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RESEARCH ARTICLE

Immunohistochemical Localization of Catalase in Geese *(Anser anser)* Liver

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Summary

The aim of the present study was to investigate immunohistochemical localization of catalase (CAT) in geese liver as well as its histological structure. Six healthy female geese (*Anser anser,* aging between 10-12 months and weighing 2-3 kg), were used in this study. Tissues were fixed in Bouin solution for histological and immunohistochemical examinations and embedded in paraffin. Serial cross-sections were taken from paraffin blocks and then stained whit Crossman's triple stain (Triple stain), Hematoxylin and Eosin (HE) and Periodic Acid Schiff (PAS) for histological examinations. After incubation cross section with for 1 hr, 1:3000 diluted anti-CAT Avidin-Biotin-Peroxidase Complex (ABC) method was applied for immunohistochemical staining. It was histologically observed that hepatocytes formed hepatic cords (also called Remark cords) mostly surrounding the central vein of goose liver. Globuler glycogen deposits were observed in some of the hepatocytes. Catalase immunoreactivity was diffusely observed in hepatocyte cytoplasms. Furthermore, some of hepatocyte nuclei were positively stained for catalase immunoreactivity. In addition, primary antibody was prepared against bovine liver catalase was immunoreactived in geese liver was detected. As a result, antioxidant defense system was carried out especially by the hepatocytes in geese liver and this study was thought to contribute to studies about antioxidant in poultry.

Keywords: *Catalase, Immunohistochemistry, Liver, Geese*

Kaz (Anser anser) Karaciğer Dokusunda Katalazın İmmunohistokimyasal Lokalizasyonu

Özet

Bu çalışmada, kaz karaciğer dokusunda katalazın immunohistokimyasal lokalizasyonu ve dokunun histolojik yapısının incelenmesi amaçlandı. Çalışmada sağlıklı, 10-12 aylık, 2-3 kg ağırlığında 6 adet dişi kaz *(Anser anser)* kullanıldı. Histolojik ve immunohistokimyasal incelemeler için Bouin solüsyonunda tespit edilen dokular rutin doku takibinden geçirildikten sonra parafinde bloklandı. Bu parafin bloklardan alınan seri kesitlere histolojik incelemeler için Crossman'ın üçlü boyaması (Triple Boyama), Hematoksilen Eosin (HE) ve Periyodik Asit Schiff (PAS) boyamaları uygulandı. İmmunohistokimyasal boyama için 1:3000 oranında dilüe edilmiş anti-CAT ile 1 sa inkubasyonda bırakılan kesitlere Avidin-Biotin-Peroksidaz Kompleks (ABC) yöntemi uygulandı. Yapılan histolojik incelemede, kaz karaciğer dokusunda hepatositlerin oluşturduğu Remark kordonları ve kordonların ışınsal olarak çevrelediği vena sentralis tespit edildi. Kaz karaciğerindeki bazı hepatositlerin sitoplazmasında globuler tarzda glikojen birikimi gözlendi. Yapılan immunohistokimyasal incelemede, kaz karaciğerindeki hepatositlerin sitoplazmasında katalaz immunoreaktivitesinin yaygın olduğu ve bazı hepatosit çekirdeklerinin katalaz pozitif olduğu görüldü. Ayrıca, sığır karaciğer katalazına karşı hazırlanmış primer antikorun kaz karaciğer dokusunda immunoreaktif olduğu tespit edildi. Sonuç olarak, kaz karaciğerinde antioksidan savunma sisteminin özellikle hepatositler tarafından yerine getirildiği ve bu çalışmanın kanatlılarda antioksidanlarla ilgili yapılacak çalışmalara katkı sağlayacağı düşünülmektedir.

Anahtar sözcükler: *Katalaz, İmmunohistokimya, Karaciğer, Kaz*

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INTRODUCTION

Liver is the largest gland that is embryonically originated from intestinal endoderm [1]. Liver process nutrients absorbed from digestive tract and store them to be used for metabolism. Liver carries out important roles such as storing and transforming metabolits as well as defusing toxic substances [2,3]. Poultry and mammalian livers are similar on account of functional and basic structural features [3]. Liver has also very crucial function against free radicals which have harmfull effects on the proteins, genes and cell entire [4]. Antioxidants are substances that prevent harmfull effect of free radicals [5,6]. Hidrogen peroxide (H_2O_2) is one of the important free radical that is produced as a product of aerobic metabolism [7]. Catalase is one of the major antioxidant enzyme that breaks H_2O_2 into water and oxygen [8]. Catalase has been found in almost all aerobic organisms [9]. Catalase was isolated from bovine liver for the first time by Sumner and Dounce [10]. Robertson et al.[11] have reported that catalase has variable enzyme activities in liver, kidney, skeletal muscle, fat tissue and pancreatic islets (the highest levels of its activity has been detected in the liver). It has been determined that some of the substrates such as Ellagic acid have increased the catalase activity in liver [12]. Güven and Yılmaz [13] have also reported the highest catalase activity in the geese liver. The main purpose of the current study was to determine immunohistochemical localization of catalase in geese liver.

MATERIAL and METHODS

This study was received approval by Experiment Ethic Committee of Kafkas University, Faculty of Veterinary Medicine (Decision no: 02, Date: 20.01.2010).

Six healthy female geese (*Anser anser*, aging between 10 and 12 months and weighing 2-3 kg), were used in this study. Liver tissues were obtained from servically dislocated animals under ether anesthesia after 12 h fasting and samples were fixed in Bouin solution for histological and immunohistochemical examinations. Liver samples were embedded in parafin for routine histological methods. Five µm sections from parafin blocks were cut and put on slides (coated with chrome-alum-gelatin) for histological and immunohistochemical examinations. Serial sections were stained whit Crossman's triple stain (Triple stain), Hematoxylin and Eosin (HE) and Periodic Acid Schiff (PAS) [14]. Slides were examined by light microscopy (Olympus BX-51) and photographed.

The sections (thickness 5 µm) taken from paraffin blocks were deparaffinizated and rehydrated for immunohistochemical analysis. Slides were incubated in 3% H_2O_2 for 10 min to block endogenous peroxidase activity and then they were washed with phosphate buffer saline (PBS). Slides were heated in citrate buffer in a microwave oven at 800 watt for 10 min followed by washing with PBS (3 times 5 min. each). Sections were incubated in species compatible serum including secondary antibodies to prevent non-specific binding and then sections were washed with PBS (3x5 min). Primary antibody was diluted in PBS solution. Section were incubated with anti-CAT (Abcam [ab1877], Cambridge MA, USA) at 1:3000 dilution for 1 h at room temperature. Avidin-Biotin-Peroxidase Complex (ABC) technique [15] was applied for visualization and then slides were washed with PBS (3x5 min). Hematoxylin staining was used for background staining. Sections were closed with lamels following the completion of staining process. To determine whether spesific catalase immunoreactivity was taken place, sections were incubated in PBS as a negative control without using primary antibody. Afterwards, sections were examined by light microscopy (Olympus BX-51) and photographed.

RESULTS

It was histologically observed that hepatocytes were found to be formed hepatic cords (also called Remark cords) mostly surrounding the central vein of goose liver. Additonally, hepatic sinusoids were detected among those cords. Some of the hepatocytes were observed to have two nuclei in their cells. Kupffer cells as various shapes were detected in hepatic cords (*Fig. 1*). As expected, Glisson's tirangles (also called Kiernan's space) containing interlobular vein, bile duct and hepatic arter were surrounded by connective tissue (*Fig. 2A-C)*. Nuclei of bile duct epithelium were very darkly stained with HE (*Fig. 2A*). Poultry specific erythrocyte nuclei were expectedly found in hepatic arter and central veins (*Fig. 2A*). Connective tissue, surrounding blood vessels were particularly stained with Triple stain (*Fig. 2B*). Globuler glycogen deposits were observed in some of the hepatocytes (*Fig. 3*).

Catalase immunoreactivity was diffusely observed in cytoplasm of the hepatocytes. Furthermore, some of hepatocyte nuclei were positively stained for catalase immunoreactivity. Catalase immunoreactivitiy was not observed in endothelia of central vein as well as erythrocytes (*Fig. 4*). The Glisson's capsule surrounding entire organ was negative for catalase antibody (*Fig. 5A*). Catalase immunoreactivity was not also observed in the walls of hepatic arter, interlobular vein and bile ducts as well as connective tissue surrounding those structures in Kiernan's space (*Fig. 5B*).

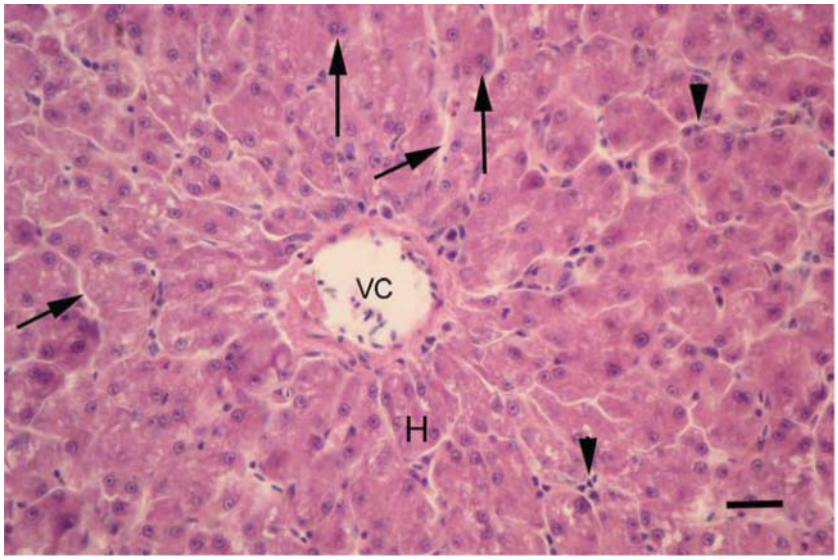

Fig. 1. Goose liver. Central vein (VC), sinusoids (short arrows), Kupffer cells (arrowheads), binucleated hepatocytes (long arrows) and hepatocytes (H) placed radialy around the central vein. HE, Bar: 50 µm

Şekil 1. Kaz karaciğer dokusu. Vena sentralis (VC), sinüzoidler (kısa oklar), Kupffer hücreleri (ok başları), çift çekirdekli hepatositler (uzun oklar) ve vena sentralisin etrafında ışınsal yerleşmiş hepatositler (H). HE, Bar: 50 µm

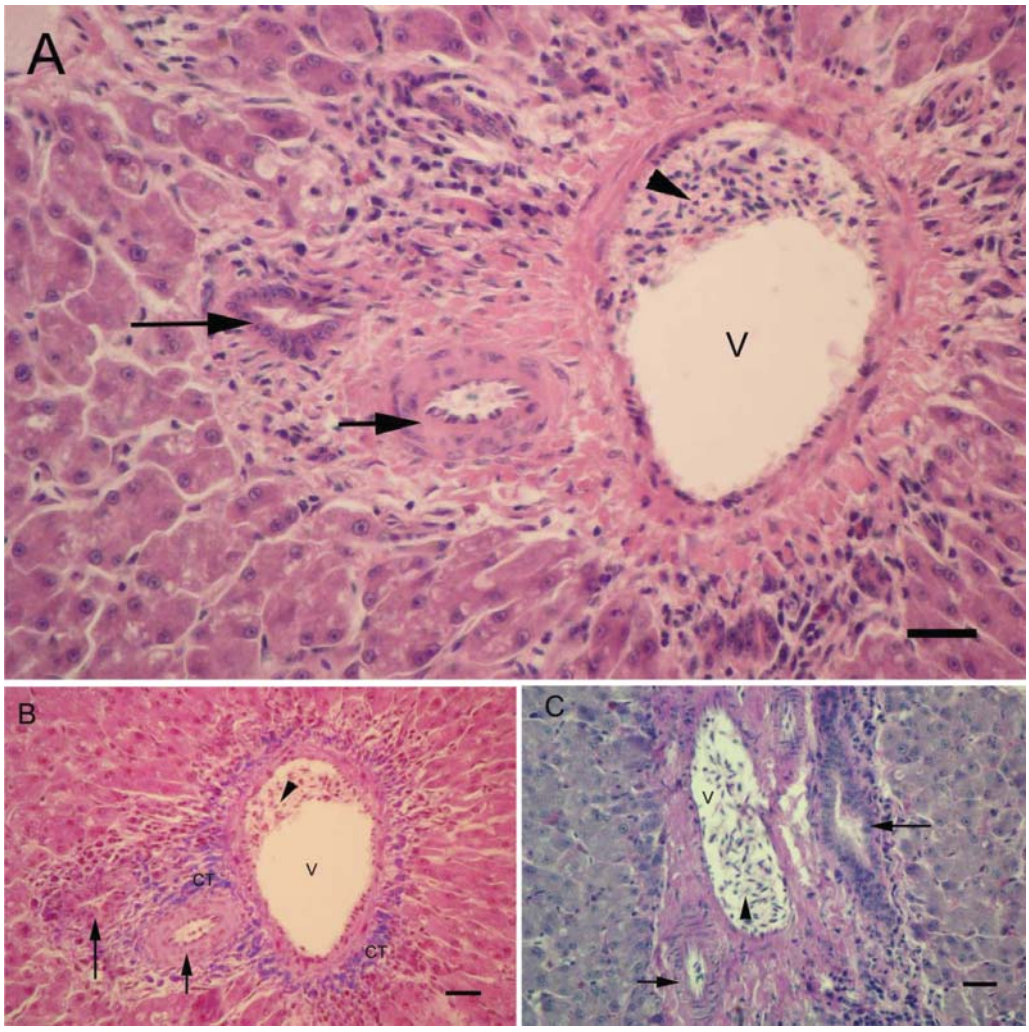

Fig. 2. Glisson's triangle (Kiernan's spaces) in geese liver. Interlobular vein (V), hepatic arter (short arrow), ductus biliferus (long arrow), erythrocytes (arrowhead) and connective tissue (CT). **A)** HE, Bar: 50 µm. **B)** Triple, Bar: 50 µm **C)** PAS, Bar: 50 µm

Şekil 2. Kaz karaciğer dokusunda Glisson üçgeni (Kiernan aralığı). Vena interlobularis (V), arteria hepatika (kısa ok), duktus biliferus (uzun ok), kanatlı eritrositleri (ok başı), bağ dokusu (CT). **A)** HE, Bar: 50 µm. **B)** Triple, Bar: 50 µm. **C)** PAS, Bar: 50 µm

DISCUSSION

In histological examination, Remark cords, central vein, Glisson's capsule, sinusoids and Kiernan's spaces in geese liver were found to be similar to the structures of mammalian liver [2,3]. Classic lobules seen distinctly in camel and pig liver [3] were not appearently seen in geese liver. In our study, we detected that some hepatocytes were binucleated in geese liver which was supported by other reports in various species [2,3]. Glycogen deposits

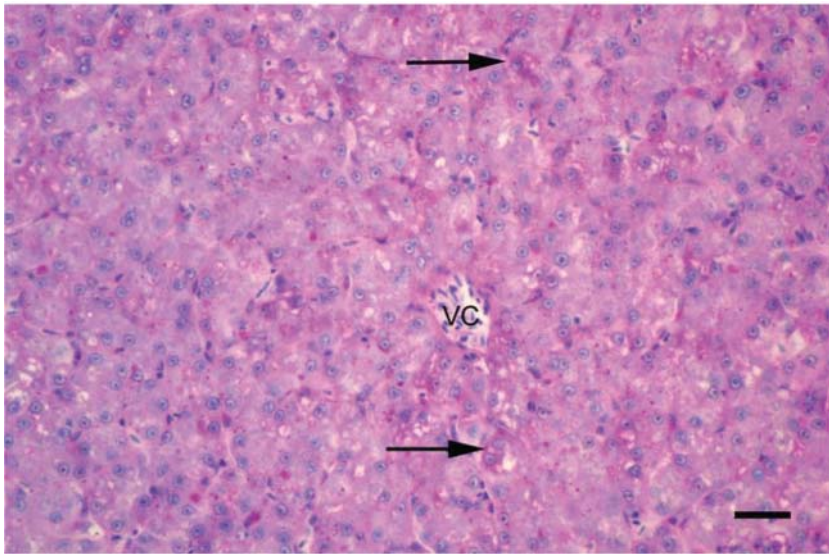

Fig. 3. Central vein (VC), PAS positive glycogen deposits in the cytoplasm of hepatocytes (arrows). PAS, Bar: 50 µm

Şekil 3. Vena sentralis (VC), hepatosit sitoplazmalarında PAS pozitif glikojen birikimi (oklar). PAS, Bar: 50 µm

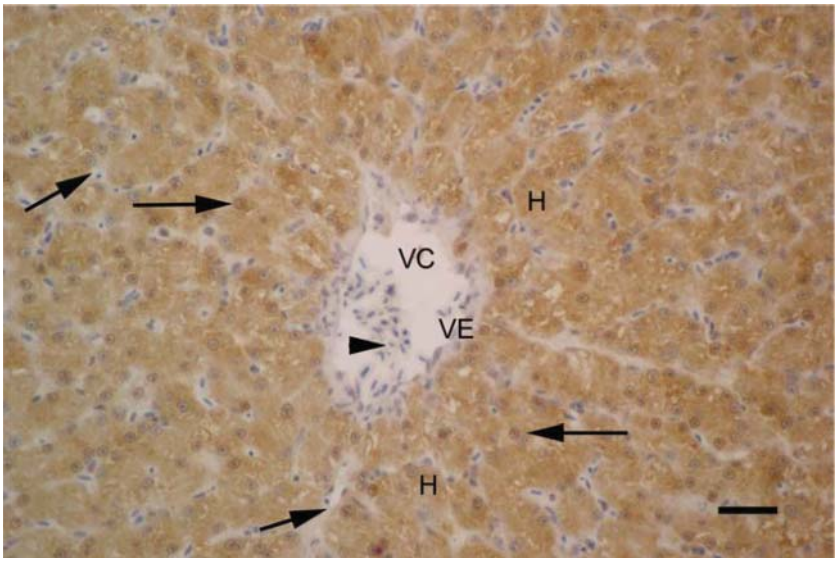

Fig. 4. Catalase positive reaction in geese liver. Diffuse catalase immunoreactivity in the cytoplasm of hepatocytes (H). Catalase positive in some of hepatocyte nuclei (long arrows), catalase negative in central vein (VC), vascular endothel (VE), erythrocytes (arrowhead) and sinusoids endothel (short arrows). Bar: 50 µm

Şekil 4. Kaz karaciğer dokusunda katalaz pozitif reaksiyon. Hepatositlerde yaygın sitoplazmik immun boyama (H). Bazı hepatosit çekirdeklerinde katalaz pozitif reaksiyon (uzun oklar). Vena sentralisin (VC) damar endoteli (VE), eritrositler (ok başı) ve sinüzoid endotelleri (kısa ok) katalaz negatif. Bar: 50 µm

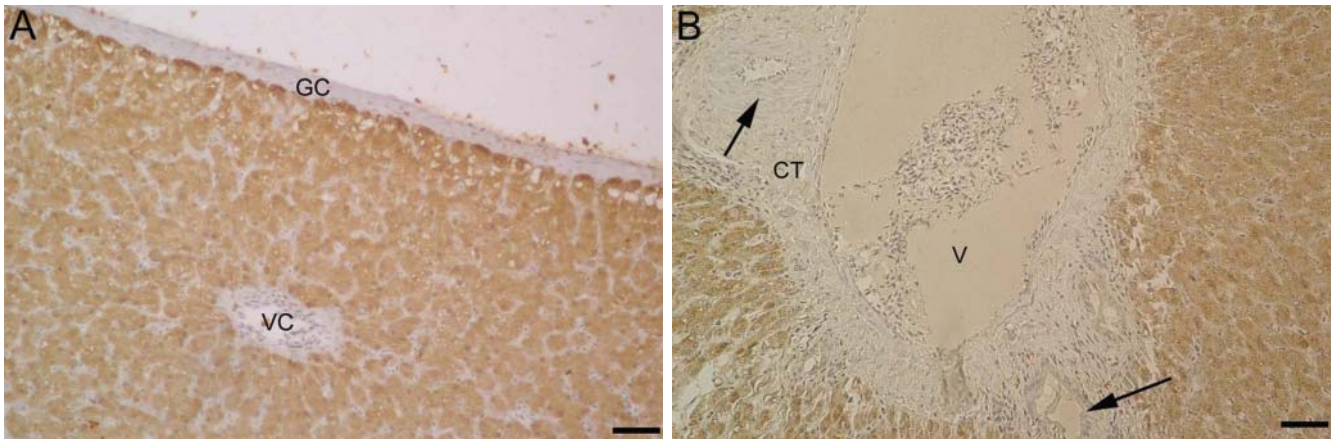

Fig. 5. Catalase immunoreactivity in geese liver. A) Glisson's capsule (GC), central vein (VC), Bar: 100 µm, B) Interlobular vein (V), ductus biliferus (long arrow), arteria hepatica (short arrow) and connective tissue (CT) were catalase negative. Bar: 100 µm

Şekil 5. Kaz karaciğer dokusunda katalaz immunoreaktivitesi. A) Glisson kapsülü (GC), Vena sentralis (VC), Bar: 100 µm. B) Vena interlobularis (V), duktus biliferus (uzun ok), arteria hepatika (kısa ok), bağ dokusu (CT) katalaz negatif. Bar: 100 µm

were detected in some hepatocytes which were in good agreement with other published results [16].

Catalase was shown to be located abundantly in the peroxisome of epithelial cells of liver [17]. Morikawa and Harada [18] reported that cytoplasm of hepatocytes had catalase as granular substances detected by immunofluorescence staining. In that study, it was also reported that specific immunofluorescence staining for catalase were observed in nuclei of hepatocytes, epithelial of bile ducts and vascular endothelium. Roels and Goldfischer [19] demonstrated that catalase immunoreactivity were not different between hepatocytes of portal and central areas in biopsy samples of human liver. Völkl et al.[20] reported that cytoplasm of hepatocytes had contained catalase as granular substances in rat liver. In this study, catalase immunoreactivity was seen in both the cytoplasm and nuclei of hepatocytes in geese liver. Immunoreactivity of catalase was not found in sinusoids, epithelial cells of bile ducts, endothelia of hepatic arter, interlobular vein and central vein, as well as Glisson's capsule and erythrocytes. Klotz et al.[21] reported that nearly 360 amino acids of catalase were structurally similar within 74 species. In this study, primary antibody prepared against bovine liver catalase showed positive reaction in geese liver, supporting the hypothesis of catalase gene significantly protected in phylogenetic process for both poultry and mammals.

In conclusion, antioxidant defense system was carried out especially by the hepatocytes in geese liver. Our findings will contribute in future studies associated with catalase contribution to poultry antioxidant defense system.

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