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Research Article

Immunohistochemical Localization of Catalase in Geese *(Anser anser)* Kidney [1]

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Abstract

The aim of this study is to observe the histologic structure of kidney tissue of geese and to determine the immunohistochemical localization of catalase in it. Six female geese were used in our study. After fixation in Bouin's solution and routine histological process, the kidney tissues taken from geese were embedded in paraffin blocks. To detect catalase immunoreactivity in the tissues, the avidin-biotin-peroxidase complex (ABC) technique was used. Crossmann's modified triple stain for histological examination was applied to the samples. It was seen that the geese kidney included numerous lobules and each lobule consists of cortical and medullary tissues. The strong immunoreactivity of catalase was observed in the proximal convoluted tubules in the cortex. Immunohistochemical reaction was also observed in intermediate tubules and the thick segment of Henle's loops. Also it was determined that the immunoreactivity in the basal epithelial cells of the secondary branches of ureters and the intercalated cells in the distal convoluted tubules and collecting duct. There was no catalase immunoreactivity in the renal corpuscles. In conclusion, localization of catalase in the proximal convoluted tubules, in the intermediate tubules, in the thick segments of Henle's loops and in the intercalated cells is considered that these parts of the geese kidney are involved in the antioxidant defence. This study is thought to contribute to the planned future studies about antioxidant system in the kidney of poultry.

Keywords: *Catalase, Goose, Kidney, Immunohistochemistry*

Kaz *(Anser anser)* Böbreğinde Katalaz'ın İmmunohistokimyasal Lokalizasyonu

Öz

Bu çalışmada amaç, kazların böbrek dokusunun histolojik yapısı ve katalazın immunohistokimyasal lokalizasyonunun belirlenmesidir. Çalışmamızda 6 adet ergin dişi kazdan alınan böbrek doku örnekleri kullanıldı. Alınan doku örnekleri Bouin solüsyonunda tespit edildikten sonra rutin histolojik doku takibi yapılıp ardından parafinde bloklandı. Dokulardaki katalaz immunoreaktivitesini incelemek için Avidin-Biotin-Peroksidaz Kompleks (ABC) tekniği kullanıldı. Dokuların genel histolojik görünümlerini incelemek için de Crossmann'ın modifikasyonu olan üçlü boyama tekniği uygulandı. Yapılan histolojik incelemelerde kaz böbreğinin her birinin ayrı ayrı korteks ve meduldadan oluşan çok lopçuklu bir yapıya sahip olduğu görüldü. Dokulardaki katalaz immunoreaktivitesi en yoğun olarak kortekstekı tubulus proksimalis konvoluta kısımlarında belirlendi. Bununla birlikte immünohistokimyasal reaksiyon intermediyer tubullerde ve çıkan Henle kısımlarında da gözlendi. Ayrıca, üreterlerin kollarındaki bazal epitel hücrelerinde, tubulus distalis konvolutalarda ve toplayıcı borucuklarda bulunan interkalat hücrelerde de yer yer reaksiyon olduğu görüldü. Korpuskulum renislerde katalaz immunoreaktivitesine rastlanmadı. Sonuç olarak, antioksidan bir enzim olan katalazın tubulus proksimalis konvoluta, intermediyer tubul, çıkan Henle bölümü ve interkalat hücrelerde lokalize olması bu kısımların kaz böbreğindeki antioksidan savunma bölgeleri olduğunu düşündürmektedir. Bu araştırmanın kanatlı böbreğinde antioksidan savunma sistemi ile ilgili yapılması planlanan başka çalışmalara katkı sağlayacağı düşünülmektedir.

Anahtar sözcükler: *Böbrek, Immunohistokimya, Katalaz, Kaz*

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INTRODUCTION

The histological structure of kidney tissue in poultry is observed as a multi-lobule structure with separate cortex and medulla. There is a thin connective tissue between the cortex and the medulla. Two different types of nephrons are present in the kidney of poultry. The first one is the nephron structure called mammalian or medullary type. In this type of nephron; renal corpuscle, proximal convoluted tubule, thin and thick segments of Henle's loop, distal convoluted tubule and collecting tubule are observed. In the mammalian type nephrons, the renal corpuscle is larger [1,2]. Another type of nephron in the poultry kidney is the reptilian type nephron which is also called as cortical type. The renal corpuscle is smaller in this nephron type. Also, there is no Henle's loop in the reptilian type nephron. There is a short part called intermediate tubule between proximal convoluted tubule and distal convoluted tubule [1-4]. In the cytoplasm of the epithelial cells located in the proximal convoluted tubule, there are plenty of mitochondria and a large number of peroxisomes [5]. Catalase is present in peroxisomes within the cell [6]. Intercalated cells are located among epithelial cells of both distal convoluted tubules and the collecting tubules [7]. There are secondary branches of the ureter in the poultry kidney [1,2].

During the normal functions of the cells, a number of molecules called reactive oxygen species are formed and they are very harmful to the cells [8]. The most important source of these molecules in the cell is the mitochondria. The harmful effects of these molecules are prevented by the antioxidant defence system [8-10]. Catalase is one of antioxidant enzymes in this system. Catalase shows antioxidant activity by detoxifying hydrogen peroxide (H_2O_2) [8,11,12]. The kidney has high oxidative metabolic activity so it has a high risk of oxidative tissue damage [13]. Degraded antioxidant defence system plays a critical role in the pathogenesis of chronic renal failure [12]. Lack or insufficiency of catalase and other antioxidant enzymes causes renal tissue damage and interstitial fibrosis, resulting in loss of renal function [12-14].

Poultry species are living organisms with high metabolic activity. However, it is stated that poultries are more resistant to oxidative damage compared to the mammals. This may be related to the production of less reactive oxygen species or a strong antioxidant defence system in the poultry [15].

The aims of this study were to investigate the histologic structure of the geese *(Anseranser)* kidney tissue and to determine the immunohistochemical localization of catalase in it.

MATERIAL and METHODS

This study was approved by the Local Ethics Commission of Experimental Animals of Kafkas University (KAÜ-HADYEK). In our study, kidney tissue samples taken from 6-12 month old, 6 female geese *(Anser anser)* butchered for consumption were used. Tissue samples taken for histological and immuno-histochemical examinations were fixed in Bouin's solution. Routine histological tissue process was performed, and the tissues were blocked in paraffin. Tissue sections were taken from paraffin blocks at a thickness of 5 µm. The sections were stained with Crossman's modified triple staining technique [16] to examine the general histological structure of the tissues after deparaffinization and rehydration procedures. Avidin-Biotin-Peroxidase complex (ABC) technique [17] was used to determine catalase immunoreactivity in tissues. Anti-polyvalent HRP kit (Thermo Scientific, TP-125-HL) was used for this purpose. Endogenous peroxidase activity was prevented by applying 3% H_2O_2 to the sections. Afterwards, the masked antigens were exposed by the application of microwave irradiated antigen retrieval (MW-AR). For this purpose, 800 watt heat was applied to the sections in citrate buffer solution (0.1 M, pH: 6.0) in microwaves for 10 min. At the end of this period, the sections were washed with PBS (Phosphate buffer solution, 0.1 M, pH 7.2). Samples had been incubated in the blocking buffer (Thermo Scientific, TA-125-UB) for 10 min, they were washed with PBS. After the sections were then incubated for 1 h at room temperature with anti-catalase primary antibody (abcam, ab1877, 1: 500 dilution). Biotinylated secondary antibody (Thermo Scientific, TP-125-BN) was applied for 30 min following incubation. Then, streptavidin peroxidase (Thermo Scientific, TS-125-HR) was applied for 30 min. In the final stage, DAB (Diaminobenzidine) (Thermo Scientific, TP-015-HD) was applied to make this reaction visible. Negative control was applied to determine the specificity of the reaction. Cross-sections were stained with hematoxylin to determine nuclei. Preparations were examined by light microscopy and photographed. Grading was performed to determine the intensity of the reaction according to regions (0: no reaction, 1: mild reaction, 2: medium reaction, 3: very intense reaction). For statistical analysis, slides were randomly selected from each subject and 120 tubulus proximalis, 120 intermadiate tubulus, 120 thick segment of Henle's loop and 120 intercalated cells were also randomly selected. Selected regions of each subject were graded in terms of the reaction intensity which ranged from 0 to 3. SPSS 16.0 for Windows was used to compare the intensity of the catalase immunoreactivity. To determine differences of the reaction intensity between different regions of geese kidney, Tamhane's T2 of Post-Hoc Multiple Comparison in One-Way ANOVA was used because of homogeneity variance result.

RESULTS

The histological examination revealed that the goose kidney had a multi-lobule structure, each consisting of cortex and medulla. Two types of nephron structure and their different tubular structures were seen in poultry kidney.

In the cortex; renal corpuscle, proximal convoluted tubule, distal convoluted tubule *(Fig. 1-A)*, sections of collecting tubule, perilobular collecting duct, intermediate tubules of reptilian type nephrons, blood vessels and connective tissue regions were identified. In the medulla; thin and thick segments of Henle's loop of mammalian type nephrons, medullary collecting duct, secondary branches of ureters, vessels and connective tissue were seen. More darkly stained intercalate cells were observed between epithelial cells in the distal convoluted tubule *(Fig. 1-B)* and collecting tubule in both cortex and medulla. In addition, partial lymphoid tissue foci were also found in the geese kidney.

In our immunohistochemical examinations to determine the localization of catalase in renal tissue *(Fig. 2-A)*, no evidence of catalase immunoreactivity was observed in the renal corpuscle. No reaction was observed in the negative controls which were performed to check whether the catalase immunoreactivity was specific or not *(Fig. 2-B)*. Cytoplasmic and granular strong catalase immunoreactivity was determined in the proximal convoluted tubules *(Fig. 3)*. Immunohistochemical reaction was also observed in intermediate tubules of reptilian type nephrons *(Fig. 4-A)*. However, the reaction observed in the intermediate tubules was found to be milder than that of proximal convoluted tubules. Generally, there was no

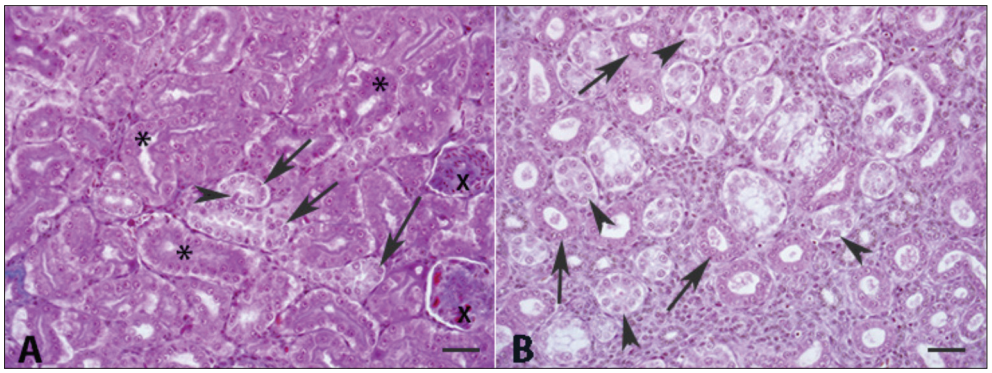
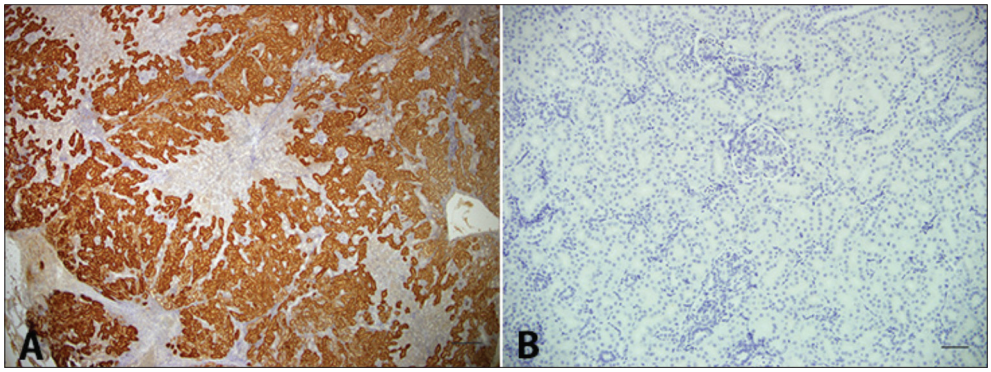


Fig 1. General view of the geese kidney. Crossman's modified triple staining. **A)** X: Renal corpuscle, * Proximal convoluted tubules, *Arrows:* Distal convoluted tubules, *arrow head:* intercalated cell. Bar: 50 µm; **B)** Medulla. *Arrows:* Thick segments of Henle's loops, *arrow heads:* medullary collecting ducts. Bar: 50 µm

Fig 2. A) General view of catalase immunoreactivity in the kidney. Bar: 500 µm; **B)** Negative control. Bar: 100 µm



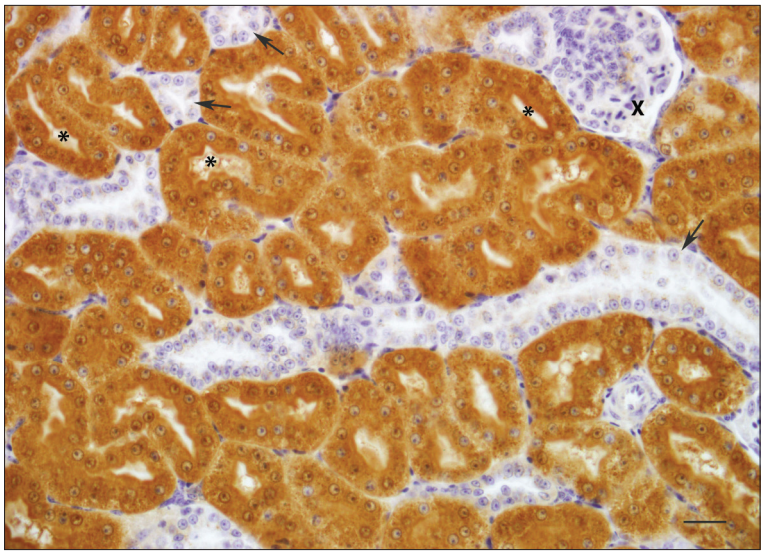


Fig 3. Catalase immunoreactivity in the renal cortex. **X:** Renal corpuscle, * Proximal convoluted tubules, *Arrows:* Distal convoluted tubules. Bar: 50 µm

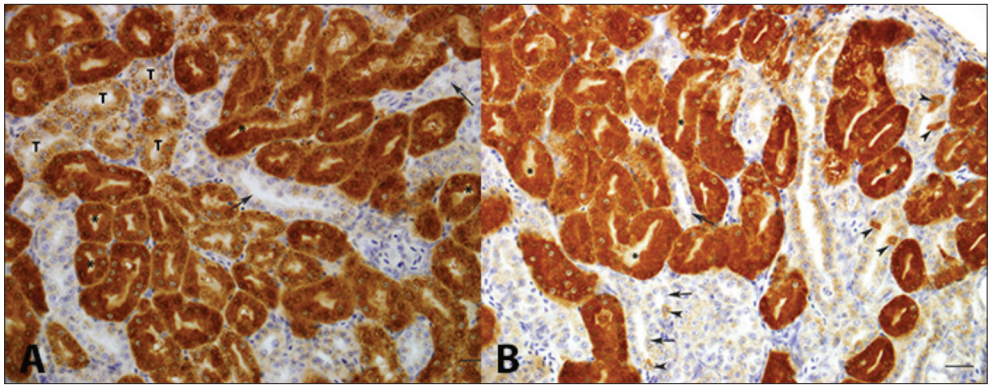


Fig 4. Catalase immunoreactivity. **A)** * Proximal convoluted tubules, *Arrows:* Distal convoluted tubules, T: Intermediate tubules. Bar: 50 µm. **B)** * Proximal convoluted tubules, *Arrows:* Distal convoluted tubules, Arrow heads: Intercalated cells. Bar: 50 µm

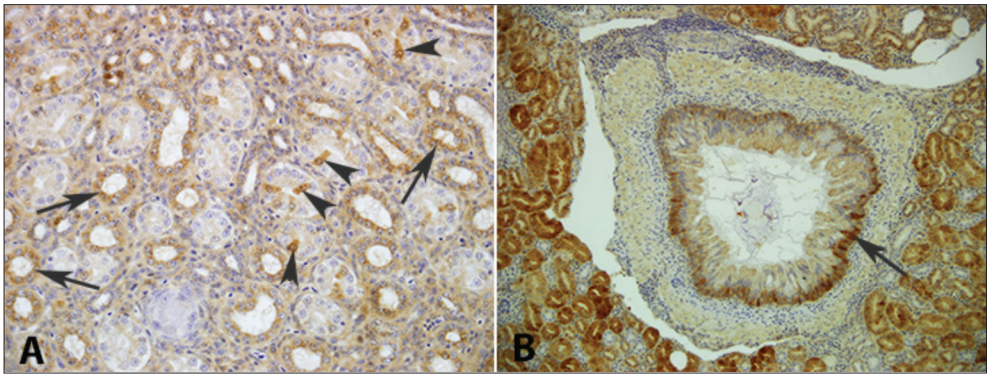


Fig 5. A) Catalase immunoreactivity in the medulla. *Arrows:* Thick segments of Henle's loops, *arrow heads:* the intercalated cells in the medullary collecting ducts. Bar: 50 µm; **B)** Catalase immunoreactivity in the secondary branch of the ureter. *Arrow:* Basal epithelial cells. Bar: 100 µm

Table 1.*The intensity of the catalase immunoreactivity according to the regions of geese kidney*

Parts of Kidney	N	Min	Max	Mean±SD	F
Proximal Convoluted Tubule	120	2	3	2.96±0.18[a]	434.15
Thick Segment of Henle's Loop	120	1	2	1.16±0.37[b]	
Intermediate Tubule	120	1	2	1.39±0.49[c]	
Intercalated Cells	120	1	3	1.46±0.57[c]	

Different superscripts mean statistically significant difference. ***SD:*** *Standart Deviation,* ***F:*** *F value*

immunoreactivity in the epithelial cells of distal convoluted tubules while a moderate reaction was observed in the intercalated cells of these tubules *(Fig. 4-B)*. Similar reactions were also observed in the intercalated cells in the collecting tubules. In the medulla, there was no reaction in the thin segment of Henle's loops of the mammalian type nephrons while mild-degree catalase immunoreactivity was observed in the thick segment of Henle's loops *(Fig. 5)*. Immunoreactivity was also observed in the intercalate cells in medullary collecting ducts *(Fig. 5-A)*.

It was found that the strongest catalase immunoreactivity was in the proximal convoluted tubules in terms of statistical analysis between different parts of the geese kidney. However, a weak immunoreactivity was found in the intermediate tubules, in the thick segments of Henle's loops and in the intercalated cells *(Table 1)*.

The catalase immunoreactivity was observed in the basal epithelial cells in the secondary branches of ureters *(Fig. 5-B)*. The catalase immunoreactivity was determined in macrophages in the connective tissue. No catalase immunoreactivity was observed in the vascular wall forming layers.

DISCUSSION

In this study, we evaluated immunohistochemical distribution of catalase enzyme and general histological structure in the geese kidney. The histological structure of the kidney shows that it is composed of nephrons and collecting tubules as parenchyma. However, kidney tissue in poultry is different from mammals because two different types of nephron are seen together [1,3]. In our study, it was seen that the histological findings of the geese kidney were parallel with the data of the mentioned literature.

Reactive oxygen species are continuously produced during the life of the cell as a result of mitochondrial oxidative metabolism. Cells are constantly fighting with these free radicals. In the antioxidant defence system, both antioxidant enzymes and natural antioxidant molecules play an

important role [18]. Catalase, which detoxifies hydrogen peroxide, is one of important antioxidant enzymes. When reactive oxygen species cannot be controlled due to deficiency of antioxidant enzymes, various damages are seen in tissues [11]. For the evaluation of these harmful effects of free radicals, not only the amount of the free radicals produced should be considered but also the effectiveness of these enzymatic and chemical antioxidant systems should be considered [18]. At this point; the low amount of free radicals produced [15] or the transcription and translation of an active gene related to oxidative stress resistance [19] or the production and activity of antioxidant enzymes in poultry may be related to the higher resistance to oxidative damage in the poultry than that in mammals.

There are few studies about catalase and antioxidant system in the tissues of the poultry [20,21]. However, no such studies could be encountered in the poultry kidney. In studies on the mammalian kidney, it was reported that catalase plays a very effective role in the maintenance of the renal functions [12-14]. In a study on the immunohistochemical distribution of catalase in mouse kidney, cytoplasmic reaction that was granular in style was reported as like our findings [22]. In our study, it was seen that the catalase was localized more intensely in proximal convoluted tubule. This result is similar to the previous studies on mouse kidney [22,23]. The most intensive catalase immunoreactivity in these tubules may be related to both production of free radicals as a result of intense metabolic activity and presence of many peroxisomes in their cells. We think that presence of reaction in intermediate tubules of cortical type nephron may be due to the same reasons.

In our study, immunoreactivity was also found in intercalate cells which are located between epithelial cells of distal convoluted tubule and collecting tubules. Intercalate cells are highly active cells that regulate acid-base balance in the kidney and have abundant mitochondria [24]. The presence of catalase immunoreactivity in these cells, in which the reactive oxygen species are produced, is an indicator of cellular antioxidant defence. Previous studies in mammals reported no catalase immunoreactivity in the Henle's loops [22,25]. Contrary to these literatures, in our study it was determined that a small amount of catalase immunoreactivity was found in the thick segments of Henle's loops in geese kidney. This may be related to the differences between species. In addition, we did not encounter any studies on catalase immunoreactivity in basal epithelial cells in the secondary branches of the ureters.

In conclusion, we investigated the immunohistochemical localization of catalase which is an important antioxidant enzyme in the kidney tissue of geese. The results showed proximal convoluted tubules, thick segments of Henle's loops and intermediate tubules as antioxidant defence regions of the poultry kidney. Intercalated cells in distal convoluted tubule and collecting tubule have also a role as antioxidant defence in the poultry kidney. In conclusion, localization of catalase in the proximal convoluted tubules, in the intermediate tubules, in the thick segments of Henle's loops and in the intercalated cells is considered that these parts of the geese kidney are antioxidant defence parts. This study will contribute to the planned future studies about antioxidant system in the kidney of poultry.

REFERENCES

1. Aslan Ş: Üriner sistem. **In**, Aslan Ş (Ed): Kanatlı Histolojisi. 85-98, Dora Basım Yayın Dağıtım, Bursa, 2018.

2. Kocamış H, Aslan Ş, Deprem T: Kazların böbrek yapısı üzerine histolojik ve histometrik inceleme. *Kafkas Univ Vet Fak Derg*, 9 (2): 157-160, 2003.

3. Hodges RD: The Histology of the Fowl. Academik Press, London, 1974.

4. Braun EJ: Osmoregulatory systems of birds. **In,** Scanes CG (Ed): Sturkie's Avian Physiology. 6th ed., 285-300, Academik Press, New York, 2015.

5. Zık B: Üriner Sistem. **In,** Özer A (Ed): Veteriner Özel Histoloji. 197-218, Dora Basım Yayın Dağıtım, Bursa, 2016.

6. Kocamış H: Hücre. **In,** Özer A (Ed): Temel Histoloji. 3. Baskı, 23-51, Dora Basım Yayın Dağıtım, Bursa, 2016.

7. Akaydın Y, Özcan Z: Hindi *(Meleagris gallopavo)* böbreğinin yapısı üzerinde ışık ve elektron mikroskobik çalışmalar. *Ankara* Üniv *Vet Fak Derg*, 52, 194-155, 2005.

8. Matés JM: Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. *Toxicology*, 153, 83-104, 2000. DOI: 10.1016/S0300-483X(00)00306-1

9. Schiffer TA, Friederich-Persson M: Mitochondrial reactive oxygen species and kidney hypoxia in the development of diabetic nephropathy. *Front Physiol*, 8:211, 2017. DOI: 10.3389/fphys.2017.00211

10. Ray PD, Huang BW, Tsuji Y: Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cell Signal*, 24 (5): 981-990, 2012. DOI: 10.1016/j.cellsig.2012.01.008

11. Nishikawa M, Hashida M, Takakura Y: Catalase delivery for inhibiting ROS-mediated tissue injury and tumor metastasis. *Adv Drug Deliv Rev,* 61, 319-326, 2009. DOI: 10.1016/j.addr.2009.01.001

12. Sindhu RK, Ehdaie A, Farmand F, Dhaliwal KK, Nguyen T, Zhan CD, Roberts CK, Vaziri ND: Expression of catalase and glutathione peroxidase in renal insufficiency. *Biochim Biophys Acta*, 1743, 86-92, 2005. DOI: 10.1016/j.bbamcr.2004.08.013

13. Kobayashi M, Sugiyama H, Wang DA, Toda N, Maeshima Y, Yamasaki Y, Masuoka N, Yamada M, Kira S, Makino H: Catalase deficiency renders remnant kidneys more susceptible to oxidant tissue injury and renal fibrosis in mice. *Kidney Int*, 68, 1018-1031, 2005. DOI: 10.1111/j.1523-1755.2005.00494.x

14. Dobashi K, Ghosh B, Orak JK, Singh I, Singh AK: Kidney ischemia-reperfusion: Modulation of antioxidant defences. *Mol Cell Biochem*, 205, 1-11, 2000.

15. Ogburn CE, Austad SN, Holmes DJ, Kiklevich V, Gollahon K, Rabinovitch PS, Martin GM: Cultured renal epithelial cells from birds and mice: Enhanced resistance of avian cells to oxidative stress and DNA damage. *J Gerontol*, 53A (4): B287-B292, 1998.

16. Luna LG: Manual of Histologic Staining Methods of Armed Forces Institute of Pathology. Mc Graw-Hill Book Comp, New York, 1968.

17. Hsu SM, Raine L, Fanger H: Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. *J Histochem Cytochem,* 29, 577-580, 1981. DOI: 10.1177/29.4.6166661

18. Michiels C, Raes M, Toussaint O, Remacle J: Importance of Se-glutathione peroxidase, catalase, and Cu/Zn-SOD for cell survival against oxidative stress. *Free Radic Biol Med*, 17 (3): 235-248, 1994. DOI: 10.1016/0891-5849(94)90079-5

19. Ogburn CE, Carlberg K, Ottinger MA, Holmes DJ, Martin GM, Austad SN: Exceptional cellular resistance to oxidative damage in long-lived birds requires active gene expression. *J Gerontol A Biol Sci Med Sci*, 56 (11): B468-B474, 2001. DOI: 10.1093/gerona/56.11.B468

20. Bingöl SA, Deprem T, Koral Taşçı S, Kocamış H: Immunohistochemical localization of catalase in geese *(Anser anser)* liver. *Kafkas Univ Vet Fak Derg,* 17 (1): 77-81, 2011. DOI: 10.9775/kvfd.2010.2423

21. Koral Taşçı S, Deprem T, Bingöl SA, Akbulut Y: The anatomical and histological structures of buzzard's *(Buteo buteo)* small intestine and liver, and immunohistochemical localization of catalase. *Kafkas Univ Vet Fak Derg,* 24 (1): 69-74, 2018. DOI: 10.9775/kvfd.2017.18333

22. Koral Taşçı S, Deprem T, Bingöl SA, Eliş Yıldız S, Aslan Ş: Effect of melatonin on catalase enzyme in mouse kidney tissue. *Kafkas Univ Vet Fak Derg,* 22 (6): 917-922, 2016. DOI: 10.9775/kvfd.2016.15659

23. Bingöl SA, Kocamış H: Sağlıklı ve diabet oluşturulmuş farelerin böbrek dokusunda katalaz enziminin RT-PCR ile gen ve immunohistokimyasal olarak protein ekspresyonu. *Kafkas Univ Vet Fak Derg,* 16, 825-834, 2010. DOI: 10.9775/kvfd.2010.1766

24. Roy A, Al-bataineh MM, Pastor-Soler NM: Collecting duct intercalated cell function and regulation. *Clin J Am Soc Nephrol*, 10 (2): 305-324, 2015. DOI: 10.2215/CJN.08880914

25. Muse KE, Oberley TD, Sempf JM, Oberley LW: Immunolocalization of antioxidant enzymes in adult hamster kidney. *Histochem J*, 26 (9): 734-753, 1994.