION ON THE DUODENUM IN RATS WITH EXPERIMENTAL HYPERTHYROIDISM

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Abstract: The aim of this study was to investigate the effects of melatonin on the intestinal motility of hyperthyroidism rats. Therefore, we determined *in vivo* and *in vitro* effects of melatonin on duodenal tissue in experimental hyperthyroid rats. 34 Wistar-Albino male rats were fed with physiological conditions, and then euthanized by cervical dislocation. The experimental animals, Group 1: Control group (n=5), Group 1B: Melatonin group *in vitro* (n=5), Group 1C: Melatonin group *in vivo* (n=6), Group 2: 2A: Hypertension group (n=6), 2B: Group 2: Hyperthyroidi sm *in vitro* melatonin group (n=6), 2C: Hyperthyroidism *in vivo* melatonin group (n=6). Acetylcholine (ACh, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2} M), potassium chloride (KCl, 20, 40, 60, 80, 100 mM) at the end of the incubation period different doses were given to the bathing environment. In *in vitro* melatonin groups, the determined submaximal doses (ACh 10^{-4} M, KCl 60 mM) and melatonin at different doses (Mel 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M) were applied. It was determined that the contraction responses of the isolated duodenal tissues induced by KCl and ACh increased significantly ($p<0.001$) in experimental rats with hyperthyroidism. In the same way, it was found that in the groups treated with melatonin *in vivo*, there was a significant ($P<0.001$) increase in the contraction responses compared to those of control group in the isolated tissue. It was found that hyperthyroidism significantly decreased the contraction responses compared with the hyperthyroidism melatonin treated groups *in vivo*. In melatonin groups, responses to different logarithmic doses of melatonin administered with subcutaneous doses of KCl and ACh were evaluated. According to the findings, contraction responses to different doses of melatonin were found to vary significantly. It was determined that *in vivo* administration of melatonin on intestinal motility decreased the contraction responses in experimental hyperthyroidism induced rats. Melatonin given in the bath environment *in vitro* was found to increase or decrease contraction responses on intestinal motility significantly in different doses. Melatonin is thought to be a positive effect of on intestinal motility.

Keywords: Hyperthyroidism, Acetylcholine, Duodenum, KCl, Melatonin, Rat

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1. Introduction

Hyperthyroidism is a condition where the thyroid gland produces too much thyroid hormone, leading to high levels of thyroid hormone in the bloodstream and low levels of thyroid-stimulating hormone (TSH). This causes the thyroid gland to grow larger than average, and increases the body's metabolism, leading to symptoms such as increased excitability, heat intolerance, sweating, weight loss, muscle weakness, psychological and nervous disorders, excessive fatigue, inability to sleep, tremor in hands, and endophthalmitis. Additionally, the increased metabolism also causes an increased oxygen consumption rate (Shahid et al., 2020). Gastrointestinal symptoms are common in both hypothyroidism and hyperthyroidism. Although the number of daily feces may be average in hyperthyroidism, the number of bowel movements may be increased (hyper defecation). This is because the increased metabolism causes an increase in appetite and nutrient intake, as well as an increase in the rate of transit through the gastrointestinal tract, leading to diarrhea. On the other hand, in hypothyroidism, the opposite effect occurs, with decreased metabolism leading to constipation (Hall, 2011; Shahid et al., 2020).

The pineal gland is a small endocrine gland located in the brain responsible for melatonin production. Melatonin is a hormone that helps regulate our sleep-wake cycle in response to changes in light exposure. The pineal gland receives signals from the environment about light levels, and adjusts the production of melatonin accordingly. Melatonin is secreted by cells in the pineal gland called pinealocytes. It acts as an inhibitory agent on various endocrine glands, including the adenohypophysis, neurohypophysis, endocrine pancreas, adrenal cortex, adrenal medulla, parathyroid, and gonads (Aulinas, 2019). Melatonin is not only produced in the pineal gland in the brain, but it is also produced by other cells in the body. Even if the pineal gland is removed, melatonin levels will decrease but not disappear entirely. Other

cells in the body that also produce melatonin include enterochromaffin cells in the digestive tract, liver, trachea, thyroid, renal tissue, adrenal cortex, thymus, and placenta, as well as cells in the immune system, such as natural killer cells and eosinophilic leukocytes. These cells can produce melatonin to communicate with each other by locally releasing the hormone; this process is called paracrine (Touitou, 2001). Melatonin helps regulate various bodily functions, including the gastrointestinal tract. It has been found to suppress the contractions of the intestines that are induced by serotonin. It also reduces sodium absorption from the gut lining, and limits the growth of cells in the small intestine. Additionally, melatonin has been found to improve the function and regeneration of the cells that line the gastrointestinal tract, reduce the tension of the muscles in the gut, and strengthen the immune system. It also has antioxidant properties that can prevent damage to the lining of the gut, reduces the production of stomach acid, and helps to renew the surface of the gut lining (Lee and Pang, 1993; Ganguly et al., 2005; Martin et al., 2005; Moezi et al., 2010).

The incidence of hyper defecation and diarrhea increases in individuals with hyperthyroidism due to increased appetite and food intake, metabolic rate, and the rate of passage in the gastrointestinal tract. Individuals with hyperthyroidism may experience increased bowel movements (hyper defecation) and diarrhea. This is because their high metabolism increases appetite and food intake, as well as an increased rate of passage through the gastrointestinal tract. This can cause the food to move through the gut more quickly, leading to more frequent bowel movements and diarrhea (Karbownik and Lewinski, 2003; Hall, 2011).

This study aimed to investigate the effects of melatonin, a neuroendocrine inhibitory hormone, on intestinal motility in rats with experimentally induced hyperthyroidism.

2. Materials and Methods

2.1. Experimental Animals and Chemicals

Thirty-four male Wistar-Albino rats (220-240 g) were used in our study. The rats were fed 12 hours at night and 12 hours daily, with rhyming rhythm in the wireframe at 22 °C temperature and 50-60% humidity. Melatonin (CAS no: 73 - 31 - 4, Santa Cruz, ABD), $MgSO_4$ (CAS No: 7487-88-9, Fluka, ABD), other chemicals used in this study were supplied by Sigma-Aldrich (Germany).

2.2. Experimental Groups

Group 1A, Control Group (n=5): The rats in this group were injected with 0.5 ml/day intraperitoneal 0.9% isotonic NaCl for 14 days. After euthanasia, the abdomen was opened, and the duodenum was taken immediately after ostium plorikum and hung in an isolated organ bath. Acetylcholine (ACh) (10^{-7}, 10^{-6}, 10^{-5}, 10^{-4},10^{-3} and 10^{-2} M) and potassium chloride (KCl) (20, 40, 60, 80, and 100 mM) in the bath medium to determine the contraction responses of duodenal tissues of rats in this group different concentrations were applied.

Group 1B, (n=5) Control + *In vitro* Melatonin Group: The rats were fasted the night before euthanasia. 0.5 ml 0.9% isotonic NaCl was administered intraperitoneally. After cervical dislocation, the abdomen was opened and the duodenum was removed without damaging the tissues. After applying 1g of tension in the isolated organ bath, the tissue was washed at 15-minute intervals and rested for 1 hour. The submaximal doses of KCl and ACh determined in group 1A (KCl 60 mM and ACh 10^{-4} M) were repeated three times and averaged. Melatonin was dissolved in 0.5 ml 0.9% isotonic NaCl solution and administered. Dose-response curves were determined by exposure to different melatonin concentrations (Mel 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} M) after the averaged responses. After the submaximal doses were tried three times and averaged, the responses of KCl 60 mM and different melatonin concentrations were obtained. After resting the tissue for 10 minutes, the coexistence curves of different ACh 10^{-4} M concentrations and melatonin were evaluated.

Group 1C, (n=6) Control + *in vivo* melatonin Group: Intraperitoneally, 3 mg/kg/day melatonin was administered for two weeks at 21:00 at night. At the end of 14 days, after the duodenal tissue of the rat whose application was completed, 1 g of tension was applied in the isolated organ bath. KCl (20, 40, 60, 80, and 100 mM) and ACh (10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, and 10^{-2} M) to the tissue that was washed with 15 minutes intervals and rested for 1-hour different concentrations were given to the medium. Dose-response curves were determined.

Group 2A, Hyperthyroidism Group (n = 6): The rats in this group were administered 0.3 mg/kg/day L-thyroxine intraperitoneally every day for two weeks to create experimental hyperthyroidism. Different concentrations of ACh and KCl were applied to the bath medium to determine the contraction responses by taking duodenal tissues.

Group 2B, *In Vitro* Hyperthyroidism Group (n=6): The rats in this group were administered 0.3 mg/kg/day L-thyroxine intraperitoneally every day for two weeks in order to create experimental hyperthyroidism. The duodenal tissue was taken and placed in an isolated organ bath. In order to determine the contraction responses of the duodenal tissues of the rats in this group, submaximal doses of ACh and KCl and different concentrations of Mel were applied to the bath environment.

Group 2C, (n=6) Hyperthyroidism + *In Vivo* Melatonin Group: L-thyroxine hormone at a dose of 0.3 mg/kg/day intraperitoneally at 08:00 in the morning and melatonin hormone at a dose of 3 mg/kg/day at 21:00 for two weeks. At the end of 14 days, after the duodenal tissue of the rat whose application was completed, 1 g of tension was applied in the isolated organ bath. KCl (20, 40, 60, 80, and 100 mM) and ACh (10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, and 10^{-2} M) to the tissue that was washed in 15 minutes intervals and rested for 1 hour. Different concentrations were given to the medium. Dose-response curves were determined (Table 1 and 2).

Table 1. Control group, *in vivo* and *in vitro* applications

Groups	Intestinal Tissue
1. Control Group (n=16)	No experimental application was done to the rats in the control group. Melatonin was applied intraperitoneally to the rats in the 1C group for 14 days.
1A. No application can be made (n=5)	ACh - (10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} ve 10^{-2} M) KCl - (20, 40, 60, 80 ve 100 mM)
1B. *İn Vitro* melatonin application (n=5)	ACh - (10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} ve 10^{-2} M) KCl - (20, 40, 60, 80 ve 100 mM) Melatonin-(Mel 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} ve 10^{-4} M)
1C. *İn Vivo* melatonin application (n=6)	ACh - (10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} ve 10^{-2} M) KCl - (20, 40, 60, 80 ve 100 mM) (*In vivo* melatonin administration was administered intraperitoneally for 14 days)

Table 2. Hyperthyroid group, *in vivo* and *in vitro* applications

Groups	Intestinal Tissue
2. Hyperthyroidism group (n=18)	Intraperitoneal L-thyroxine injection was applied for 14 days. After the disease was created, the tissues were suspended in the isolated organ bath, while L-thyroxine was applied only in the 2C group, while melatonin was administered *in vivo* for 14 days.
2A. No application was made after hyperthyroidism was created (n=6)	ACh - (10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} ve 10^{-2} M) KCl - (20, 40, 60, 80 ve 100 mM)
2B. *In vitro* melatonin administration was performed after hyperthyroidism was created (n=6)	ACh - (10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} ve 10^{-2} M) KCl - (20, 40, 60, 80 ve 100 mM) Melatonin - (Mel 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} ve 10^{-4} M)
2C. *In vivo* melatonin application was performed while creating hyperthyroidism (n=6)	ACh - (10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} ve 10^{-2} M) KCl - (20, 40, 60, 80 ve 100 mM) (*In vivo* melatonin administration was administered intraperitoneally for 14 days)

2.3. Test Protocol

After euthanasia, the abdomens of the rats were opened along the median line. The duodenum tissues, determined after ostium pyloricum, which is the intestine of the small intestine with the stomach, were taken with the help of scissors. The isolated duodenum tissue was removed from the connective tissue around it by taking the cold tyrode solution. The lumen content of the duodenum was cleaned with a tyrode solution using an injector. After all of these procedures, sections 1-1.5 cm long were taken from the isolated duodenum tissue. The connective tissues were ligated and fixed in a 10 ml isolated organ bath, with the cleaned duodenum tissues in a continuously ventilated tyrode solution (Tyrode solution contains 119 mM NaCl, 4.75 mM KCl, 1.5 mM $MgSO_4$, 1.2 mM $NaHCO_3$, 2.5 mM $CaCl_2$, and 1.1 mM glucose) (Lucchelli et al. 1997) at 37 °C and a mixture of 5-95% carbon dioxide-oxygen. Tissue fragments were kept in a solution of tyrode every 15 minutes for 1 hour. After the adjustment period, KCl, ACh and melatonin were added into the solution in different concentrations. After each response, the tyrode solution was changed three times to reach the previous tone of the tissues by changing the solution two times, and the tissue was rested for 10 min before starting the other chemical doses.

3.4. Statistical Analysis

SPSS 24 was used to analyze the data we obtained at the end of our study. Post-hoc Tukey test was used to evaluate the results and the One Way ANOVA test. Values are given as mean (X) ± Standard deviation (SD). Values of $P<0.05$ were considered significant (Genç and Soysay, 2018).

3. Results

This study seeks how melatonin affects the duodenum (a section of the small intestine) of rats with hyperthyroidism. The researchers will be studying the effects of melatonin both inside the body (*in vivo*) and outside the body (*in vitro*) on the duodenal tissue of these rats.

Duodenal tissues isolated from rats in Control (1A), Control + *in vivo* melatonin (1C), Hyperthyroidism (2A), and Hyperthyroid + *in vivo* melatonin (2C) groups were induced with different doses of KCl. Dose-response curves by evaluating the obtained contraction responses are shown in Figure 1. It was determined that the contraction responses of the isolated duodenal tissues of the rats in the Control group were significantly lower than other groups, and the contraction responses of the duodenal tissues in the hyperthyroid group were higher

than the others. In both groups who were administered intraperitoneal melatonin for 14 days, it was found that the contraction responses of duodenal tissues were higher than the control group. However, these responses were lower than the Hyperthyroid group.

Isolated duodenal tissues in Control, Control + *in vivo* melatonin, Hyperthyroid, and Hyperthyroid + *In vivo* melatonin groups were induced with different doses of ACh. Dose-response curves are shown in Figure 2 by evaluating the contraction responses of ACh given to the isolated organ bath at different concentrations. It was determined that the contraction responses of the rats in the control group obtained from the duodenal tissues were significantly lower than the other groups, and the contraction responses of the tissues in the hyperthyroid group were higher than the other groups. The contraction responses of duodenal tissues were higher in both groups, in which *in vivo* melatonin was applied compared to the Control group. In Control + *in vivo* melatonin group, the contraction responses of the tissue were higher at low ACh concentrations (10^{-7}, 10^{-6}, and 10^{-5} M) compared to the Hyperthyroid group but lower at other doses than in the same group. The Hyperthyroid + *in vivo* melatonin group was higher in the first two doses (10^{-7} and 10^{-6} M) compared to the Hyperthyroid group and lowered in the other doses.

In the Control + *in vitro* melatonin (1B) and Hyperthyroid + *in vitro* melatonin (2B) groups, the submaximal dose of KCl was applied three times and the mean values were determined. By applying a submaximal dose of KCl and doses of different concentrations of melatonin together, the contraction responses of the isolated duodenal tissues of the rat were evaluated.

Figure 3A. (a, b; a, c; b, c; P<0.0001) and Figure 3B. It was observed that there was a statistically significant difference in columns with different letters in (a, bc; a, c; ab, c; P<0.0001, ab, c; P<0.05). The submaximal dose of ACh was administered three times in the Control + *in vitro* melatonin (n=5) and Hyperthyroid + *in vitro* melatonin (n=6) groups. The mean values of the three submaximal doses administered were calculated. The contraction responses of duodenal tissues isolated from rats were evaluated by giving a submaximal dose of ACh and different melatonin concentrations to the bath environment. Figure 3C. (a, c; P<0.0001, ab, c; P<0.001, a, bc; P<0.001) and Figure 3D. It was observed that there was a statistical difference in columns with different letters in (a, bc; a, c; p <0.0001, a, bcd; ab, c; P<0.001).

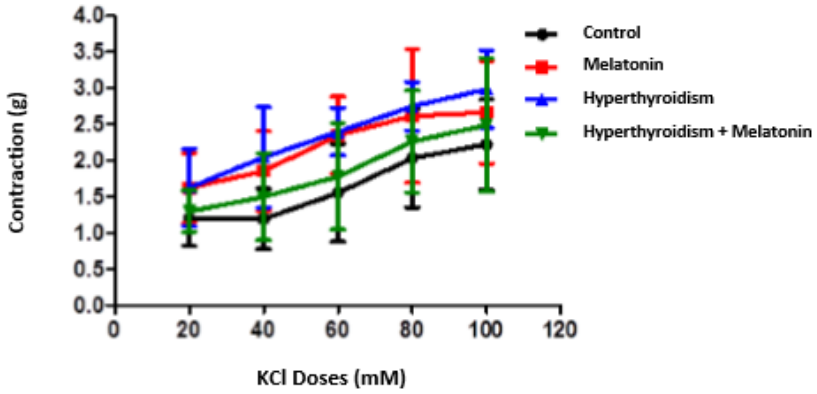

Figure 1. KCl-induced isolated duodenal tissue dose-response curves in Control (1A), Control + *in vivo* melatonin (1C), Hyperthyroidism (2A), Hyperthyroidism + *in vivo* melatonin (2C) groups.

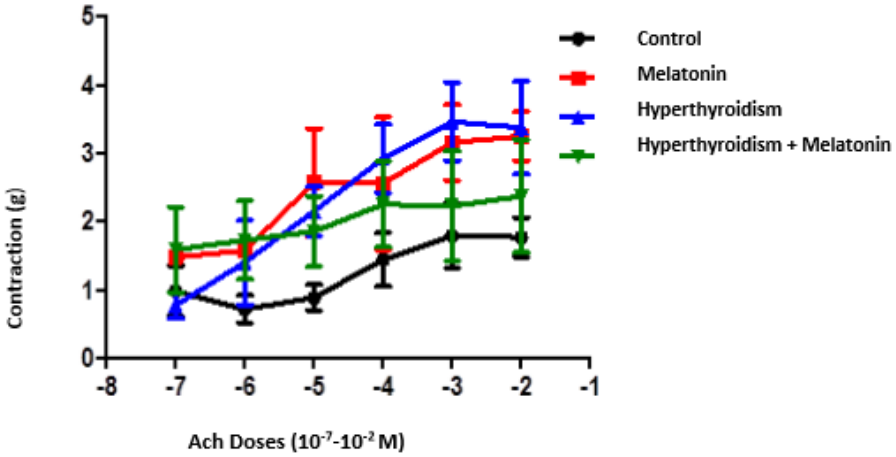

Figure 2. ACh-induced isolated duodenal tissue dose-response curves in Control (1A), Control + *in vivo* melatonin (1C), Hyperthyroidism (2A) and Hyperthyroidism + *in vivo* melatonin (2C) groups.

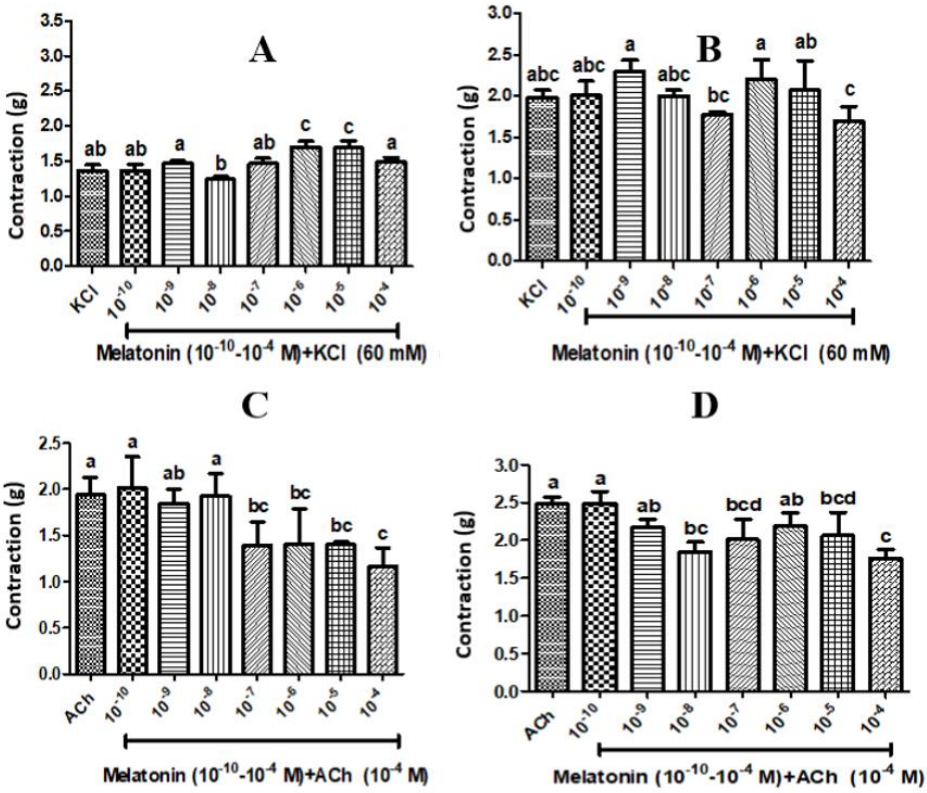

Figure 3. A: The contraction responses of the control + *in vitro* melatonin group to melatonin applied with submaximal KCl (60 mM) to duodenal tissues. B: The contraction responses of hyperthyroid + *in vitro* melatonin group to melatonin applied with submaximal KCl (60 mM) to duodenal tissues. C: The contraction responses of the control + *in vitro* melatonin group to melatonin administered with submaximal ACh (10^{-4} M) on duodenal tissues. D: The contraction responses of hyperthyroid + *in vitro* melatonin group to melatonin applied with submaximal ACh (10^{-4} M) to duodenal tissues.

4. Discussion

This research aims to understand the impact of melatonin on duodenum (a section of the small intestine) of rats with hyperthyroidism. This hormone has inhibitory effects on certain bodily functions, on the duodenum (a part of the small intestine) of rats induced with hyperthyroidism. The researchers observed the effects of melatonin on the duodenal tissue of these rats by applying it both inside the body (*in vivo*) and outside the body (*in vitro*) and comparing the results.

Thyroid hormones, which increase metabolic activity in almost all tissues and organs in the body, have an effect on all systems. Hyperthyroidism is a clinical condition that results from the excessive secretion of thyroid hormones (Shahid et al., 2020). The small intestines digest the nutrients taken in the blood and absorb the blood. Intestinal motility, the contents of the stomach, bile, pancreas, and small intestine secretions by mixing the turn into a column turns into kimus. This transition in the intestinal tract varies between about 2-4 hours (Daher et al., 2009). In diseases of the thyroid gland, changes in bowel movement and digestion may occur due to variations in the time it takes for food to move through the digestive system, changes in the body's metabolism, and differences in the absorption of nutrients.

According to the study by Drago et al. (2002), it was determined that low doses of melatonin injected intraperitoneally increase intestinal transit and high doses decrease this transition. In the 1B group, where we gave melatonin to the bathing environment, there was a significant increase in the doses of submaximal KCl (60 mM) and melatonin 10^{-6} and 10^{-5} M in combination with the submaximal dose of KCl. It was determined that the contractility decreased significantly.

Although hyperthyroidism was detected in the 2B group with submaximal KCl and melatonin combined with the bathing environment, there was an increase in the concentration of melatonin 10^{-9} M, 10^{-6} M, 10^{-5} M, and submaximal KCl according to the contraction response to a submaximal dose of KCl. Reduction of contraction responses in concentrations of 10^{-7} M and 10^{-4} M melatonin was detected. In the duodenum tissue induced by the submaximal dose of ACh, the contraction responses were not changed in the tissue in which submaximal ACh and melatonin 10^{-10} M, and 10^{-8} M concentrations were applied in the 1B group. In contrast, the other doses of melatonin were decreased. In the 2B group, 10^{-10} M melatonin and ACh were found to be decreased in all doses except the submaximal dose of ACh according to the contraction response to the submaximal dose of ACh.

Mogulkoç et al. (2006) found that oxidative damage to the brain, liver and heart tissues of hyperthyroid rats was high in the Hyperthyroidism group. They observed that melatonin reduced the negative effect of this oxidative damage. In some studies, melatonin has been reported to be protective in the gastrointestinal tract. Brzozowski et al. (1997) observing that melatonin reduced gastric lesions due to stress and ischemia-reperfusion (Bandyopadhyay et al. 2002).

In a study, melatonin was shown to protect the gastric mucosa from indomethacin-induced damage in diabetic rats (Pradeepkumar Singh et al., 2011). The study observed that the symptoms decreased after melatonin treatment in patients with constipation and intestinal bowel syndrome (IBS) (Chojnacki et al., 2013). Their studies found that the ethanol-induced rat permeability of the duodenal mucosa reduced melatonin. In addition, they demonstrated that melatonin abolished ethanol-induced duodenal hypermotility but had no effect on basal motor activity. In our study, the hyperthyroidism model that causes increased intestinal motility was created in rats in similar with the researches in the literature (Sommanson et al., 2013; Sommanson et al., 2014). Tissue contractility was determined by inducing the duodenum suspended in the isolated organ bath system with different doses of KCl and ACh. It was found that contractions increased significantly in the Hyperthyroid group compared to the Control group, and these increases were significant.

Similarly, in the *In vivo* melatonin-applied groups, it was observed that contraction increased compared to the Control group, but when compared with the Hyperthyroid group, contractility decreased, and that the contraction response in the Control + *in vivo* melatonin group was higher than in the Hyperthyroid + *in vivo* melatonin group.

Bondarenko et al. (2011) reported a significant relationship between the thyroid gland and the pineal gland. Baltaci et al. (2017) found that plasma melatonin levels were significantly higher in the experimental hyperthyroid group compared to the control group. In general, it was reported that there was a significant relationship between thyroid and pineal glands, and impaired thyroid function could alter the release of melatonin (Rom-Bugoslavskaia et al., 1984; Bondarenko et al., 2011; Baltacı et al., 2017). Drago et al. (2002) According to their study, we have mentioned above that high doses of melatonin injected intraperitoneally reduce intestinal passage. Bondorenko et al. (2011) and Rom-Bugoslavskaia et al. (1984) Plasma melatonin level of hyperthyroidism was found to increase and melatonin supplementation increased the level of plasma melatonin in hyperthyroid rats. Lucchelli et al. (1997) observed that spontaneous and serotonin-induced contractions of rat duodenum *in vitro* were determined by high-dose melatonin. In parallel with these studies, it was determined that the contact responses of rat-isolated duodenal tissues in the Hyperthyroid + *in vivo* melatonin group to different concentrations of KCl and ACh were lower than the contraction responses of the Control + *in vivo* melatonin group and the Hyperthyroid group.

Our study determined that contraceptive responses of duodenum tissues induced by different logarithmic melatonin and submaximal KCl and ACh *in vitro* melatonin groups were different. In the Control group of melatonin treated *in vitro*, melatonin combined with a submaximal dose of KCl was found to be significantly decreased in 10^{-8} M rat tissue. However, there was a significant increase in contraction responses to melatonin administered at doses of 10^{-6} and 10^{-5} M in combination with a submaximal dose of KCl in the same group. In 14 days, hyperthyroidism was induced by L-thyroxine, and melatonin was given to the bathing environment. An increase in KCl and melatonin levels of 10^{-9} and 10^{-6} M was observed according to submaximal KCl-induced groups. It was observed that 10^{-7} and 10^{-4} M concentrations decreased significantly compared to the other doses. The responses to different logarithmic doses of melatonin in rats' isolated tissues induced by the submaximal dose of ACh (10^{-4} M) were found to differ from those given to the submaximal dose of KCl.

This study investigated the effects of *in vivo* and *in vitro* application of melatonin on duodenal tissues isolated from rats with experimental hyperthyroidism.

Duodenal tissues isolated from rats in the Control group, Control + *in vivo* melatonin group, Hyperthyroid group, and Hyperthyroid + *in vivo* group were induced with different doses of KCl and ACh, and dose-response curves were determined according to the contraction responses of the contractions. It was found that the contraction responses obtained from the isolated duodenal tissues obtained from the experimental Hyperthyroid group by administering 0.3 mg/kg/day L-thyroxine in all doses of ACh and KCl for 14 days increased significantly compared to the contraction responses obtained from the other groups. It was found that the contraction responses to KCl in Control + *in vivo* melatonin group increased significantly compared to the contraction responses of the duodenal tissues in the Control and Hyperthyroid + *in vivo* melatonin groups, whereas these responses were found to be lower than the Hyperthyroid group. Responses of isolated duodenal tissues to ACh in the same group were significantly higher than in the control group. According to the Hyperthyroid group, the responses of the Control + *in vivo* melatonin group to ACh 10^{-7} M, 10^{-6} M, and 10^{-5} M doses were found to be lower than the other high doses in the Hyperthyroid group. The responses of tissues in the Hyperthyroid + *in vivo* melatonin group to different doses of KCl were found to be higher than the Control group and lower than the Control *in vivo* melatonin group. The responses to ACh were observed to be higher than all other groups in their responses to 10^{-7} M and 10^{-6} M concentrations. In contrast, responses to other doses of ACh were lower than those of tissues in the Hyperthyroid and Control + *in vivo* melatonin groups.

Submaximal doses of KCl and ACh were administered three times with logarithmic doses of melatonin in the *In vitro* melatonin-applied groups and averaged. Then, the contraction responses were evaluated by giving the determined submaximal doses and logarithmic doses of melatonin together. According to the responses of isolated duodenal tissues to KCl in the Control + *in vitro* melatonin group, there was a significant decrease in KCl + melatonin dose of 10^{-8} M, while a significant increase in KCl + melatonin 10^{-6} M and 10^{-5} M was observed. In the responses given to ACh, it was determined that the responses of ACh to the submaximal dose of ACh + melatonin doses of 10^{-7} M, 10^{-6} M, 10^{-5} M, and 10^{-4} decreased significantly. In the Hyperthyroid + *in vitro* melatonin group, KCl + melatonin responses were found to be increased by 10^{-9} M and 10^{-6} M. However, according to the responses given to KCl in the same group, it was determined that the responses of KCl + melatonin to 10^{-7} M and 10^{-4} M doses were low, and the same responses were obtained at other concentrations. In the responses of the Hyperthyroid + *in vitro* melatonin group to submaximal ACh, a significant decrease was found in the contraction responses of all concentrations except ACh + melatonin 10^{-10} M. Our findings from this study show that melatonin has therapeutic potential in treating digestive disorders caused by hyperthyroidism.

5. Conclusion

This research aims to underline the impact of melatonin on the duodenum of rats with hyperthyroidism. Melatonin was applied both *in vitro* and *in vivo*, and its effects on the duodenal tissue were compared with control groups. Previous studies have shown that melatonin has protective effects on the gastrointestinal tract and reduces oxidative damage to various organs. In the present study, it was observed that the contractility of the duodenum increased in the hyperthyroid group compared to the control group, and *in vivo* melatonin application decreased this contractility. A significant relationship exists between the thyroid gland and the pineal gland, and impaired thyroid function can alter melatonin release. Different doses of melatonin have been shown to reduce intestinal transit.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	E.Ş.	F.Ç.
C	50	50
D	60	40
S		100
DCP	80	20
DAI	70	30
L	80	20
W	50	50
CR	60	40
SR	70	30
PM	40	60
FA	40	60

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Approval/Informed Consent

This study was conducted under the approval of the Ethics Committee of Atatürk University (approval date; January 05, 2017; protocol code: 75296309-050.01.04-E.1700034113.

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