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A Histological Study on Liver of Near Eastern Fire Salamander, *Salamandra infraimmaculata* Martens, 1885 (Urodela: Salamandridae)

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Abstract: The aim of the present study is to examine the histological structure and to determine melanin-containing cells in liver for a better understanding of the role of these cells in *Salamandra infraimmaculata*. The surface of the liver is covered with haematopoietic tissue. The liver is divided into lobules. The boundaries between lobules are not clearly defined. Hepatocytes are radially arranged around the central vein. Melanin-containing cells are localised in the liver parenchyma. Although it is known that melanin-containing cells are present in various organs of heterothermic vertebrates such as kidney, liver, spleen and lung, their function in the visceral organs of lower vertebrates is still unclear.

Key words: amphibian, liver, histology, melanomacrophages

Introduction

The liver is a vital organ with prominent functions including protein synthesis and production of enzymes necessary for digestion. It also plays a role as a hub for carbohydrate, lipid and amino acid metabolism. Additionally, the liver actively participates in defence, detoxification and elimination of foreign particles from the body. All these hepatic functions are essential for maintaining the body's metabolic homeostasis and life (Kuntz & Kuntz 2008). Amphibian liver is important model for evaluating interactions between environmental factors and hepatic structures. Therefore, studies on microanatomy of amphibian liver are important for assessing pollution in both aquatic and terrestrial systems (Barni et al. 1999, Fenoglio et al. 2005, Rohr et al. 2008). In addition, phylogenetic study on microanatomy of amphibian liver may serve as an optimal model for liver ontogenesis in vertebrates to clarify the correlation between liver structures and phylogenetic relationships (Akiyoshi & Inoue 2012). Several studies have been carried out related to microanatomy of amphibian liver, which has been regarded as good environmental indicators. However, reports on microanatomy of amphibian liver are still scarce.

The genus *Salamandra* Laurenti, 1768 consists of six species and has a wide geographical distribution. Near Eastern Fire Salamander, *Salamandra infraimmaculata* Martens, 1885 is a thick-bodied, robust salamander with maximum total length up to 32 cm. The females are usually larger than males and cloacal region is swollen in males. This salamander species is black with yellow spots on its back but it has no coloration on the belly area. Adults are active at twilight, spending most of the day under snags and stones. During rainy weather, salamanders leave their hiding places. The adults feed on insects, earthworms, slugs and snails (Budak & Göçmen 2008, Amphibia Web 2017).

The aim of the present study is to assess histological and histomorphological characteristics of the liver of *Salamandra infraimmaculata* as well as to determine melanin-containing cells in liver for a better understanding of their role.

Materials and Methods

Four specimens of *Salamandra infraimmaculata* (two adult males and two adult females) were used in the present study. Animals were captured from their natural environment in Hatay, Turkey (36°49′25.16″ N, 36°20′35.58″ E), anaesthetised with ether and euthanised. Then the body cavity was opened and the liver was immediately fixed in Bouin's fluid overnight at 4°C. Liver fragments were processed according to the standard histological techniques for paraffin embedding. Five micrometers thick sections were stained with Mayer's haematoxylin-eosin (HE) (Humason 1962) and Periodic acid-Schiff (PAS) (Lillie 1951). Sections were examined and photographed using Zeiss Axio Scope A1 microscope that was equipped with a digital camera Axiocam Erc5s.

Results

The surface of liver in *Salamandra infraimmaculata* was covered with haematopoietic tissue (Fig. 1A). The liver parenchyma consisted of hepatocytes arranged into cords, which were separated by vascular sinusoids. Sinusoids were capillary networks and varied widely in size. The lumen of sinusoids contained erythrocytes and macrophages. The hepatocytes (epithelial cells of the liver) were polyhedral and had high nuclear : cytoplasmic ratio. The nucleus was spherical, rich in euchromatin and occupied mainly central position in hepatocyte (Fig. 1B). The liver was divided histologically into lobules in which the hepatocyte sinusoidal structures were formed. The interlobular connective tissue was undeveloped and, therefore, the boundaries between lobules were not distinct. Hepatocytes were radially arranged around the central vein. Portal areas, also called portal triads, were located at the corners of liver lobules (Fig. 1C). The parenchyma structure was a combination of several-cell-thick and two-cell-thick plate types.

The melanin-containing cells were localised in both parenchyma (at the sinusoidal level) and perihepatic subcapsular tissue (Fig. 1A). The accumulation of these pigmented macrophages in the liver formed melanomacrophage centres. Large glycogen deposits were identified in the melanomacrophage centres by PAS-staining procedure (Fig. 1D).

Discussion

The present study was carried out to examine histological and histomorphological characteristics of the liver in *Salamadra infraimmaculata.* Haematopoietic tissue was observed on the surface of the liver. Golub et al. (2004) reported that the liver and spleen in both larval and adult urodeles could serve as haematopoietic organs. Henry & Charlemagne (1977) have suggested that the perihepatic layer of urodelan amphibian *Pleurodeles waltlii* may be equivalent to the avian bursa of Fabricius. Additionally, the foetal liver of murine rodents is the initial site of haematopoiesis. Haematopoietic stem cells and colony-forming progenitor cells are believed to migrate from liver to bone marrow around the time of birth, where they remain throughout the animal's life (Wolber et al. 2002). Based on these studies, we could speculate that the liver of *S. infraimmaculata* probably acts as haematopoietic organ like in other urodeles and foetal murine rodents.

The liver of *S. infraimmaculata* consisted of hepatocytes arranged into cords, separated by vascular sinusoids. In higher vertebrates, hepatic plates line the simple-layered hepatocytes, the so-called one-cell-thick plates. In teleosts, hepatic plates line the multi-layered hepatocytes, or the so-called two-cell-thick or several-cell-thick plates (Elias & Bengelsdorf 1952, Akiyoshi & Inoue 2004). Akiyoshi & Inoue (2012) reported that the hepatocyte-sinusoidal structures of amphibian livers could be classified into three different types: several-cell-thick plate type, two-cell-thick plate type and one-cell-thick plate type. In *S. infraimmaculata*, the arrangement of liver parenchyma was a combination of the several-cell-thick and two-cell-thick plate types.

The melanin-containing cells were observed in both parenchyma (at sinusoidal level) and perihepatic subcapsular tissue of *S. infraimmaculata* liver. In heterothermic vertebrates, melanin-containing cells were reported in various organs such as kidney, liver, spleen, lung and skin (Zuasti et al. 1990, Agius et al. 2003, Akat et al. 2014). Studies on *Rana esculenta* L. revealed that pigment cells in liver (Pintucci et al. 1990, Guida et al. 1998, Corsaro et al. 2000) and spleen (Gallone et al. 2002) derived from haematopoietic stem cells. These pigmented cells are known as melanomacrophages due to their phagocytic capacity (Christiansen et al. 1996, Rund et al. 1998). In addition to their phagocytic activity, these cells in the liver parenchyma of lower vertebrates act against endogenous and exogenous cytotoxic substances (Scalia et al. 1990, Sichel et al. 1994, 1997).

Melanin synthesis as well as hypertrophy and proliferation of melanophores during prehibernation are associated with an increase of the pigmented area of the liver (Barni et al. 1999). Enzymatic studies showed that the pigmented macrophages from liver and spleen of *Rana esculenta* L. possess dopa-oxi-

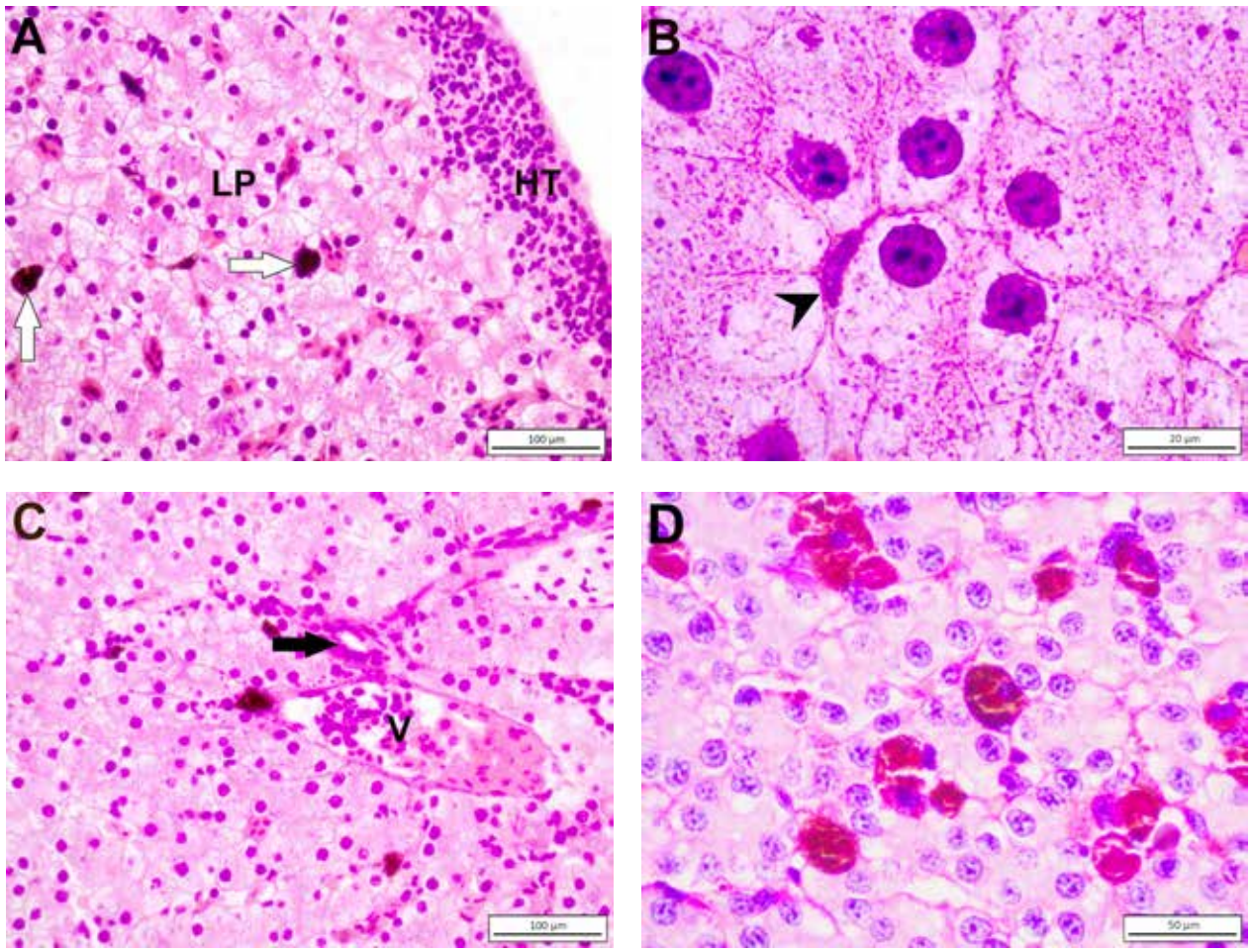

Fig. 1. Histomorphology of the liver of *Salamandra infraimmaculata*. **A.** Histological structure of liver and melanin-containing cells (white arrow), liver parenchyma (LP), haematopoietic tissue (HT), haematoxylin-eosin (HE), scale-bar=100 µm. **B.** Polyhedral hepatocytes with large round nucleus, Kupffer cell (black arrow head), HE, scale-bar=20 µm. **C.** Light microscopic view of portal area, portal vein (V), bile duct (black arrow), HE, scale-bar=100 µm. **D.** Melanomacrophage centres, PAS, scale-bar=50 µm

dase and peroxidase activities (Gallone et al. 2002). It was reported that the dopa-oxidase was directly related to the liver melanogenesis because its activity levels varied seasonally, in accordance with the liver melanin content, showing maximum level in winter and minimum in summer (Corsaro et al. 1990, Gallone et al. 2007). In another study, the amount and distribution of melanin in liver were reported in three amphibian species (*Rana esculenta*, *Triturus a. apuanus* and *T. carnifex*) during two periods of the annual cycle (summer activity and winter hibernation) (Barni et al. 1999). The content of melanin in liver was not stable in amphibians during the year (Corsaro et al. 1990, Barni et al. 1999).

The degree of the melanomacrophage centres (MMC) aggregation in spleen of *Prochilodus lineatus* was significantly different in all seasons. The number of MMC aggregation was high when the water temperature was below 20ºC and the minimum of MMC aggregation was observed at 30ºC in summer (Balamurugan et al. 2012). In another study, after treating *Pelophylax ridibundus* samples with Reldan 40EC insecticide (0.01 ml/g) and keeping them at 4-6ºC, an increase in the number of MMC in the liver was reported. However, when frogs were treated with Reldan 40EC (0.01 ml/g) and kept at 22-24ºC, results showed a decrease in the number MMC of liver (Paunescu *et al.* 2010). Paunescu *et al.* (2010) reported that an increase in the number of MMC during prehibernation phase but the reasons of the increase in the number of MMC were not well known.

Generally, the presence of melanin is often explained as cell protection against cytotoxic substances (Scalia et al. 1990, Sichel et al. 1997). Several authors pointed out connection between the activation of visceral melanogenesis and enzymatic activities such as the decline of antioxidant enzymatic activities during hibernation (Barni et al. 1999) or the maximum level of dopa-oxidase in winter (Corsaro et al. 1990, Gallone et al. 2007).

The function of melanomacrophages in the visceral organs of lower vertebrates has been the focus interest. Despite this interest, the questions of melanomacrophage functions in the visceral organs remain unclear.

As a result of falling of the tissue temperatures below the freezing point, water within the cell forms ice crystals and expands. This expansion can cause organelle damage and loss of membrane integrity. Strategies for the prevention of cell damage caused by freezing include the use of either permeating or non-permeating cryoprotectants. Permeating agents are small molecules which enter the cells and prevent the formation of ice crystal. Non-permeating agents remain extracellular and draw out the cellular water, thus preventing formation of intracellular ice crystal via dehydration (Woodruff & Snyder 2007).

In *Salamandra infraimmaculata*, large glycogen deposits were observed in the melanomacrophage centers by application of periodic acid-Schiff (PAS) staining method. Taking into account the above data, these glycogen deposits probably serve as non-permeating cryoprotectant. The glycogen deposit area in the liver provides the spontaneous movement of water molecules through a semi-permeable membrane of hepatocytes into extracellular region as a result of the concentration gradient. Thus, formation of intracellular ice crystals may be prevented by dehydration. Consequently, the histological and histochemical properties of *S. infraimmaculata* liver may be considered a model for assessing cryoprotection mechanism in heterothermic vertebrates.

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