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Research Article

Effect of Melatonin on Catalase Enzyme in Mouse Kidney Tissue

Serap KORAL TAŞÇI [1] Turgay DEPREM [1] Seyit Ali BİNGÖL [2]
Sevda ELİŞ YILDIZ [2] Şahin ASLAN [1]

[1] Kafkas University, Faculty of Veterinary Medicine, Department of Histology and Embryology, TR-36100 Kars - TURKEY
[2] Kafkas University, Faculty of Health Sciences, Department of Midwifery, TR-36100 Kars - TURKEY

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Abstract

In this study, we aimed to immunohistochemically investigate the effects of melatonin administration on catalase in the kidney tissue. In our study, 18 male Swiss albino mice were used and they were divided into three groups as control (n=6), sham (n=6) and experimental (n=6). The experimental group received 10 mg/kg dose of melatonin for 28 days (i.p.). Tissue samples were embedded in paraffin. To examine catalase immunoreactivity, avidin-biotin-peroxidase complex (ABC) technique was performed to tissue sections. Based on the results of the immunohistochemical staining, reaction was showed in proximal tubules in all three groups. The reaction in inner cortical proximal tubules was found to be more intense. There was no immunoreactivity in distal tubules, Malpighian bodies, vascular endothelium and structures in the medulla. Catalase immunoreactivity was determined to be more intense in the experimental group than other groups. Our study supports the idea that melatonin could have a direct effect on the kidney tissue and that it could be used as an antioxidant therapeutic agent by strengthening antioxidant mechanism in oxidative stress.

Keywords: *Catalase, Immunohistochemistry, Kidney, Melatonin, Mouse*

Melatoninin Fare Böbrek Dokusunda Katalaz Enzimi Üzerine Etkisi

Özet

Bu çalışmada, melatonin uygulamasının böbrek dokusunda katalaz üzerine etkisinin immunohistokimyasal olarak araştırılması amaçlandı. Çalışmamızda, 18 adet erkek Swiss albino fare kullanıldı ve bunlar kontrol (n=6), sham (n=6) ve deneme (n=6) olarak üç gruba ayrıldı. Deneme grubuna 28 gün 10 mg/kg dozda melatonin uygulandı (i.p.). Alınan doku örnekleri parafinde bloklandı. Katalaz immunoreaktivitesini incelemek için doku kesitlerine avidin-biotin-peroksidaz kompleks (ABC) tekniği uygulandı. Yapılan incelemeler sonucunda immunohistokimyasal boyamada, üç grupta da reaksiyonun tubulus proksimalislerde olduğu belirlendi. Korteksin iç kısmındaki tubulus proksimalislerde reaksiyonun daha yoğun olduğu görüldü. Tubulus distalislerde, Malpighi cisimciklerinde, damar endotellerinde ve medullada bulunan yapılarda immunoreaktiviteye rastlanmadı. Katalaz immunoreaktivitesinin deneme grubunda, kontrol ve sham grubuna kıyasla daha yoğun olduğu belirlendi. Çalışmamız, melatoninin böbrek dokusu üzerine doğrudan etkisinin olabileceği ve antioksidan mekanizmayı güçlendirerek oksidatif stres durumlarında antioksidan bir terapötik madde olarak kullanılabileceği düşüncelerini desteklemektedir.

Anahtar sözcükler: *Katalaz, İmmunohistokimya, Böbrek, Melatonin, Fare*

INTRODUCTION

Melatonin, first defined by Lerner et al.[1], is an endogenously secreted hormone by pinealocytes in pineal gland [2]. Melatonin has effects on circadian rhythms, sleep, psychology, sexual development and reproduction [3,4]. In addition, melatonin has been reported to affect organs in many systems such as cardiovascular, gastrointestinal, respiratory and renal system [2]. The most important feature discovered in recent years is being a powerful antioxidant [5].

Melatonin was first reported by Ianas et al.[6] to be an antioxidant in 1991. Melatonin is a powerful antioxidant that eliminates hydroxyl (OH) radical, the most harmful radical amongst free radicals. Melatonin is known to be more effective than other antioxidants as it can enter many organelles and cell nucleus and protect DNA from oxidative damage [7,8]. Melatonin shows its antioxidant effects in three ways: Directly binding hydroxyl (OH) radical [9], inhibiting some pro-oxidant enzymes [10] or increasing gene expression and activity of antioxidant enzymes [5,11,12]. Catalase, which is one of the most important antioxidant enzymes, was first isolated in 1937 [8,13]. The task of this enzyme, localized in peroxisomes in the cells, is to transform hydrogen peroxide, which is harmful, into oxygen and water [8,14,15]. This enzyme has been reported to be extensively found

İletişim (Correspondence)
+90 536 3044630
serapkoral@hotmail.com

in hepatocytes in the liver [16] and proximal tubules in the kidney [17]. There are important tasks of the kidney tissue on the oxidative metabolism. Although kidneys constitute less than 1% of total body weight, an average of 10% of total oxygen consumption occurs here [18]. Renal proximal tubular cells exposed to high concentrations glucose and reactive oxygen species (ROS) [19]. For this reason, they are always at risk of oxidative damage. Catalase is found in proximal tubules that is protecting kidney functions efficiently [20,21]. It has been stated that lack of catalase could cause situations that may lead to loss of kidney functions such as oxidative tissue damage and renal fibrosis [18,19]. Melatonin administration has been shown by biochemical methods to increase the levels of antioxidant enzymes such as catalase, glutathione reductase and glutathione peroxidase in liver and kidney [12,22]. Also in a study on pigs, the presence of binding regions of iodomelatonin has been shown at pig kidney cortex [23]. However, there is no study showing the effect of melatonin on catalase in kidney tissue immunohistochemically. In our study, it was aimed to immunohistochemically investigate the effects of exogenous administration of melatonin on catalase in mouse kidney tissues.

MATERIAL and METHODS

This study was performed in accordance with approval from Kafkas University Animal Experimentation Local Ethics Committee (KAÜ-HADYEK/2014-007). In our study, 18 male *Swiss albino* mice were used as experimental animals. Experimental animals were divided into three groups as control (n=6), sham (n=6) and experimental (n=6). The experimental group received 10 mg/kg dose of melatonin (Sigma) dissolved in ethanol and diluted by normal saline for 28 days (i.p.). The same injection procedure applied to sham group, but melatonin was not used. The control group received no applications. At the end of the experimental period, the mice were euthanized and kidney tissues were removed. The tissues were processed with Bouin solution and embedded in paraffin following routine histological phases. 5 µm sections from these blocks were passed through deparaffinization and rehydration process. Triple staining [24] and periodic acid-Shiff (PAS) staining [25] was performed in order to examine the tissues histologically. 3% H_2O_2 application to block endogenous peroxidase activity and microwave application in citrate buffer to unleash antigens were performed in order to examine catalase immunoreactivity. The tissues were washed with PBS (Phosphate buffer solution, 0.1 M, pH 7.2). The tissues were allowed to incubation with anti-catalase antibody (Abcam, 1: 3.000 dilution) for 1 h at room temperature and avidin-biotin-peroxidase complex (ABC) technique [26] was applied. For this, ultravision detection system anti-rabbit, HRP/DAB (Thermo Scientific) kit was used. Finally, counter staining was performed with haematoxylin. Extent of immunoreactivity was assessed semi-quantitatively according to the density of the reaction (No reaction; 0, Weak; 1, Moderate; 2, Strong; 3). Negative control application was performed to determine whether the catalase immunoreactivity was specific. Ten areas in outer cortex and ten areas in inner cortex, total twenty areas of each slide are selected randomly and scored aspect of density of catalase immunoreactivity. One way ANOVA was used to compare catalase immunoreactivity among groups and t-test was used to compare catalase immunoreactivity between inner and outer cortex in SPSS (SPSS version 18.0 for Windows; SPSS Inc.,Chicago, IL, USA).

RESULTS

Histological and Histochemical Results

Similar results were observed all groups regarding histological appearances with triple staining. Nephrons, the functional units of the kidneys, structures forming nephrons, collector tubes and connective tissue areas were observed to be similar *(Fig. 1)*.

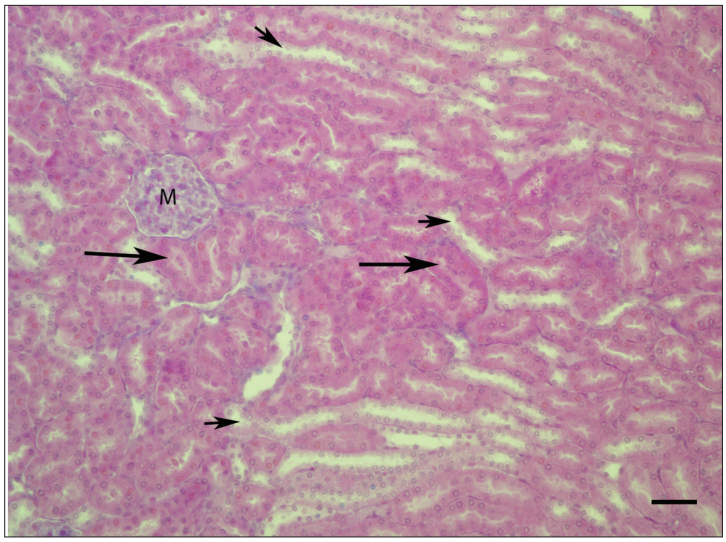


Fig 1. General appearance of the renal tissue. M: Malpighi corpuscle, *long arrow:* tubulus proximalis, *short arrow:* tubulus distalis. Triple staining. Bar: 100 µm

Şekil 1. Böbrek dokusunun genel görünümü. M: Malpighi cisimciği, *uzun ok:* tubulus proksimalis, *kısa ok:* tubulus distalis. Triple boyama. Bar: 100 µm

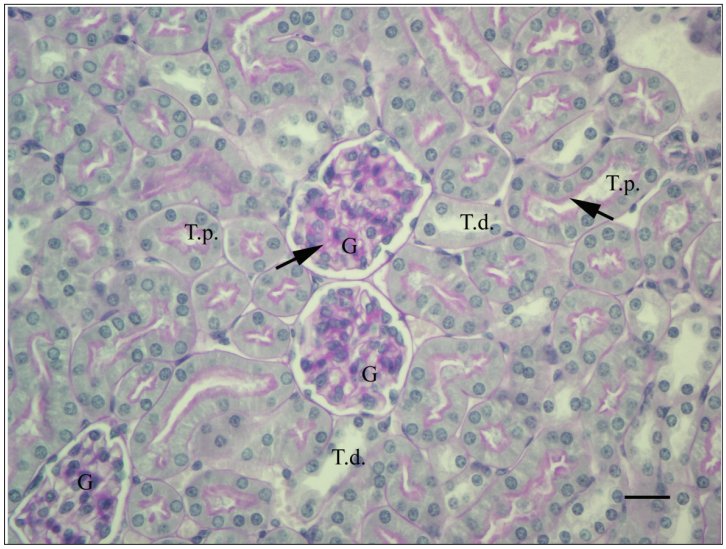
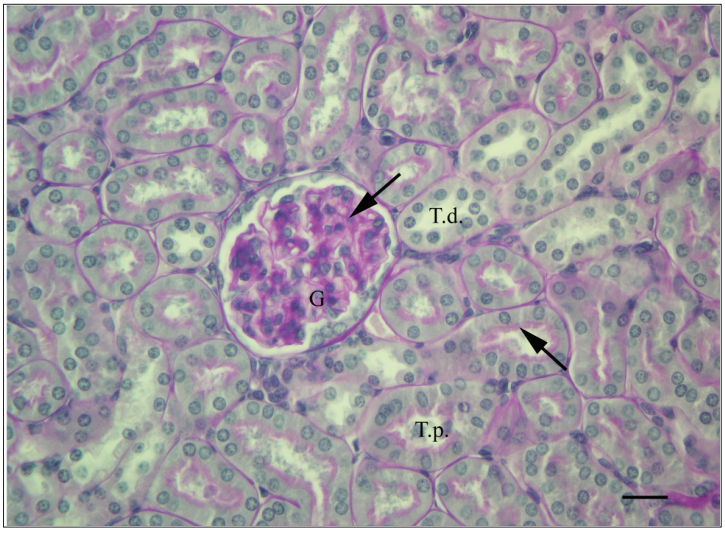


Fig 2. PAS staining in the renal tissue. Control group. G: glomerulus, T.p.: tubulus proximalis, T.d.: tubulus distalis, *arrows:* PAS positive staining. Bar: 50 µm

Şekil 2. Böbrek dokusunda PAS boyama. Kontrol grubu. G: glomerulus, T.p.: tubulus proksimalis, T.d.: tubulus distalis, *oklar:* PAS pozitif boyanma. Bar: 50 µm

Fig 3. PAS staining in the renal tissue. Experimental group. G: glomerulus, T.p.: tubulus proximalis, T.d.: tubulus distalis, *arrows:* PAS positive staining. Bar: 50 µm

Şekil 3. Böbrek dokusunda PAS boyama. Deneme grubu. G: glomerulus, T.p.: tubulus proksimalis, T.d.: tubulus distalis, *oklar:* PAS pozitif boyanma. Bar: 50 µm



There were also no differences between the groups in PAS staining. PAS-positive staining was observed in all groups in glomeruli, Bowman's capsule, apical portions of cells of proximal tubuli and basement membrane. Staining in the inner part of the cortex was determined to be more intense than the outer cortex in proximal tubuli. Strong staining was seen in the basement membrane of distal tubuli *(Fig. 2, Fig. 3)*.

Immunohistochemical Results

Regarding the results of immunohistochemical evaluation, catalase immunoreactivity was observed only in proximal tubuli in all three groups. No immunoreactivity was observed in Malpighian bodies in the cortex, distal tubuli, vascular endothelium and other connective tissues *(Fig. 4a,b)*. No immunoreactivity was also found in the structures in medulla. Immunoreactivity was also observed to be more intense in the inner part of the cortex. These were differences statistically significant for all groups ($P<0.05$). The immunoreactivity was observed usually in the cytoplasm and rarely in the nucleus, and the staining in the cytoplasm was granular in style *(Fig. 4b)*. Catalase immunoreactivity in proximal tubuli was found to be more intense in the experimental group *(Fig. 5)* compared to control *(Fig. 6)* and sham *(Fig. 7)* groups *(Table 1)*. No significant difference was observed between control and sham groups *(Fig. 6, 7)*. The negative control did not show any reaction *(Fig. 8)*.

DISCUSSION

Antioxidant system includes antioxidant enzymes (SOD, CAT, GPX etc.), which can prevent from oxidative stress [8]. Melatonin is an antioxidant that increases the activity of antioxidative enzymes [5,12].

It was determined that the findings from the histological examination of the kidney tissue were consistent with normal histology, in accordance with classical literature [2,27] and that there is similar for all groups.

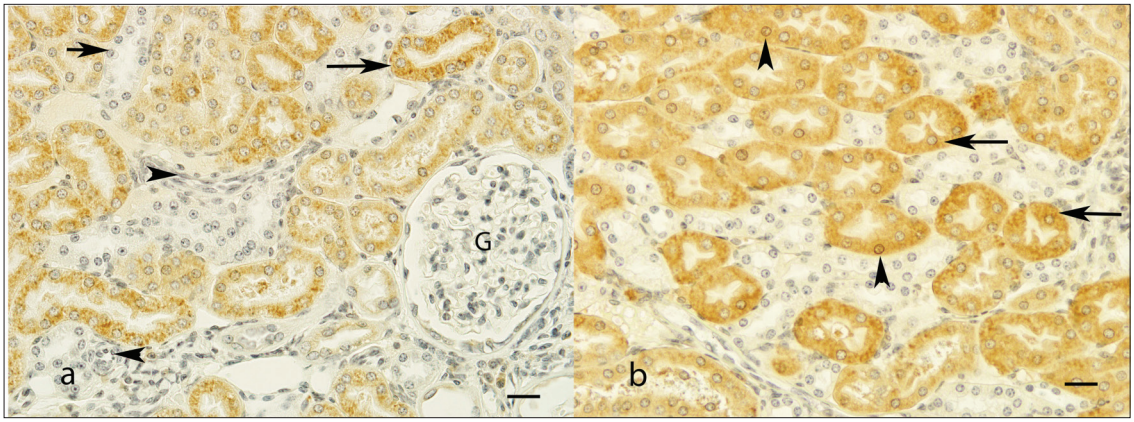
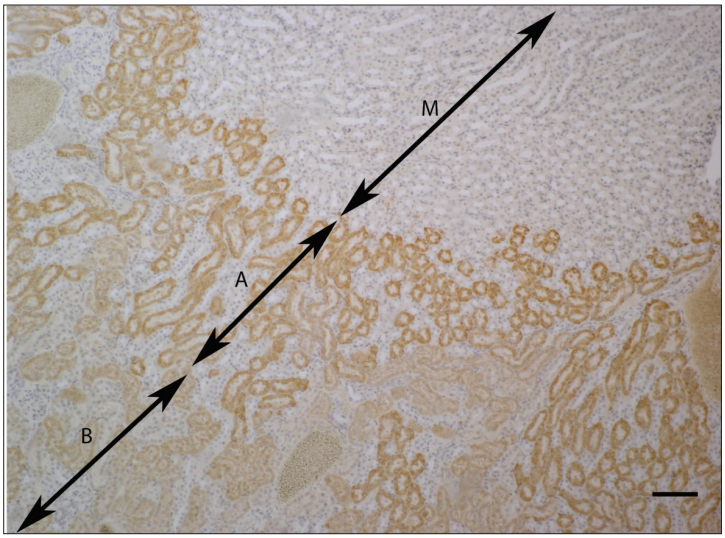


Fig 4. Catalase immunoreactivity in the cortex section of the kidney. Control group. **a)** G: glomerulus, *arrow head:* vascular endothelium, *short arrow:* tubulus distalis, *long arrow:* tubulus proximalis. Bar: 50 µm, **b)** *Arrow:* cytoplasmic immunoreactivity, *arrow head:* immunoreactivity in the cytoplasma and nucleus. Bar: 50 µm

Şekil 4. Böbreğin korteks kısmındaki katalaz immuno-reaktivitesi. Kontrol grubu. **a)** G: glomerulus, *ok başı:* damar endoteli, *kısa ok:* tubulus distalis, *uzun ok:* tubulus proksimalis. Bar: 50 µm, **b)** *Ok:* sitoplazmik immunoreaktivite, *ok başı:* sitoplazma ve çekirdekte immunorektivite. Bar: 50 µm

Fig 5. Catalase immunoreactivity in the renal tissue of the experimental group. M: medulla, A: inner cortex, B: outer cortex. Bar: 200 µm

Şekil 5. Deneme grubu böbrek dokusunda katalaz immuno-reaktivitesi. M: medulla, A: iç korteks, B: dış korteks. Bar: 200 µm



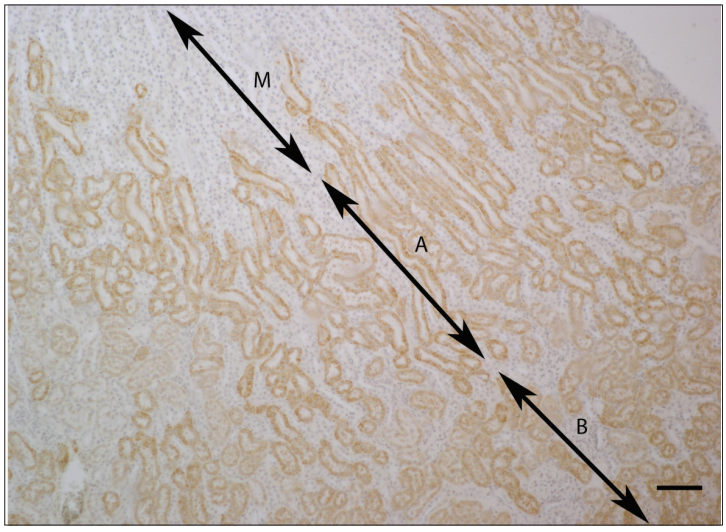


Fig 6. Catalase immunoreactivity in the renal tissue of the control group. M: medulla, A: inner cortex, B: outer cortex. Bar: 200 µm

Şekil 6. Kontrol grubu böbrek dokusunda katalaz immuno-reaktivitesi. M: medulla, A: iç korteks, B: dış korteks. Bar: 200 µm

Ergin and Başaloğlu [28] studied the effect of chronic melatonin injections in kidney tissue and reported that there is no difference histologically, similarly to our findings.

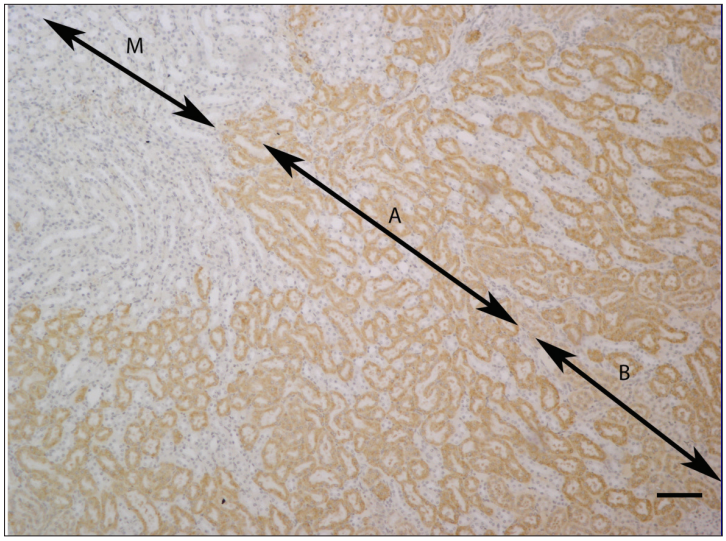


Fig 7. Catalase immunoreactivity in the renal tissue of the sham group. M: medulla, A: inner cortex, B: outer cortex. Bar: 200 µm

Şekil 7. Sham grubu böbrek dokusunda katalaz immunoreaktivitesi. M: medulla, A: iç korteks, B: dış korteks. Bar: 200 µm

Fig 8. Negative control. Control group. Bar: 100 µm

Şekil 8. Negatif Kontrol. Kontrol grubu. Bar: 100 µm

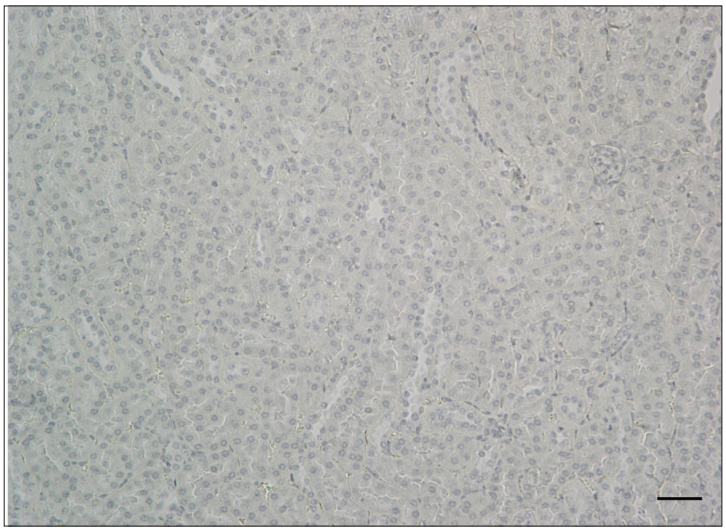


Table 1. *Comparison of catalase immunoreactivity among groups in proximal tubules (P<0.05)*

Tablo 1. *Proksimal tubullerdeki katalaz immunoreaktivitesinin gruplar arası karşılaştırılması (P<0.05)*

Regions of Cortex	Control	Sham	Experimental
Outer cortex	1.250±0.056 [a]	1.166±0.048 [a]	2.033±0.088 [b]
Inner cortex	2.033±0.091 [a]	2.066±0.081 [a]	2.850±0.046 [b]

[a,b] Differences between values having different letters in the same line are statistically significant (P<0.05)

In a study by Bingöl and Kocamış [17] on immunohistochemical examination of catalase enzyme in kidney tissues of healthy and diabetic rats, they have reported that catalase immunoreactivity is intense only in proximal tubuli in the cortex of the kidney and quite weak in the medulla, whereas there is no immunoreactivity in structures such as glomeruli, vascular endothelium, distal tubuli. In the same study, they stated that immunoreactivity in the cortex varies by region and immunoreactivity in the inner part of the cortex in the entire groups is more intense. Similarly in their study, Morikawa et al.[29] studied immunohistochemical localization of catalase in tissues of mammals and they reported that the reaction is in the cytoplasm of proximal tubuli cells of the kidney and that no specific reaction is present in distal tubule epithelium, connective tissue and blood vessels. Zhou and Kang [30] studied immunohistochemical localization of catalase in various tissues in transgenic mice and stated that there is cytoplasmic reaction in proximal tubuli of kidney. They have observed that this present reaction in proximal tubuli is weaker in the outer part of the cortex, stronger in the inner part and not present in distal tubuli, glomeruli, loop of Henle, collector tubes and medulla [30]. Immunohistochemical localization of catalase in geese liver has been reported to be mostly cytoplasmic and that some staining is present in the nuclei of a few hepatocytes [16].

Our findings are parallel to the findings of Bingöl and Kocamış [17], Morikawa et al.[29], Zhou and Kang [30] regarding presence of immunoreactivity in the cortex of the kidney, especially in proximal tubuli in the inner cortex portion and absence of reaction in distal tubuli, vascular endothelium and connective tissue. Kidney and especially proximal

tubular cells always expose to reactive oxygen species (ROS). Catalase is an antioxidant enzyme and locates in peroxisomes [15]. Peroxisomes is the typical organelles of the proximal tubule cells [2], it shows that antioxidant defence and catalase immunoreactivity are seen in these cells intensely. Moreover, in our study, reaction being mostly cytoplasmic and less nuclear is consistent with the findings of similar studies [16,29,30]. However, immunoreactivity in the medulla observed by Bingöl and Kocamış [17] could not be observed in our study.

Bharti et al.[22] have reported that melatonin administration increases catalase, glutathione reductase and glutathione peroxidase in the liver and kidney, thus activates antioxidant mechanisms and has protective and therapeutic effect in oxidative stress. In our study, immunoreactivity being more intense in the experimental group compared to other groups is consistent with the biochemical study of Bharti et al.[22].

In conclusion, our study supports the opinion that melatonin might have a direct effect on the kidney tissue [23]. However, this effect was observed not to cause a change in the histology at light microscopic level. In our study, it was concluded that melatonin administration increases catalase immunoreactivity in kidney. Catalase immunoreactivity being more intense in the experimental group supports the idea that melatonin could be used as a therapeutic agent in oxidative stress by supporting antioxidant mechanism. We believe our study will contribute to studies to be done on renal, melatonin and antioxidant systems.

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