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Histomorphological, Histochemical and Ultrastructural Studies on the Healthy Liver of Yemen Veiled Chameleon (*Chamaeleo calyptratus*) in Southern Saudi Arabia

Estudios Histomorfológicos, Histoquímicos y Ultraestructurales en el Hígado Sano del Camaleón Velado de Yemen (*Chamaeleo calyptratus*) en el Sur de Arabia Saudita

Amin A. Al-Doaiss[1]; Mohammed A. Alshehri[1]; Ali A. Shati[1]; Mohammad Y. Alfaifi[1]; Mohammed A. Al-Kahtani[1]; Ahmed Ezzat Ahmed[1]; Refaat A. Eid[2]; Laila A. Al-Shuraym[3]; Fahd A. Al-Mekhlafi[4]; Mohammed Al Zahrani[5] & Mohammed Mubarak[5]

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SUMMARY: The livers of reptiles are being studied as a model for the link between the environment and hepatic tissue. There have been few investigations on the histology of reptile livers, and very few or no studies have examined the histology of liver of veiled chameleon (*Chamaeleo calyptratus*). This paper describes the histomorphological, histochemical and ultrastructural characterization of the liver of veiled chameleons in southern Saudi Arabia. Seven *Chamaeleo calyptratus* were captured in the summer season in Abha City, Aseer region, southern Saudi Arabia. Chamaeleon liver samples were processed for histomorphology, histochemistry and ultrastructure analyses. Morphologically liver of *Chamaeleo calyptratus* was observed as a large dark brown organ with lighter speckles, which represent melanin deposits. It located at the ventral part of abdominal cavity forward of the stomach. Its dimensions approximately were 3.7×2 cm. The liver was a bilobed organ divided into two lobes, right and left lobes. The right one was bigger than the others. The gallbladder was well developed and had an elongated shape, situated between the two lobes and contained the bile for the digestion. Microscopically, the liver was found to be covered by a thick layer of connective tissue, which formed the hepatic capsule. Hepatic parenchyma probably appeared in cross sections as hepatic glandular-like alveoli “acini” or follicular structures with various diameters, each acinus contains approximately four to six hepatocytes, surrounded by sinusoidal capillaries filled with abundant melanomacrophages, which are absent in birds and mammals. Melanomacrophages are common in the hepatic parenchyma's perisinusoidal areas, particularly near portal spaces. Hepatocytes are polyhedral or pyramidal with and mostly contained large, rounded nuclei mostly peripherally located, with prominent dark oval nucleoli. Some of nuclei are eccentric or central position. The cytoplasm appeared spongy or vacuolated and more eosinophilic when stained by hematoxylin-eosin and strongly reactive to PAS staining technique, indicating abundant glycogen content. The reticular fibers that surround hepatocytes, blood arteries, and sinusoids supported the hepatic parenchyma. The blood sinusoids are seen interspersed among hepatocytes of varying sizes. The sinusoidal lumen was bordered by flattened endothelial cells and includes elliptical nucleated erythrocytes and liver macrophages as phagocytes, which are also known as Kupffer cells. Branches of the portal vein, hepatic artery, small bile duct, and lymph vessels were detected in the hepatic portal area “tract” or triad which made up of connective. Hematopoietic tissue was observed in subcapsular region and portal triads. Ultrastructurally, the hepatocyte appeared polyhedric containing a single large rounded basal or eccentric vesicular nucleus with prominent nucleolus. Extensive network of rough endoplasmic reticulum (RER) often arranged in an array parallel to the nuclear membrane with many mitochondria, and Golgi apparatus were described. The cytoplasm contained glycogen granules, vesicles or vacuoles scattered throughout the cytoplasm especially at the apical region were reported. The bile canaliculi and the hepatic "Kupffer" cells were also discussed. This is the first study on the histological characterization of the healthy liver of Yemen veiled chameleon in southern Saudi Arabia. The findings reported here should be used as a reference to compare with the pathological abnormalities of the liver in this animal.

KEY WORDS: Liver; *Chamaeleo calyptratus*; Yemen veiled chameleon; Histomorphology; Reptiles; Hepatocytes.

[1] Biology Department, College of Science, King Khalid University, P.O. Box 9004, Abha 61413, Saudi Arabia.
[2] Pathology Department, College of Medicine, King Khalid University, P.O. Box 62529, Abha 61421, Saudi Arabia.
[3] Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, PO Box 88428 Riyadh 11671, Saudi Arabia. laalshuraym@pnu.edu.sa
[4] Department of Zoology, College of Science, King Saud University, PO Box 2455, Riyadh 11451, Saudi Arabia. falmekhlafi@ksu.edu.sa
[5] Department of Biology, College of Science, Imam Mohammad Ibn Saud Islamic University (IMSIU), Riyadh 11623, Saudi Arabia.

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INTRODUCTION

Reptiles are the planet's oldest fully terrestrial vertebrates, dating back millions of years (Laslie, 2018). Reptiles include Crocodilia, Rhynchocephalia, Testudinata, and Squamata. The Squamata order comprises lizards, which are terrestrial creatures with well-defined feeding patterns (Frimiano *et al*., 2011). The Chamaeleonidae lizard reptile family is distinguished by morphological features like as a prehensile tail, independently spinning eyeballs, a ballistic tongue, and the ability to change colors. Chameleons or chamaeleons (family Chamaeleonidae) are a group of saurian that have piqued the interest of biologists, veterinarians, and herpetology enthusiasts due to their unique anatomical and physiological properties, as well as their aesthetics, as of 2015, there were 202 species described (Glaw, 2015; Melero Jurado, 2020; Al-Shehri, M. A. & Al-Doaiss, 2021). Chameleons have many morphological and physiological characteristics as with all ectothermic reptiles, with an optimal temperature zone ranging from 20 to 38 °C (Melero Jurado, 2020). The chameleon, like other reptiles, plays a crucial role in the ecology, by acting as a predator for various non-vertebrate and tiny vertebrate creatures (Ahmed *et al*., 2018).

Chamaeleon calyptratus (veiled chameleon), also known as Yemen chameleons due to its origin. It is the most well-known chameleon in the world and the most commonly seen in veterinary medicine (Melero Jurado, 2020). The veiled chameleons are diurnal and arboreal lizard species that are highly independent. In nature, *Chamaeleon calyptratus* is a colorful reptile, indigenous to the Western Yemen, Asir Province, Saudi Arabia's southwest of the Arabian Peninsula. It lives primarily in humid coastal lowlands, coastal slopes, and high plateaus (Gillette & Krysko, 2012). The average lifespan of the veiled chameleon is 5 to 8 years, depending on sex. Males have an average lifespan of 8 years, whereas females have a shorter lifespan 5 years, due to producing eggs over time. body (Melero Jurado, 2020). ("Veiled Chameleon Care Sheet [2022 Updated]," n.d.). Like other lizards in nature, males are brighter and banded in colors of bright green, yellow, and brown, while females are green with no color pattern. The tall casque (helmet) on top of the head, is the simplest method to recognize the veiled from other chameleons. If they are the same size or age, the male casque is usually visibly bigger than the female, but females have a larger body. Tarsal spurs on the back foot of the male, are fleshy triangles that seem to be ankles (Melero Jurado, 2020). The chameleon's body is laterally flattened. Males may grow to be 62 cm (18 to 24 inches) in length, while females can grow to be 45 cm (10 to 13 inches). At the age of 5-6 months, they acquire sexual maturity. Males live an average of five years in captivity, while females live an average of three years, with males living up to ten years. *Chamaeleo calyptratus* is typically a quiet, solitary species, but males are extremely territorial and will engage in combat with other males (Melero Jurado, 2020). Like other chameleons, they rarely drink from a water container. At least three times a day, a drip or artificial rain system should be installed, or water should be sprayed on the leaves. They are one of just a few chameleon species that can endure temperatures ranging from 23 to 35 °C. usually, they can withstand temperatures ranging from 24 to 26 °C during the day and 18 to 20 °C at night. If the chameleons kept in captivity, you need to create a surrounding that can mimic the natural environment. They require direct sunshine, UV light designed for reptiles, a photoperiod of 12-14 hours of light and 10-12 hours of night to prevent metabolic bone disease and vitamin D deficiency, and a humidity level at about 60 to 80 %. Veiled chameleons are primarily tree-dwellers. You may provide branches and woods for them to climb inside the cage. In the wild, the veiled chameleon is a solitary reptile. As a result, when a veiled chameleon achieves maturity at roughly 8 to 10 months of age, it should always be housed in a single cage (Melero Jurado, 2020; ReptilesWeb, 2023). Veiled chameleons have large eyeballs that rotate on turrets and can swivel 180 degrees and look in any direction without rotating. their tongues are swift and lengthy, measuring nearly 2.5 times the length of their bodies when fully extended. The prehensile tail serves as a fifth limb for balancing and stabilizing while grasping trees. The reptile is an omnivore, which means it eats insects as well as fruits and vegetables (Eid *et al*., 2011; Gillette & Krysko, 2012; Lustig *et al*., 2013; Melero Jurado, 2020).

Chamaeleo calyptratus is currently classified as follows: Kingdom Animalia; Phylum Chordata – chordates; Subphylum Vertebrata; Infraphylum Gnathostomata; Superclass Tetrapoda; Class Reptilia – Reptiles; Order Squamata – Lizards; Suborder Iguania – Iguanas; Family Chamaeleonid – Chameleons; Subfamily Chamaeleoninae; Genus Chamaeleo – Chameleons; Species *Chamaeleo calyptratus*; Infraspecies *Chamaeleo calyptratus calyptratus* – Chameleons; Species *Chamaeleo calyptratus*; Infraspecies *Chamaeleo calyptratus calyptratus* (Barten & Simpson, 2019; Melero Jurado, 2020).

The liver is the largest vital accessory gland accompanied with the digestive system of the vertebrates including the lizards. It plays a role in numerous and complex functions: metabolic processes, bile secretion for fat emulsification and digestion, carbohydrate storage, production of plasma proteins, fetal hematopoiesis and detoxification of the blood (Akat & Göçmen, 2014). The liver participates in the storage of glycogen as an energy source that can be used in a variety of animal activities such as sexual reproductive efficiency and metabolic variations throughout the year, particularly in species influenced by cold climatic fluctuations "hibernation period" (Frimiano *et al*., 2011; Akat & Göçmen, 2014: Kardong, 2018: Pawlina & Ross, 2018). It is one of the most essential organs in the reptile's body. Because it collects all the material received by the gut, secretes bile and plasma proteins, and plays an important function in the body, it is the site of several metabolic activities (Moura *et al*., 2009). The livers of reptiles have been characterized macroscopically and microscopically by a few authors. A limited number of studies have discussed the anatomy of the digestive system including the liver (Laslie, 2018). Reptile livers are used as a model for studying the relationship between the environment and hepatic tissue. There have been few investigations on the histology of reptile livers (Ahmed *et al*., 2018). The reptile liver is a good model for investigating the links between environmental influences and hepatic architecture. Thus, research on reptile liver is critical, especially in the field of problems affectingboth aquatic and terrestrial systems (Akat & Göçmen, 2014; Ahmed *et al*., 2018). The approach to animal organ histology is centered on using this knowledge effectively understand the pathophysiology of animal diseases (Moawad *et al*., 2017).

Although numerous studies on reptile livers in general have been conducted, and numerous researchers have investigated the morphology and histology of Chamaeleon organs such as the tongue, skin, and eyes, and few studies have examined the infected liver. Furthermore, no studies of the healthy liver of *Chamaeleo calyptratus*, particularly its histomorphology and ultrastructure, have been conducted (Laslie, 2018). As a result, the purpose of this study was to show the morphology, histology, histochemistry, and ultrastructure characterization of the liver of the veiled chameleons in southern Saudi Arabia.

MATERIAL AND METHOD

Animals "Chameleons". The present study was carried out on seven 1-year-old, 175-190 g, adult, mature male *Chamaeleo calyptratus* (Fig. 1). During the summer of 2021, the animals were seized in Abha City, Aseer area, Saudi Arabia. The caught animals were taken to the reptilian laboratory at King Khalid University's Biology Department, College of Science, where all experimental procedures were carried out. Before their euthanasia and dissection, the animals were kept in adequate cages at ambient temperatures (23 ±1.5 °C) with natural photoperiods (12 hours of a light-dark cycle). Individuals were ether-anesthetized and subjected to gross, histological, and histochemical examinations.

Gross examination. The gross morphological features of the liver of adult male veiled chameleons were demonstrated. Before being sacrificed, the animals were anesthetized with ether. According to (Jacobson, 2007; Terrell & Stacy, 2021), the animals were opened through a medium incision from the cloaca to the anterior limbs, exposing all the visceral organs including the livers for gross morphological examinations by removing the rib cage and coelomic wall on one side (Fig. 2).

Histological and histochemical studies. Fresh pieces of each chameleon's liver (5 mm) were cut quickly and preserved in neutral buffered formalin (10 %) and Bouin's solution for 48 hours. The fixed liver specimens were processed for paraffin techniques using an Automatic Tissue Processor (ATP 1000, Histo-line, Italy) and dehydrated with grades of ethanol (70, 80, 90, 95, and 100 %), cleared in two changes of xylene, and impregnated with two changes of molten paraffin wax. Modular Tissue Embedding Center (Myr, EC350, Spain) was used to embed and block out the specimens, and a series of section thicknesses (4-5 μm) were cut using a rotary microtome (MR2258, Histo-line, Italy). The liver sections were stained with the following stains: hematoxylin and eosin (H&E) for general histology, periodic acid-Schiff (PAS) for neutral carbohydrates, and Masson trichrome for collagen fibers, as well as mercuric bromophenol blue (MBPB) using an Automated Slide Stainer

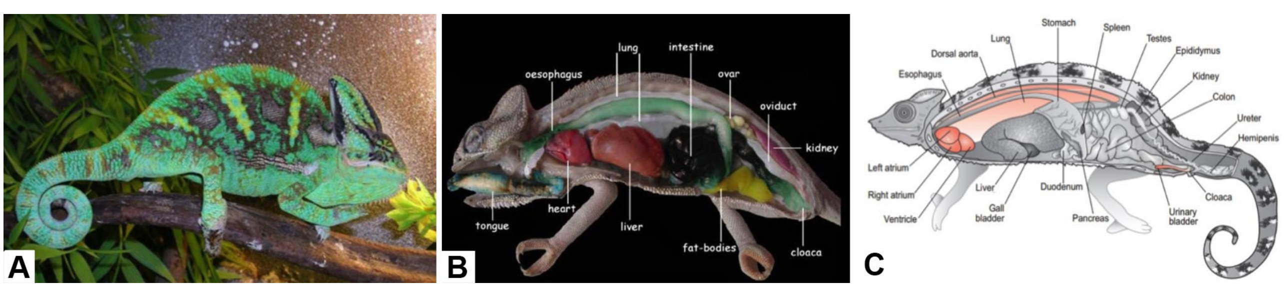


Fig. 1A-C. (A): Image of an adult male veiled chameleon (*Chamaeleo calyptratus*) adapted from Melero Jurado (2020). Note: Inset our catched *Chamaleo calyptratus.* (B-C): lateral midsagittal skitchs of an adult male veiled chameleon (*Chamaeleo calyptratus*) adapted from O'Malley (2005) and Melero Jurado (2020).

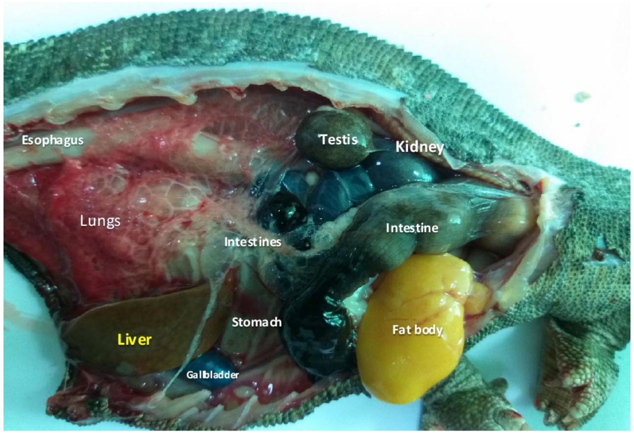


Fig 2. Topography of male of the veiled chameleon, *Chamaeleo calyptratus* showing left lateral midsagittal view of the body, showing the coelomic cavity of the animal after removing the skin and ribs. The chameleon has large dark brown liver with two lobes (right & left) and large gallbladder, anatomically the liver is located in the ventral side of the coelomic cavity and surrounding the pancreas, intestine, stomach and the lungs. Bar: 20 mm.

(Myr, SS30, Spain). An optical microscope (Olympus Microscope BX53 with DP73 Digital Camera, Japan) was used to inspect and photograph all stained sections. The techniques of Bancroft (2008) and Suvarna *et al*. (2018) were used for staining.

Transmission electron microscopy. For the transmission electron microscopy (TEM), small portions (3.0 mm) of liver samples of chameleons were fixed in in 2.5 % glutaraldehyde solution in phosphate buffer (pH 7.2–7.4) overnight at 4 ºC, and then in 1 % osmium tetroxide. Samples were dehydrated at increasing concentrations of ethanol before being embedded in Epon resin. Polymerization of resin-embedded samples was allowed to occur at oven temperature (60 ºC). A glass knife was used to cut semithin (0.5 -1µm) and a diamond knife to make ultrathin (80-100 nm) sections. To select the suitable areas representing the desired observations, semithin sections were stained with toluidine blue and studied with light microscopy. Ultrathin sections were stained with 5 % uranyl acetate and lead citrate on copper grids. Finally, these grids were analyzed with the TEM (JEOL1011, Japan) at King Khalid University's Electron Microscope Unit, Abha, Saudi Arabia.

RESULTS

Morphology of the liver. At the gross anatomy level, the liver of male *Chamaeleo calyptratus* is a large dark brown organ, located at the ventral part of the coelomic cavity, surrounding the stomach, lungs, intestines, and fat bodies. The liver is measured approximately 3.7 × 2 cm. (Fig. 1). As with all lizards, the chameleon's liver is bilobed organ composed of right and left lobe. It was easy to distinguish between the right and left hepatic lobes because the right lobe was larger and more defined than the left, which was viewed as a triangular form structure. The structure of the left hepatic lobe was fusiform. The liver is connected to the dorsal body wall by a strong falciform ligament. The liver possesses large dark green gallbladder which positioned between the two lobes in the notch on the inner surface of the right elongated lobe of the liver and aids in fat digestion. The bile duct links the liver to the duodenum. The hilus is the center location of the liver, through which the portal vein and the hepatic artery travel (Figs. 2 and 3).

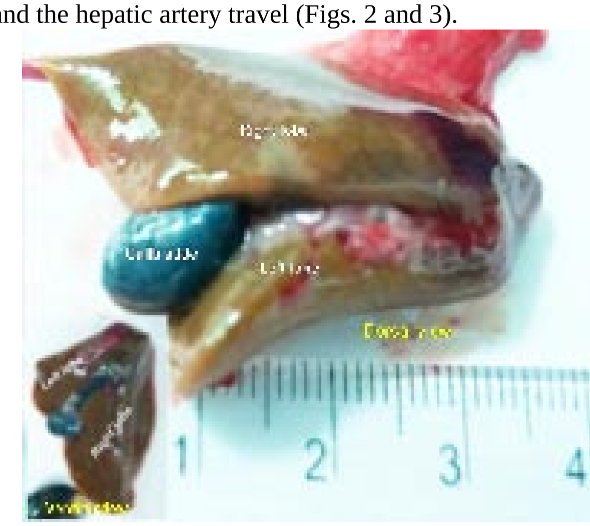


Fig 3. Photograph of liver of the male Chameleon (*Chamaeleo calyptratus*) showing left and right lobes with dark brown color associated with big dark green pear-shaped gallbladder lying under the right lobe.

Microscopic analysis of the liver

The hepatic capsule and hepatic organization. The liver of male *Chamaeleo calyptratus* is coated by a thin layer of connective tissue that forms the hepatic capsule, according to microscopic analysis. The hepatic capsule is found in all livers of vertebrates. this capsule composed of dense irregular connective tissue" collagen fibers" and containing smooth muscle fibers. Thin trabecular connective tissue descending from the hepatic capsule helps to divide the hepatic parenchyma into structural units known as hepatic lobules in most of the vertebrates (Fig. 4A, B). The liver of *Chamaeleo calyptratus* does not appear to be organized into lobules. Furthermore, the hepatic plates are not positioned radially around the central vein. The hepatic plates arranged as irregular follicles or alveoli "acini" surrounded by connective tissue and melanomacrophage (Fig. 5A, D). The

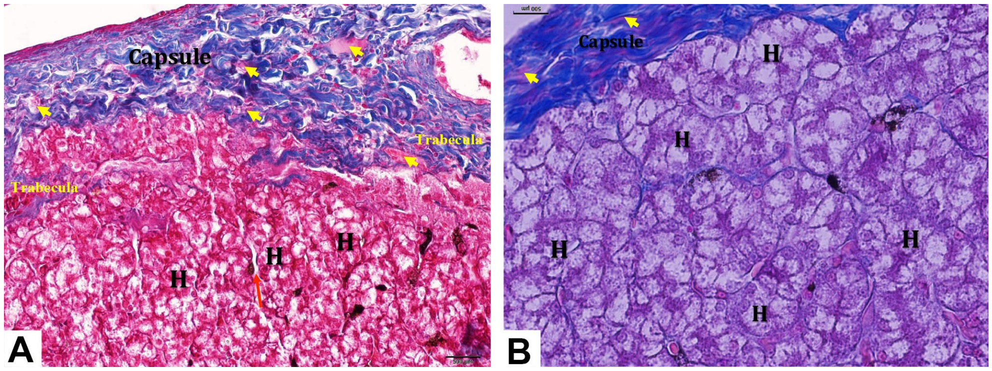
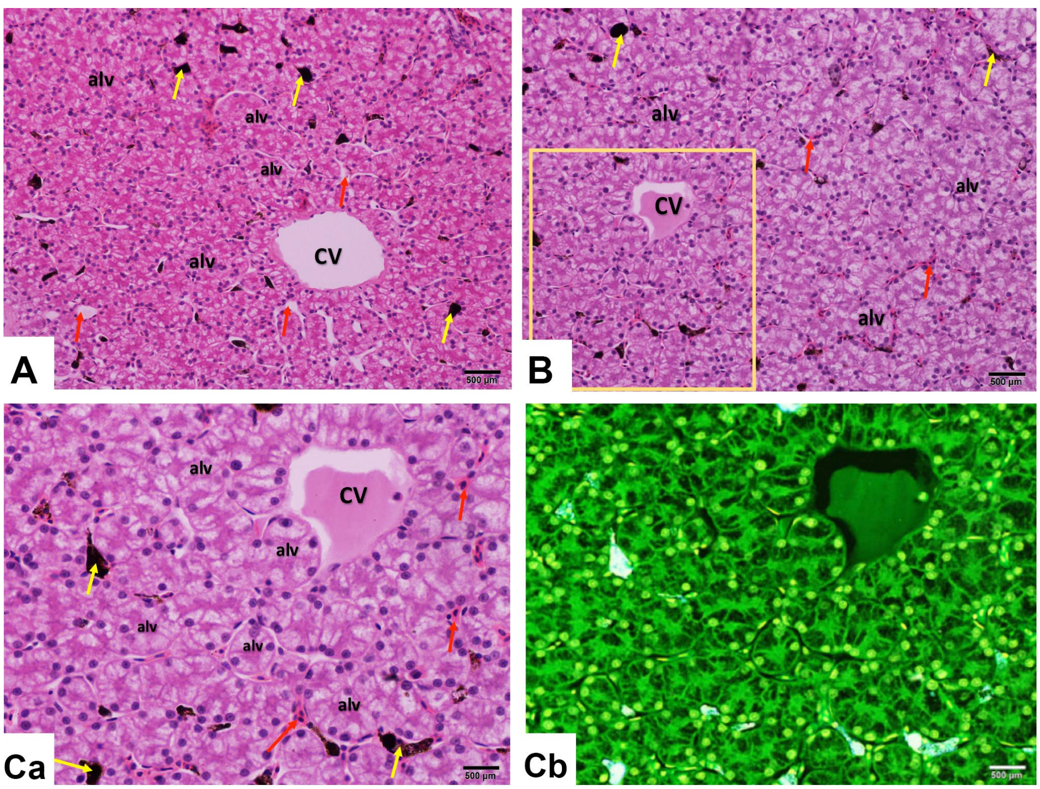


Fig 4A, B. Light photomicrographs of the liver of male Chameleon (*Chamaeleo calyptratus*) showing a thick hepatic capsule (Glisson's capsule) composed of dense fibrous connective tissue and trabeculae "blue color". The capsule invested the hepatic parenchyma (H). In both subcapsular areas and portal triads, hematopoietic tissue structures were detected in the connective tissue (arrow heads; A): Mallory trichrome stain, (B): Masson trichrome stain), 200×.



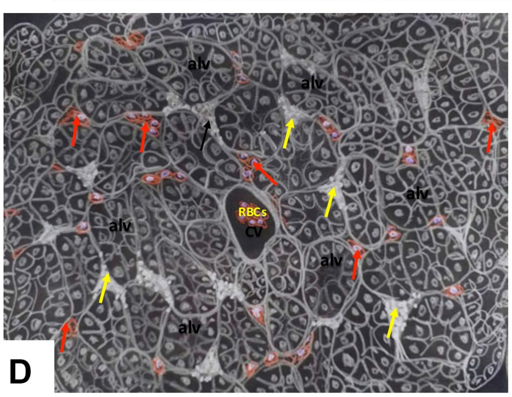


Fig.5A-D. Photomicrographs of a cross section through the liver of a male *Chamaeleo calyptratus*. (A-B): There is no apparent lobular organization. Hepatic cords are not radially arranged around the central veins (CV) they arranged as irregular hepatic follicles or alveoli "acini" (alv) surrounded by connective tissue, blood sinusoids (red arrows), and melanomacrophages (yellow arrows) containing a brown-stating materials. 200×. H&E stain. (Ca): Photomicrograph of high magnification of area in the square in the last figure, showing more details about hepatic organization and arrangement of hepatic plates (H) around the central veins (CV) as irregular follicles or tubules. 200×. H&E stain. (Cb): negative image to show more details. (D): skitch of a cross section through the liver of a male *Chamaeleo calyptratus* showing the hepatic organization.

bile ducts, portal vein and hepatic artery branches are showed in the connective tissue of portal area of the hepatic tissue (Fig. 6). The liver of a male *Chamaeleo calyptratus* has good central vein (CV) filled with nucleated RBCs in the lumen. It lined by endothelial cells and surrounded by layer of collagen fibers (Fig. 7A, B).

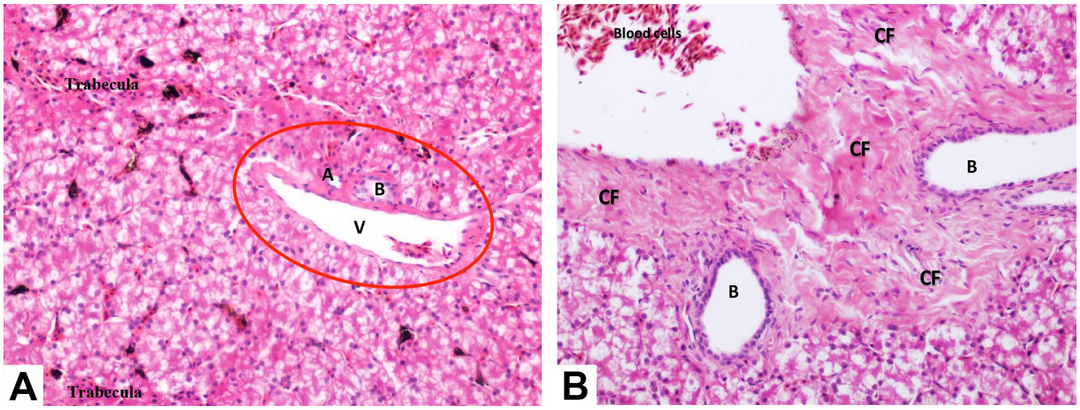


Fig 6A, B: (A): Photomicrographs of liver of male Chameleon (*Chamaeleo calyptratus*) showing the portal area or “triad" of the liver (in the circle; B): portal area or triad containing a portal vein (V), hepatic artery (A) and small bile ductules (B) embedded in large amount of collagen fibers connective tissue (CF).

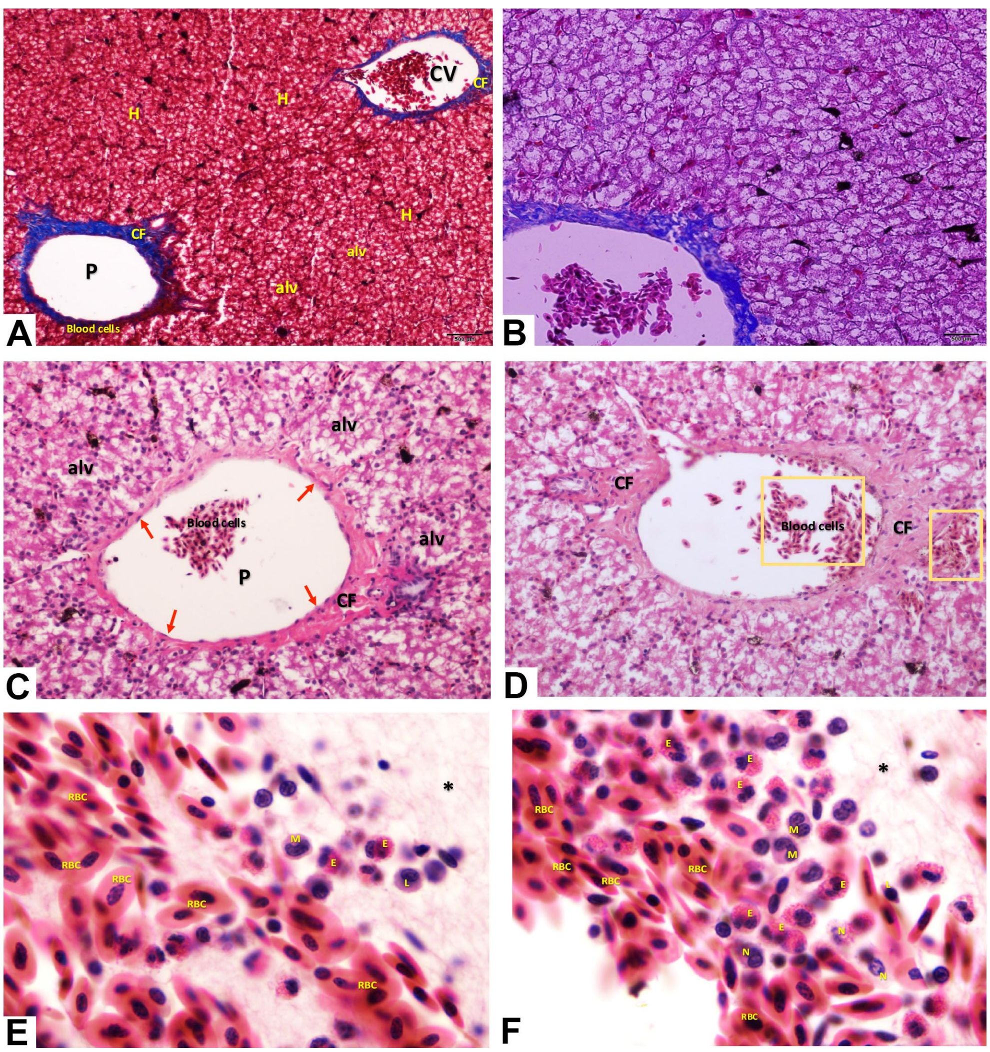


Fig.7A-F. Photomicrographs of a cross section through the liver of a male *Chamaeleo calyptratus* showing hepatic tissue (H) and irregular hepatic follicles or alveoli “acini” (alv). It contains central vein (CV) and portal triad (P) filled with nucleated blood cells in the lumen. Central vein and portal vein lined by endothelial cells (red arrows) and surrounded by a layer of the collagenous fibers (CF) (A): Masson trichrome stain, (B): Mallory trichrome stain, (C-D): H&E stain, 200 ×. (E-F): Photomicrographs of high magnification of area in the squares in the last figure, showing large elliptical nucleated erythrocytes with oval nuclei and different types of leukocytes (monocytes” M”, lymphocytes ”L”, eosinophils “E”, lymphocytes “L”, neutrophils “N”) contained within the blood plasma (*) in the lumen of blood vessels and blood sinusoids H&E stain, 1000 ×.

Hepatocytes. The hepatocytes of *Chamaeleo calyptratus* are large polyhedric and come in varied sizes with large rounded vesicular nuclei and prominent nucleoli. The nuclei are displaced toward the periphery of the cells. The hepatocytes are separated from the sinusoids by a narrow subendothelial space called the Disse space. The sinusoids are capillaries coated by endothelial cells and macrophages, which are known as Kupffer cells in the liver. Light microscopy examinations revealed that hexagonal hepatic lobules cannot be seen, hepatocytes are not organized in cords along the sinusoids, but only the tubular or alveolar (acinar) arrangement is apparent with narrow lumens (Fig. 8A, B).

The cytoplasm of the hepatocytes of *Chamaeleo calyptratus* appeared highly vacuolated and lightly stained by H&E (Fig. 8C) and reacted weakly to PAS (Fig. 8D). These findings indicated an abundance of glycogen in the cytoplasm. This is a characteristic commonly found in healthy individuals, since the stock of hepatic glycogen is the main reserve energy source. The parenchyma is supported by delicate reticular fibers that surround the hepatocytes and sinusoids.

Melano-Macrophage centers "melano-macrophages cells". All the staining techniques utilized in *Chamaeleo calyptratus* liver parenchyma allowed for the detected of a considerable number of melano-macrophage centers (MMCs), which are unique groups of pigment-containing cells known as melano-macrophages (MMs). They are normally found in the hepatic tissues of amphibians, reptiles, and some fish, but absent in birds and mammals. The hepatic cells of *Chamaeleo calyptratus* are similar to those of other vertebrates, has abundant Melanomacrophages centers or macrophage aggregates (Figs. 9A, B and 10). The melano-macrophages are numerous in amphibians and reptiles, except among snakes, in which they are less plentiful. These cells have various functions among them synthesis of melanin, phagocytosis and neutralization of free radicals.

Gallbladder. The gallbladder is a big dark green pear-shaped sac lying under the right lobe (Fig. 3). It is contained within a notch, and it clearly protrudes beyond the surface of the liver. The biliary canaliculi unite randomly to generate bile ducts, the biliary system is comparable to that of mammals. The bile is carried by these channels to the gallbladder, where it is stored and concentrated.

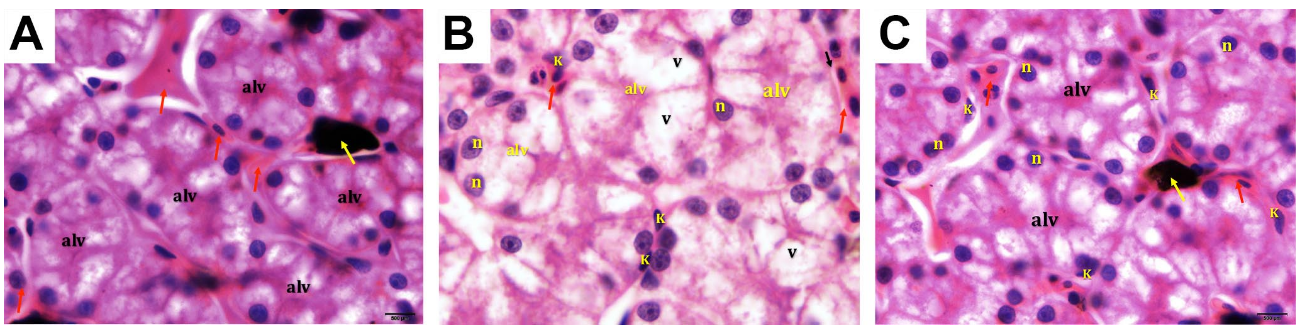


Fig.8 A-C. Photomicrographs of a cross section through the liver of a male *Chamaeleo calyptratus* demonstrates the sponge-like appearance of the parenchyma, which is composed of polyhedral hepatocytes with large peripherally located spherical nuclei having prominent nucleoli. Hepatocytes are not organized in cords along the sinusoids, but only the follicular or alveolar (acinar) arrangement is apparent and separated by sinusoids (red arrows) containing erythrocyte. The sinusoids are capillaries coated by endothelial cells and macrophages, which are known as Kupffer cells (K) in the liver. The hepatocytes are separated from the sinusoids by a narrow subendothelial space called the Disse space (black arrows). H&E stain, A: 200×, B-C: 1000×.

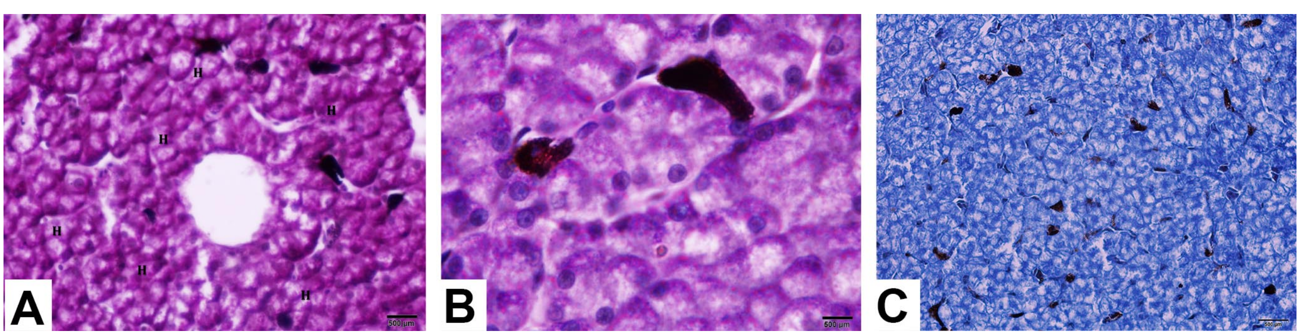


Fig.9A-C. Photomicrograph of a cross section through the liver of a male *Chamaeleo calyptratus* displaying (A,B): the hepatic cells (H) with PAS positive granules, which signify the cells' glycogen content. The granules predominantly concentrated at one side of the cells, 200×, 400×. (C): reacted weakly to mercuric bromophenol blue stain (MBPB), 200×.

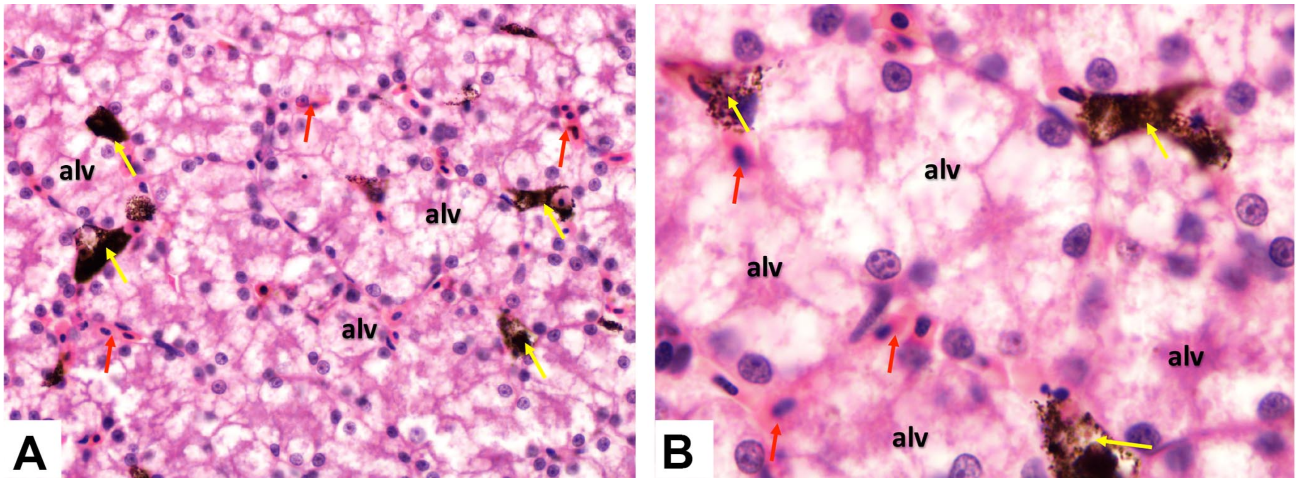


Fig.10 A,B. Photomicrograph of a cross section through the liver of a male *Chamaeleo calyptratus* showing massive Melanomacrophage Centers "MMC" (yellow arrows) and melanomacrophage harboring brownish-black staining materials. MMC as a histological marker of immune function in reptiles, fish, and other poikilotherms. MMC is found in the blood sinusoids (red arrows) between the hepatic alveoli (alv). The parenchyma is supported by delicate reticular fibers that surround the hepatocytes and sinusoids. In the hepatic blood sinusoids, elliptical erythrocytes with centrally placed nuclei and homogeneous cytoplasm are seen (red arrows). H&E stain, 200×, 1000×.

Histochemical studies of the liver. Light microscopy of the liver revealed that the hepatocyte glycogen granules stained intensely and positively with magenta color to periodic acid and Schiff's reagent (PAS+) for neutral carbohydrates (Fig. 9A, B). Hepatocytes stained faintly for proteins using the mercuric bromophenol blue method (MBPB+) (Fig. 9C).

Transmission electron microscopic examination. The ultrastructural investigation of the liver of *Chamaeleo calyptratus*' showed large hepatocytes separated by cell membranes that form the boundaries between the hepatocytes. Ultrastructurally, the hepatocyte appeared polyhedral in the shape containing a single large rounded basal or eccentric vesicular nucleus with prominent high electronic density nucleolus. The chromatin is granular, euchromatin and heterochromatin distributed through the space of the nucleus. Extensive network of rough endoplasmic reticulum with many mitochondria, and Golgi apparatus. (Fig. 11A). The perinuclear region of hepatocyte showed, nuclear envelope composed of inner and outer nuclear membrane fused at nuclear pores. The heterochromatin distributed on the inner nuclear membrane and around the nucleolus while the heterochromatin diffused among the

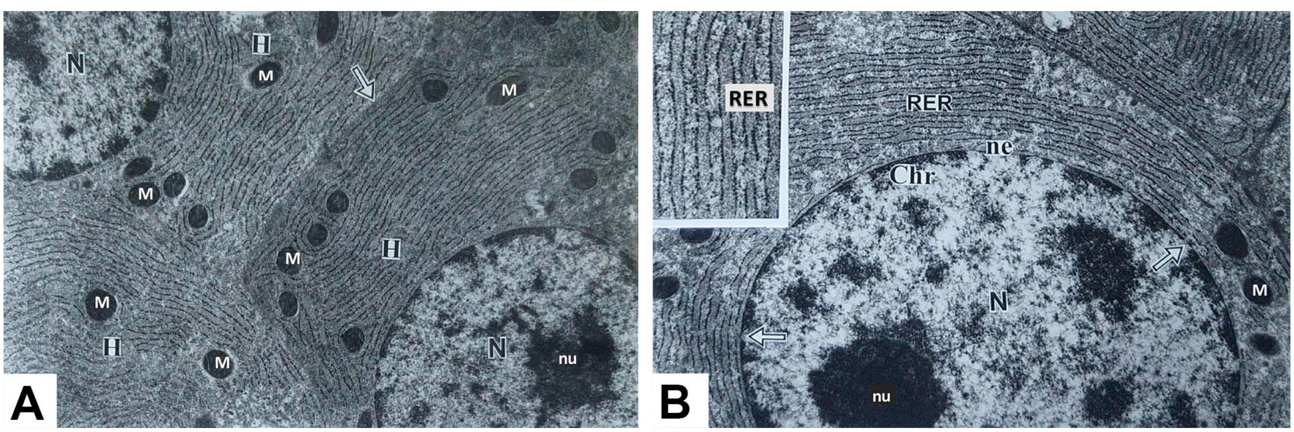


Fig. 11A, B. Transmission Electron micrographs of the hepatic tissue of the male *chamaeleo calyptratus* showing (A): general morphology of hepatocytes (H) contain large rounded basal or eccentric nuclei (N) with distinct nucleoli (nu), containing extensive well-developed rough endoplasmic reticula (RER), mitochondria (M), distinct cell membranes with boundaries between the hepatocytes (arrow), Bar = 0.1 µm. (B): high magnification of the perinuclear region of hepatocyte showing large rounded basal or eccentric nuclei (N) with prominent nucleolus (nu) and hetero/euchromatin granules (Chr). The nucleus has a distinct nuclear envelope (ne) with nuclear pores (arrows). Abundant extensive rough endoplasmic reticula (RER) often arranged in an array parallel to the nuclear membrane, near to the mitochondria (M), Bar = 0.1 µm;

center of the nucleus. There are abundant and extensive rough endoplasmic reticula associated often arranged in an array parallel to the nuclear membrane. Mitochondria come in a variety of shapes, from spherical to elongated, and are linked to the rough endoplasmic reticulum (Fig. 11B). The supranuclear part of hepatocyte cytoplasm contained glycogen granules, vesicles or vacuoles scattered throughout the cell cytoplasm especially at the apical region, Golgi apparatus has slightly flattened cisternae, bile canaliculus, cell membrane of lateral surfaces of two hepatocyte, sealed off with desmosomes/tight junctions (Fig. 12A). The mitochondria vary in shape and size from rounded to elongated and are surrounded by extensive network of rough endoplasmic reticulum cisternae. The mitochondrion has double mitochondrial membrane "inner/ outer", intact thick cristae embedded within the mitochondrial matrix (Fig. 12B). Although bile canaliculi between hepatocytes are found in apical cell areas, at the middle of tubules or alveoli. The bile canaliculus formed at the junction of hepatocytes boundary by cell membrane. The canalicular lumen is filled by microvilli of parenchymal cells which exhibited apically located microvilli which are protruded inside a bile canaliculus. The desmosomes and tight junctions occur around the bile canaliculi between hepatocytes, contributing to complete pericanalicular junctional complexes (Fig. 12C). The hepatic macrophage "Kupffer" phagocytic cell has irregular shaped and pseudopodia with irregular nucleus. The Kupffer cell extended from the sub-sinusoidal space "space of Diss" between the sinusoid and the hepatocyte. The Kupffer cell contains many phagocytosed materials and numerous lysosomes (Fig. 12D).

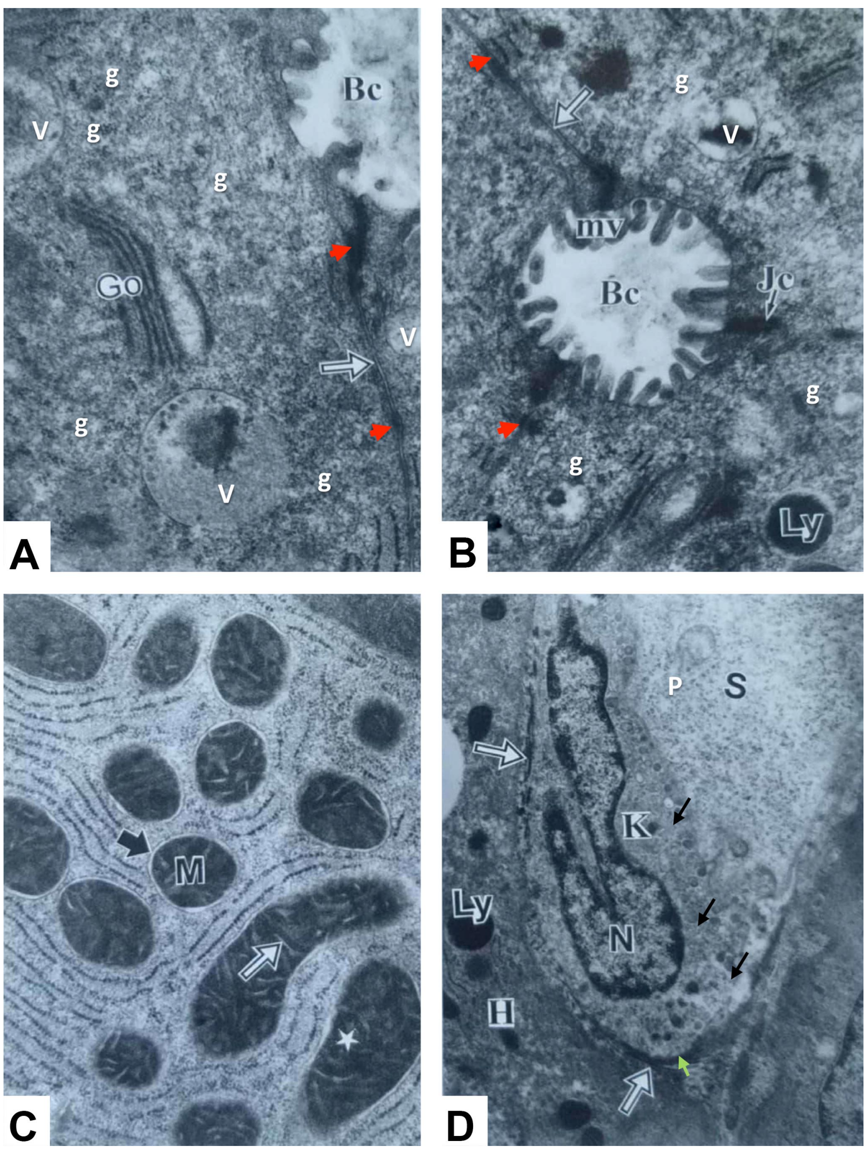


Fig. 12A-D Transmission Electron micrographs of the hepatic tissue of the male *Chameleo calyptratus* showing (A): the supra nuclear part of hepatocyte cytoplasm contained vesicles or vacuoles (V) scattered throughout the cell cytoplasm especially at the apical region, Golgi apparatus (Go) has slightly flattened cisternae, bile canaliculus (Bc), glycogen granules (g), cell membrane of lateral surfaces of two hepatocyte (arrow), sealed off with desmosomes/tight junctions (red arrowheads); vacuole (V), Bar = 0.5 µm. (B): distribution of mitochondria (M) through the cytoplasm of hepatocyte and surrounded by rough endoplasmic reticulum cisternae (RER). The mitochondrion has double mitochondrial membrane "inner/ outer" (black arrow), intact thick cristae (white arrow) embedded within the mitochondrial matrix (X). (C): the bile canaliculus (Bc) formed at the junction of hepatocytes boundary by cell membrane (arrow), microvilli of hepatocytes (mv) protruded inside a bile canaliculus, Desmosomes, and tight junctions of junctional complexes (Jc) occur at bile canaliculus, note the lysosomes (Ly) and vacuole (V). Bar = 0.5 µm; (D): The hepatic macrophage "Kupffer" phagocytic cell (K) extended from the sub-sinusoidal space "space of Diss" (white arrows) between the sinusoid (S) and the hepatocyte (H) and having many pseudopods. The Kupffer cell has irregular shaped, irregular nucleus (N), cytoplasm containing numerous electron-dense lysosomes (ly) and many phagocytosed materials (black arrow). Note the blood sinusoid contains blood plasma (P), Bar = 0.5 µm.

DISCUSSION

There are still few studies that have studied the liver of reptiles both morphologically and microscopically. Little is known about the histomorphological aspects of most reptiles including turtles, lizards and snakes (Ahmed *et al*., 2018).

The liver is the largest accessory digestive gland in vertebrates, including reptiles. It performs numerous essential activities and serves as metabolic organ of early processing of substances absorbed by intestinal capillaries and transmitted to the liver via the hepatic portal vein (Frimiano *et al*., 2011). It is a site of many metabolic activities and nutrients absorbed in the digestive tract are processed and stored for use by other parts of the body (Akiyoshi & Inoue, 2012; Akat & Göçmen, 2014). Because it metabolizes stores, synthesizes, and removes the chemicals received, the organ is one of the most essential in the organism. It also generates bile, an exocrine secretion of the liver cells that aids in fat breakdown and absorption (Frimiano *et al*., 2011).

In healthy adult reptiles, the liver is bilobed, dark brown to black in color. Chameleon livers are brownish gray in hue and have two lobes, with the right lobe being bigger and possessing a greenish-colored gallbladder between the lobes of the liver. This finding is consistent with Ahmed *et al*. (2018), who reported similar findings on the Nile monitor's liver, which was a huge dark brown bi-lobed organ with the right lobe being larger than the left. The microscopic examination revealed that the liver of chameleon was covered by a thick hepatic “Glisson’s” capsule. The hepatic capsule is a connective tissue layer and some smooth muscle fibers. Similar results were reported about the liver of lizard *Acanthodactylus boskianus* (Nafady & Awadalla, 2019). This capsule like the “Glisson” capsule in the mammals, which aids by the trabeculae in the partition of the hepatic parenchyma into lobules (Beddard, 1907; Ross & Pawlina, 2003; Necˇas, 2004). The thickness of the capsule may be due to consumption of stored materials during hibernation. There are considerable morphological changes occurred in the liver parenchyma and thickness of the hepatic fibrous capsule during hibernation (Marycz *et al*., 2009).

Histological analysis demonstrated that the polyhydric hepatocytes were arranged in tubular or acinar structures and surrounded by sinusoids and portal triads. The portal triad in the liver tissue consisted of a bile duct, a portal vein, and a hepatic arteriole. However, no hepatic lobules were observed. This conclusion is consistent with the results of Ahmed *et al*. (2018), on reptilian animal “the Nile monitor, and with Nafady & Awadalla (2019) who reported that the portal triad comprises branches of the portal vein, hepatic artery, and bile duct, as well as lymph arteries and neurons. Gardner & Oberdorster (2016) discovered that the anatomical pattern of the liver split into lobules is not evident in all reptiles and may even be missing in others, such as lizards.

Furthermore, the chameleon liver has some connective tissue stroma around the major vein, as well as the sinusoidal walls and portal triads. Other researchers observed that the parenchyma is maintained by a connective tissue stroma that surrounds the hepatocytes, blood sinusoids, and portal region in the livers of some lizards (Moura *et al*., 2012; Nafady & Awadalla, 2019).

The liver possesses large dark green gallbladder which positioned between the two lobes in the notch on the inner surface of the right elongated lobe of the liver and aids in fat digestion. The bile duct links the liver to the duodenum. The hilus is the center location of the liver, through which the portal vein and the hepatic artery travel (Hamdi *et al*., 2014; Barten & Simpson, 2019; Melero Jurado, 2020).

According to the findings of the current study, few hepatocyte nuclei are eccentric located, while the majority are displaced peripherally. Similar results were reported about the liver of lizard *Acanthodactylus boskianus* (Nafady & Awadalla, 2019), the liver of the *Podocnemis expansa* (Moura *et al*., 2012), the liver of the lizard *Tropidurus torquatus* (Frimiano *et al*., 2011) and the liver of dwarf crocodile *P. geoffroanus* (Moura *et al*., 2009). However, our findings disagree with the results recorded in the liver of *Chamaeleo calyptratus* (Eid *et al*., 2011), and the liver of *Osteolaemus tetraspis* (Storch *et al*., 1989), which reported that the nuclei are found in the center of the cells.

The *Chamaeleo calyptratus* liver hepatic cords were not observed; instead, the hepatic parenchyma consisted of hepatocytes organized in glandular-like alveoli "acini" or tubular structures. In some fishes, the hepatocytes are arranged as glandular acini surrounded by biliary canaliculi (Hampton *et al*., 1985). These findings are consistent with what we observed in the liver of *Chamaeleo calyptratus*, where the hepatocytes are most likely organised as acini surrounded by twisted sinusoidal capillaries in cross section. This finding contrasted from that of Moura *et al*. (2012), who found that the hepatocytes of *P. expansa*'s liver resembled twin strings of cells wrapped by twisted sinusoidal capillaries. Frimiano *et al*. (2011) discovered polyhedral lobules separated by a thin layer of interlobular connective tissue in the liver of the lizard *Tropidurus torquatus*. These features have been identified by Gardner & Oberdorster (2016), who proposed a tubular configuration of hepatocytes with laminar threads. Most animals, including reptiles, have two cells in these tubules (Storch *et al*., 1989). In mammals, the hepatic cells arranged in different ways as one-cell-thick plates, two/several-cell-thick plates or as a solid or tubular kinds, or as the multi-layered hepatocytes in teleosts (Akiyoshi & Inoue, 2012). Most hepatocytes of *Chamaeleo calyptratus* liver were polyhedric or pyramidal form which come in varied size (Eid *et al*., 2011). Hepatocyte contains one rounded nucleus which was located mostly in the periphery, but few nuclei are eccentric. Similar observation was reported by Nafady & Awadalla (2019) for the liver of lizard *Acanthodactylus boskianus*, Ahmed *et al*. (2018) for liver of Nile monitor, Moura *et al*. (2009) for *Phrynops geoffroanus*, and Firmina *et al*., 2011 for liver of the lizard *T. torguatus*, while Petcoff *et al*., 2006 showed the hepatocyte in fish liver varying from polyhedral to rounded shape. Each hepatocyte contain large, rounded and central nucleus with a prominent dark nucleolus (Ahmed *et al*., 2018). A finding differs from that reported by Eid *et al*. (2011) for the liver of Chameleon and with for *Osteolaemus tetraspis*, who found nuclei located in the center of the hepatocytes. Chameleon liver showing elliptical erythrocytes with typically centrally located nuclei and homogenous cytoplasm in hepatic blood sinusoids, the portal area and central vein, this finding was confirmed by Eid *et al*. (2011). the central vein surrounded by a layer of collagenous fibers, the same results was reported in the liver of lizard *Acanthodactylus boskianus* by Nafady & Awadalla (2019).

All the staining techniques used here detected large quantities of melanomacrophages in the hepatic parenchyma. The similar result was reported about the liver of lizard *Acanthodactylus boskianus* (Nafady and Awadalla, 2019). According to Frye (1991) and Nafady & Awadalla (2019), these cells are abundant in amphibians and reptiles, with the exception of snakes, where they are less abundant. The melanomacrophages are probably involved in many functions in organs of poikilotherms vertebrate as synthesis of melanin phagocytosis and protection against pathogen in addition to neutralization of free radicals (Gallone *et al*., 2007). Since, melanomacrophages are not present in birds or mammals, they are normally found in the liver of amphibian, reptiles, and some fishes (Ribeiro *et al*., 2011; Kardong, 2018; Nafady & Awadalla, 2019). These results are similar to those found by Moura *et al*. (2009), liver of the *Phrynops geoffroanus*. Also, Akat & Göçmen (2014), was demonstrated these cells in liver parenchyma of *L. arikani*. The hepatic parenchyma was separated by sinusoids that are lined with endothelial cells and Kupffer cells. Kupffer cells commonly known as stellate hepatic sinusoidal macrophages. They are monocyte-derived cells that migrate into the liver sinusoids. They are important in immune defense because they eliminate harmful and foreign chemicals, remove degenerated blood cells, and hemoglobin (Ross & Pawlina, 2003; Naito *et al*., 2004; Jacobson, 2007).

The livers of snakes and certain lizards are thin, but thick and compact in many other reptiles. Lizard hepatic cells are similar to those of other vertebrates, with the exception of melanomacrophages, which are missing in birds and mammals (Agius & Roberts, 2003; Nafady & Awadalla, 2019).

Usually, the portal triads of vertebrates are found in the portal gaps between the hepatic lobules and are made up of connective tissue-encased branches of the portal vein and hepatic artery, bile duct, and lymph vessels (Akiyoshi & Inoue, 2012). Chameleon liver showing elliptical erythrocytes with typically centrally located nuclei and homogenous cytoplasm in hepatic blood sinusoids (Eid *et al*., 2011; Nafady & Awadalla, 2019).

Glycogen

The cytoplasm was appeared as eosinophilic and spongy vacuolated found when analyzed by the hematoxylin-eosin staining. Moreover, it was strong reactive to PAS, indicating the presence of glycogen. The similar result was reported about the liver of lizard *Acanthodactylus boskianus* (Nafady & Awadalla, 2019). The liver is involved in the storage of glycogen, as an energy source that may be used in a range of animal activities, such as sexual reproductive efficiency and metabolic variations throughout the year, especially in species that are influenced by cold climatic fluctuations where hibernation occurs. It was weak reactive to MBPB, indicating inadequate of mucopolysaccharides and protein (Frimiano *et al*., 2011; Akat & Göçmen, 2014; Kardong, 2018; Pawlina & Ross, 2018).

The ultrastructural investigation of the liver of *Chamaeleo calyptratus* reported that, the hepatocytes are similar to those of other vertebrates with the exception of melanomacrophages. Previous findings revealed that the lizard hepatic cells are identical to those of other vertebrates, with the exception of melanomacrophages, which are missing in birds and mammals (Agius & Roberts, 2003; Bertolucci *et al*., 2008; Nafady & Awadalla, 2019).

The ultrastructural investigation of the liver of *Chamaeleo calyptratus* revealed large hepatocytes separated by cell membranes that form the boundaries between the hepatocytes. The hepatocyte appeared polygonal or pyramidal in the shape containing a single rounded basal or eccentric vesicular nucleus with prominent high electronic density prominent nucleolus. The similar result was reported about the liver of lizard *Acanthodactylus boskianus* (Nafady & Awadalla, 2019), for *Phrynops geoffroanus* (Moura *et al*., 2009), for *Tropidurus torquatus* (Frimiano *et al*., 2011), and with the results of *Astyanax astyanax* (Bertolucci *et al*., 2008), which referred to the same ultrastructure.

There are abundant and extensive rough endoplasmic reticula associated with outer nuclear membrane and with many mitochondria, and Golgi apparatus. RER is often arranged in an array parallel to the nuclear membrane. Mitochondria come in a variety of shapes, from spherical to elongated, and are linked to the rough endoplasmic reticulum. Our findings corroborated previous research that showed an increase in the number of mitochondria in the liver and muscles of animals during hibernation. Furthermore, animals housed at a lower temperature had a better formed rough endoplasmic reticulum, as evidenced by an increase in the endoplasmic reticulum as well as the number of mitochondria and peroxisomes (Bertolucci *et al*., 2008; Nafady & Awadalla, 2019).

Glycogen was abundant in the cytoplasm of hepatocytes as tiny granules scattered throughout the cell cytoplasm especially at the apical region. This result was similar to those found in *Trachomys scripta elegans* (Marycz *et al*., 2009) and the liver of the lizard *Acanthodactylus boskianus* (Nafady & Awadalla, 2019). The major reserve energy source in healthy animals is hepatic glycogen, which is acclimated to low temperatures and exhibits a high level of glycogen as the temperature drops (Nafady & Awadalla, 2019).

Bile canaliculi between hepatocytes are found in apical cell areas, at the middle of tubules or alveoli and few perisinusoidal canaliculi do occur. The bile canaliculus formed at the junction of hepatocytes boundary by cell membrane. The canalicular lumen is filled by microvilli of parenchymal cells which exhibited apically located microvilli which are protruded inside a bile canaliculus. These findings are consistent with findings of Bertolucci *et al*. (2008) for livers of *Astyanax altiparanae* and supported by Nafady & Awadalla (2019) for liver of the lizard *Acanthodactylus boskianus*, who mentioned ultrastructurally the polygonal hepatocyte has three surfaces, the sinusoidal surface of the hepatocyte plasma membrane is covered with many irregular microvilli, which increases hepatocyte surface area; the lateral surface is specialized for adhesion via junctional complexes; the canalicular surface that faces the canaliculi and coated with microvilli. The canaliculus is an intercellular gap separated by junctional complexes between two neighboring hepatocytes (Nafady & Awadalla, 2019).

The hepatic macrophage “Kupffer” phagocytic cell has irregular shaped and pseudopodia with irregular nucleus. The Kupffer cell extended from the sub-sinusoidal space “space of Diss” between the sinusoid and the hepatocyte. The Kupffer cell contains many phagocytosed materials and numerous lysosomes, because the Kupffer cell acts as a hepatic macrophage that scavenges free radicals (Nafady & Awadalla, 2019).

CONCLUSION

This is the first study on the histological characterization of the healthy liver of *Chamaeleo calyptratus* in southern Saudi Arabia. The morphological, histological, and ultrastructural descriptions of the liver of *Chamaeleo calyptratus* lead to the conclusion that this species is an excellent model for studies of this class, and the findings presented here should be used as a reference to compare with the pathological abnormalities of the liver in this animal. Morphologically, the liver of *Chamaeleo calyptratus* is described morphologically as a bilobed dark brown organ with rectangular form, occupying the cranial region of the coelomic cavity and surrounding by the pancreas, intestine, and stomach. Microscopically, the hepatic parenchyma surrounded by thick capsule with short trabeculae. The hepatocytes were polyhydric cells, arranged in tubular or alveolar structures and surrounded by sinusoids and portal triads. However, no clear hepatic lobules were observed. The hepatocytes showed strong react with PAS stain due to the high content of glycogen and MBPB indicating the low content of mucopolysaccharides and protein respectively. Ultrastructurally, the hepatocyte appeared polyhedric containing a single large rounded basal or eccentric vesicular nucleus with prominent nucleolus. Extensive network of rough endoplasmic reticulum with many mitochondria, and Golgi apparatus were observed. The cytoplasm contained glycogen granules, vesicles or vacuoles scattered throughout the cytoplasm especially at the apical region were showed. The bile canaliculi with microvilli and the hepatic "Kupffer" cells were also reported.

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AL-DOAISS, A. A.; ALSHEHRI, M. A.; SHATI, A. A.; ALFAIFI, M. Y.; AL-KAHTANI, M. A.; AHMED, A. E., EID, R. A.; AL-SHURAYM, L. A.; AL-MEKHLAFI, F. A.; AL ZAHRANI, M. & MUBARAK, M. Estudios histomorfológicos, histoquímicos y ultraestructurales en el hígado sano del camaleón velado de Yemen (*Chamaeleo calyptratus*) en el sur de Arabia Saudita. *Int. J. Morphol., 41(5)*:1513-1526, 2023.

RESUMEN: En este trabajo se analizaron los hígados de los reptiles como modelo de la relación entre el medio ambiente y el tejido hepático. Hay pocas investigaciones sobre la histología del hígado de los reptiles, y muy pocas o ningún estudio que ha examinado la histología del hígado del camaleón velado (*Chamaeleo calyptratus*). Este artículo describe la caracterización histomorfológica, histoquímica y ultraestructural del hígado de camaleones velados en el sur de Arabia Saudita. Siete *Chamaeleo calyptratus* fueron capturados en la temporada de verano en la ciudad de Abha, región de Aseer, al sur de Arabia Saudita. Se procesaron muestras de hígado de camaleón para análisis de histomorfología, histoquímica y ultraestructura. Morfológi-camente, el hígado de *Chamaeleo calyptratus* se observó como un órgano grande de color marrón oscuro con motas más claras que representan depósitos de melanina. El hígado se encuentra en la parte ventral de la cavidad abdominal, delante del estómago. Sus dimensiones son aproximadamente de 3,7 × 2 cm. El hígado es un órgano bilobulado dividido en dos lóbulos, el derecho y el izquierdo. El de la derecha es más grande. La vesícula biliar estaba bien desarrollada y de forma alargada, situada entre los dos lóbulos y contenía la bilis para la digestión. Microscópicamente, se encontró que el hígado estaba cubierto por una capa gruesa de tejido conectivo, que formaba la cápsula hepática. El parénquima hepático probablemente apareció en secciones transversales como "acinos" de tipo glandular hepático o estructuras foliculares con varios diámetros; cada acino contenía aproximadamente cuatro a seis hepatocitos, rodeados por capilares sinusoidales con abundantes melanomacrófagos, los que no se encuentran en aves y mamíferos. Los melanomacrófagos son comunes en las áreas perisinusoidales del parénquima hepático, particularmente cerca de los espacios porta. Los hepatocitos son poliédricos o piramidales y en su mayoría contienen núcleos grandes y redondeados, ubicados periféricamente, con nucléolos ovalados oscuros prominentes. Algunos de los núcleos estaban en posición excéntrica o central. El citoplasma apareció esponjoso o vacuolado y más eosinofílico cuando se tiñó con hematoxilina-eosina y fuertemente reactivo a la técnica de tinción PAS, lo que indica un contenido abundante de glucógeno. Las fibras reticulares que rodean los hepatocitos, las arterias y los sinusoides sostienen el parénquima hepático. Los sinusoides sanguíneos se ven intercalados entre hepatocitos de diferentes tamaños. La luz sinusoidal está rodeada por células endoteliales aplanadas y contiene como fagocitos eritrocitos nucleados elípticos y macrófagos hepáticos, también conocidos como células de Kupffer. Se detectaron ramas de la vena porta, la arteria hepática, el pequeño conducto biliar y los vasos linfáticos en el "tracto" o tríada del área porta hepática formado por tejido conectivo. Se observó tejido hematopoyético en la región subcapsular y tríadas portales. Ultraestructuralmente, el hepatocito parecía poliédrico y contenía un único núcleo vesicular excéntrico o basal redondeado de gran tamaño con un nucléolo prominente. Se describió una extensa red de retículo endoplásmico rugoso, a menudo situada en una disposición paralela a la membrana nuclear con muchas mitocondrias, y el complejo golgiense. El citoplasma contenía gránulos de glucógeno. Se reportaron vesículas o vacuolas diseminadas por todo el citoplasma, especialmente en la región apical. También se discutieron los canalículos biliares y los macrofagocitos estrellados (células de Kupffer). Este es el primer estudio sobre la caracterización histológica del hígado sano del camaleón velado de Yemen en el sur de Arabia Saudita. Los hallazgos aquí reportados deben usarse como referencia para comparar con las anomalías patológicas del hígado en este animal.

PALABRAS CLAVE: Hígado; *Chamaeleo calyptratus*; Camaleón velado de Yemen; Histomorfología; Reptiles; Hepatocitos.

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Corresponding author:
Amin A. Al-Doaiss
Biology Department
College of Science
King Khalid University
P.O. Box 9004
Abha 61421
SAUDI ARABIA

E-mail: aaldoaiss@kku.edu.sa