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A HISTOLOGICAL STUDY ON HEPATIC STRUCTURE OF *Lyciasalamandra arikani* (URODELA: SALAMANDRIDAE)

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The liver is one of the most important internal organ in the body. It plays a prominent role in many processes in the body, particularly those concerned with its metabolism (protein synthesis, storage metabolites, bile secretion and detoxification). This report represents the histological characterization of liver *Lyciasalamandra arikani*, endemic salamander to Turkey. Hematopoietic tissue structures were examined in the connective tissue of the perihepatic regions. The liver of *L. arikani* probably possesses immunologic capabilities due to the presence of lymphocytes in the perihepatic regions. The hepatic lobules consisted of both hepatocytes and sinusoidal blood capillary networks, in which hepatocyte-sinusoidal structures were formed. The hepatocytes were polygonal and had a rounded nucleus. There is a lot of differing content of melanin containing cells in the hepatic parenchyma. Periodic acid-Schiff (PAS) staining method showed large glycogen deposits in the clusters of melanin granules. These melano-macrophage centers probably act significant role in organs of heterothermic vertebrates including providing energy and protection against pathogens due to fact that heterothermic vertebrates can produce energy slowly and so are torpid related the blood flow rate at low temperatures.

Keywords: liver; histology; amphibian; hepatocyte; Kupffer cell; melano-macrophage centers.

INTRODUCTION

The liver is the largest internal organ in the body and many important functions are ascribed to the liver, which serves as a hub for carbohydrate, lipid, and amino acid regulation. These hepatic functions play a central role into maintaining the body's metabolic homeostasis and life. In addition to its metabolic activity, the liver actively participates defense, inactivating toxins and xenobiotics absorbed by the intestine and eliminating foreign particles from the body (Rappaport, 1967).

Amphibian liver is very important model for the study of interactions between environmental factors and hepatic structures. Thus research on amphibian liver is important, especially in the field of problems induced by pollution of both aquatic and terrestrial systems (Barni et al., 1999; Fenoglio et al., 2005; Rohr et al., 2008). Additionally, Akiyoshi and Inoue (2012) reported that a phylogenetic study of amphibian livers may be valid as an optimal model for liver ontogenesis in vertebrates to demonstrate the correlation between liver structures and phylogenetic status.

The genus *Lyciasalamandra* Veith and Steinfartz, 2004 contains ten allopatric species and subspecies from an area stretching for approximately 420 km along the Mediterranean coast of Turkey between Kaplanhanı Plateau (Alanya, Antalya) and Marmaris (Muğla) and some adjacent islands (Budak and Göçmen, 2005; Akman et al., 2011; Göçmen et al., 2011; Göçmen and Akman, 2012). The ground color of the dorsum in both sexes is honey yellowish, with yellowish-whitish green upper eyelids and yellowish white flecks on the flanks, not forming a distinct line. The ground color of the parotoids and the front side of the eyes, around the snout is typically lighter and unspotted than the other parts of the dorsum, being more orange in color. Over the ground color of dorsum, scattered and indistinct, especially in adults, small whitish spots are present (Göçmen and Akman, 2012).

The aim of the present study was to perform a histological and histomorphological characterization of liver tissue in *Lyciasalamandra arikani* Göçmen and Akman, 2012 and to compare the histological characteristics of the livers of other vertebrates.

MATERIAL AND METHODS

The present study was carried out according to the animal ethical committee of Ege University, Faculty of

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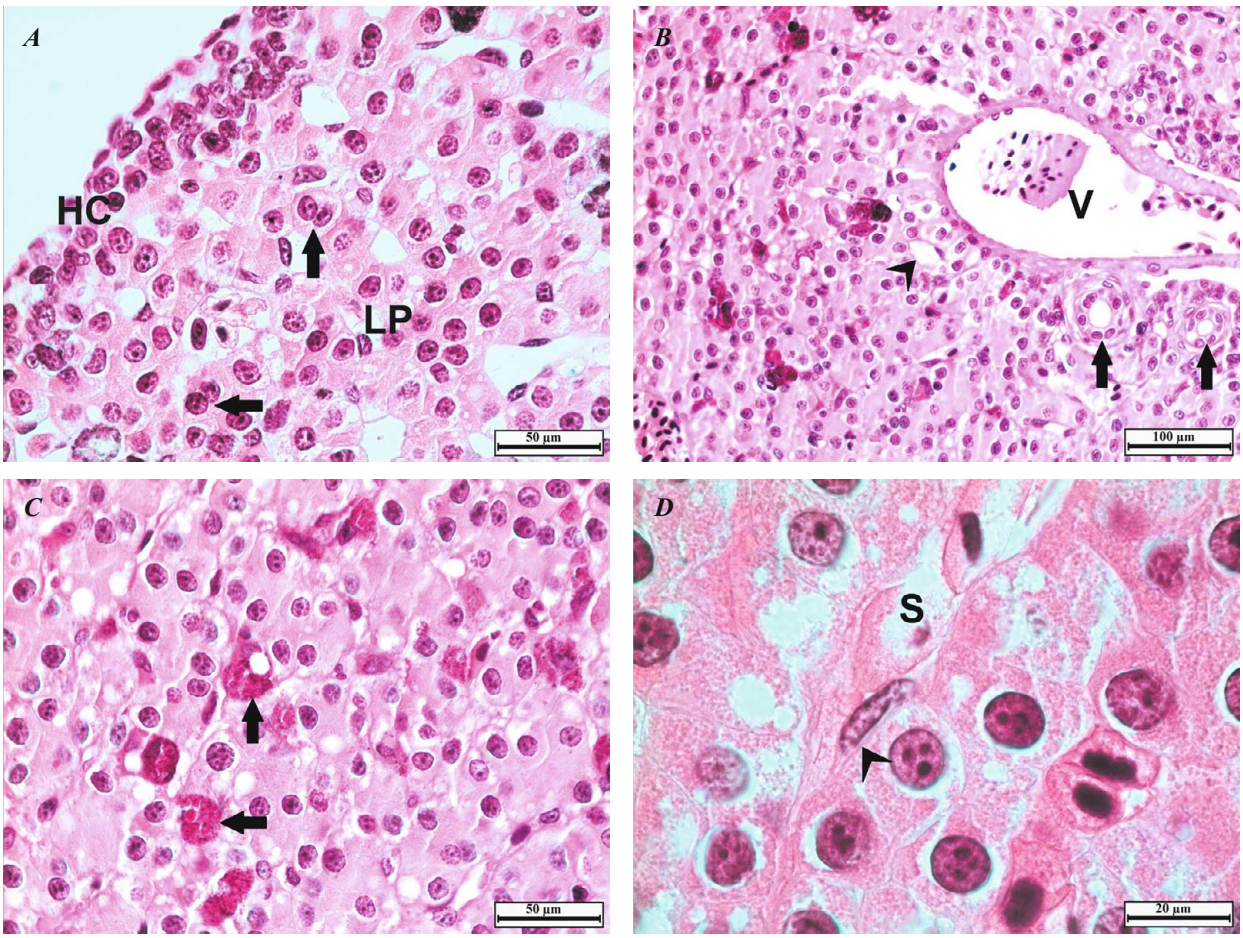

Fig. 1. (*A*) Histological structure of liver in *Lyciasalamandra arikani* HE: LP, liver parenchyma; HC, hematopoietic subcapsular tissue; binuclear hepatocytes (arrow). (*B*) Light microscopic view of a portal triad PAS; hepatic arterial (arrow head), portal vein (V), and bile duct (arrow). (*C*) Different content of the melanin containing and large glycogen deposits in the clusters of melanin granules (arrow), PAS. (*D*) The polyhedral hepatocytes and Kupffer cell (arrow head) from *L. arikani* HE: S, sinusoid.

Medicine (2011-091) and approved by Ministry of Forestry and Water Affairs (date: 11 April 2011, number: 42694). Four specimens (two adult males and two adult females) of *Lyciasalamandra arikani* were captured from Antalya/Turkey (Göçmen and Akman, 2012). Animals were anaesthetized with ether, and euthanized by decapitation. For light microscopic examinations livers were fixed in Bouin's fluid for 48 h at 4°C. Thereafter, the samples were washed with ethanol 70% for 24 h, and processed according to the standard histological protocols for paraffin embedding. Five micrometer serial sections were stained with Gill's hematoxylin-eosin (HE) and Periodic acid-Schiff (PAS). After the stain with PAS, counterstaining was carried out with HE. Sections were evaluated and photographed using a Leica DM3000 microscope (Leica Microsystems) that was equipped with a Leica digital camera (DFC290).

RESULTS AND DISCUSSION

The superficial liver of *L. arikani* covered with hematopoietic subcapsular tissue which was responsible for its support and protection (Fig. 1*A*). The liver of *L. arikani* may serve as the hematopoietic organ. The fetal liver of mice and human has the initial site of fetal hematopoiesis (Enzan et al., 1980; Medlock and Haar, 1983) and B cell development in mammals (Moore, 2004). Akiyoshi and Inoue (2012) studied on the 46 amphibian livers and reported that they showed a variety of histological features, but anurans were the same as in mammalian livers. The hepatocyte-sinusoidal structures of the amphibian livers were classified into three different types: (I) several-cell-thick plate type, (II) two-cell-thick plate type, and (III) one-cell-thick plate type, depending on the percentage extension of sinusoidal areas per unit

area. Hematopoietic tissue structures were observed in the connective tissue of both the perihepatic subcapsular regions and portal triads in Urodela and Gymnophiona, but were not observed in Anura (except for the genus *Bombina* Oken, 1816 and *Xenopus* Wagler, 1827). As phylogenetic relationships are branched from urodeles to anurans, the parenchyma arrangement progressed from the combined several- and two-cell-thick plate type to one-cell-thick plate type as seen in the mammalian liver type (Akiyoshi and Inoue, 2012).

In the hepatic portal area of *L. arikani* interlobular artery, vein and bile duct were observed. The lumen of interlobular artery was smaller than vein. The interlobular bile duct was composed of simple cuboidal epithelium (Fig. 1*B*). The liver of *L. arikani* was several-cell-thick plate type (Fig. 1*A*).

Hepatocytes of *L. arikani* were polyhedral and each hepatocyte contained large, round, centrally situated nucleus like in *Cynops orientalis* (David, 1873) and *Hynobius stejnegeri* Dunn, 1923 (Akiyoshi and Inoue, 2012; Xie et al., 2013). Additionally, binuclear hepatocytes were also observed (Fig. 1*A*), but not frequent. Sinusoids are interspersed within hepatocytes, which vary widely in size. The lumen of sinusoids contains erythrocytes and macrophages. Large cells resting on the luminal surface of sinusoid endothelium are present, these cells are known as Kupffer cells (Fig. 1*D*). Kupffer cells (macrophagocytus stellatus), also called stellate sinusoidal macrophages, are derived from monocytes and reside in liver sinusoids (Ross et al., 2003). They play a prominent role in host defense by eliminating toxic and foreign substances, removing degenerated red and white blood cells and degrading hemoglobin (McCuskey and McCuskey, 1990; Dini et al., 2002; Naito et al., 2004). Çakıcı and Akat (2013, 2013a) reported that an increase in the number of Kupffer cells were found in the liver of diazinon and propanil exposed mice.

A large number of melanin granules were gathered into clusters and distributed in liver parenchyma of *L. arikani* (Fig. 1*C*). Melanophores with developing melanosomes are situated throughout the hepatic parenchyma. Additionally, melanophores were observed in the dermis beneath the basement membrane of dorsal skin of *Lyciasalamandra billae* (Franzen and Klewen, 1987), *L. luschani basoglui* (Baran and Atatür, 1980), and *Hyla orientalis* Bedriaga 1890 (Akat and Arıkan, 2013; Akat et al., 2014). However the melanins of the liver pigment cells are considered belonging to the reticulohistiocytic system (also defined as the mononuclear phagocytic system) and deriving from macrophagocytus stellatus based on their localization and phagocytic capacity (Rund et al., 1998; Barni et al., 1999). It seems to play on important role as scavengers of cytotoxic substances such as ions and free radicals (Barni et al., 1999; Frangioni et al., 2005). Melano-macrophage centers, also known as macrophage aggregates, are distinctive grouping of pigment-containing cells within the spleen, kidney and liver of heterothermic vertebrates (Agius and Roberts, 2003). Ali et al. (2011) reported that the prominent improve in growth in addition the hyperactivation of melanophores and melanomacrophage centers in spleen and liver of *Oreochromis niloticus* (Linnaeus, 1758) treated by *Azotobacter* sp.

In *L. arikani*, large glycogen deposits were determined in the clusters of melanin granules, after Periodic acid-Schiff (PAS) staining method (Fig. 1*C*). These melano-macrophage centers probably act significant role in organs of heterothermic vertebrates including providing energy and protection against pathogens due to fact that heterothermic vertebrates can produce energy slowly and so are torpid related the blood flow rate at low temperatures.

Consequently, the histological and histochemical properties of the liver of *L. arikani* may be considered important features for taxonomic, phylogenetic and toxicological studies.

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