Original Article

The Study of the Liver structure in the Egyptian Tomb Bat, Taphozous Perforatus (Histological, Histochemical and Electron Microscope)

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ABSTRACT

Introduction: Bats display unique lifestyle; these mammals may be frugivores, insectivores, or hematophages depending on morphological, biological, and anatomical features. Egyptian tomb bats, Taphozous perforatus, are insectivores.

Aim of the Work: This study investigated the histology and ultrastructure of the liver of T. perforatus to assess its adaptation to its diet and way of life.

Material and Methods: Ten healthy adult T. perforatus of each sex were captured from Aborawash, Gizza Governorate, Egypt. Liver specimens were fixed for light and transmission electron microscopy to examine hepatic architecture. Histochemical and special stains were also used.

Results: Hematoxylin and eosin stained sections showed that the hepatocytes arranged in hepatic plates. A radial arrangement of hepatic cords around central veins is generally not apparent, and hepatocytes are separated by blood sinusoids. Histochemical studies showed the presence of collagen fibers around portal triads and glycogen granules scattered throughout the cytoplasm of some hepatocytes. Glycogen accumulated at the ends of other hepatocytes indicating a glycogen flight phenomenon. T. perforatus hepatic cells under TEM are similar to cells from birds and mammals, but with peripheral nuclei, numerous mitochondria, a large number of irregularly shaped lymphocyte aggregations, and dark, oval, elongated nuclei with fenestrated nuclear membranes and very little cytoplasm.

Conclusion: The histological, histochemical, and ultrastructural features of the liver of T. perforatus bats are identical to features of other mammals, with a few exceptions based on lifestyle and energy demand during flight.

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Key Words: Bile canaliculi, liver, hepatocyte, space of Disse, Taphozous perforatus.

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INTRODUCTION

Bats are particularly interesting mammals because of their unusual lifestyle. They are the only mammals that can fly and feed while in flight[1,2]. Further, bats demonstrate powered flight, suggesting that they have higher daily energy demands than other mammalian species[3-5]. Bats can be frugivores, insectivores, or hematophages; these feeding styles are directly connected to numerous morphological, biochemical, and anatomical characteristics[6-11]. Egyptian tomb bats, Taphozous perforatus, are sac winged species in the Emballonuridae family, order Chiroptera, and suborder Microchiroptera[12,13]. The nutrition of Emballonuridae is dependent on insects. Taphozous is an insectivorous genus with species that feed on a variety of beetles, ants, flies, and bugs[14,15]. The bat can be found all over northern and sub-Saharan Africa, the Arabian Peninsula, and all the way east to the Indian subcontinent. When compared to New World bats, this species has received little attention, particularly in terms of gastrointestinal morphology. Understanding the histology of an animal's liver is crucial for understanding its nutritional ecology[16]. The liver of a bat performs various critical activities in the storage and conversion of metabolites[17]. It is dark red or reddish-brown due to a plentiful blood supply. Liver tissue is divided into lobules, each of which is encased in a connective tissue sheath that forms a thin stiff capsule containing elastic fibers[18]. The tissue is characterized by a series of interconnected hexagonal hepatic lobules; polygonal lobules exhibit a central vein and portal peripheral canals at its corners. Hepatic cells are polygonal, with spherical or ovoid nuclei with a regular surface. Nuclei vary in size from cell to cell. Each nucleus is vesicular, with noticeable dispersed chromatin granules and one or more nucleoli[19]. Hepatic cells are separated from the wall of the vascular channel by a thin perisinusoidal space at the surface close to a sinusoidal space. The plasma membrane of hepatic cells is covered by numerous long microvilli[18]. In Meriones hurrianae and Tatera indica, the portal area consists of branches of portal veins, hepatic artery, bile duct, and a lymphatic vessel lying in a small amount of connective tissue. The apical face of adjacent hepatic cords forms slender bile canaliculi that drain centrifugally to a nearby portal canal[20]. A scant collagen III fiber meshwork supports hepatocytes and sinusoids in mammals[21]. Throughout the liver parenchyma, sparse irregularly shaped lymphatic aggregations containing mostly lymphocytes are observed. However, little information on the architecture of the liver

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of Microchiroptera[22] is available, and none is published regarding the liver of the Egyptian tomb bat. Few studies on this species, in general, have been published. This study examined histological, histochemical, and ultrastructural properties of the liver in adult T. perforatus to assess their adaptation to feeding type and lifestyle.

MATERIAL AND METHODS

Collection of bat samples

The livers of ten healthy adult Egyptian tomb bats (T. perforatus, Saint-Hilaire,[12]) of each sex were examined in a collaborative effort with a team of researchers from the Tanta University Faculty of Science, Comparative Anatomy Laboratory. Animals were collected from Aborawash, Gizza Governorate, Egypt. Specimens were managed and processed following the Tanta University local ethical board requirements.

Histological examination

After anesthesia with Anahal solution, liver specimens of healthy adult male and female Egyptian tomb bats were used in histological studies. Livers were divided into several segments and immediately fixed in a solution in Bouin's fluid and 10% neutral buffered formalin. After 24 hr, tissue samples were dehydrated in an ascending series of ethyl alcohol solutions, cleared with xylene until transparent, and embedded in paraffin. Five-µm sections were mounted using Canada balsam and stained with hematoxylin and eosin[23]. All preparations were examined under a microscope OLYMPUS (CX33).

Histochemical examination

Alcian blue – PAS

Identification of the full complement of tissue proteoglycans and differentiation between neutral, acidic mucosubstances and acidic mucins were stained with Alcian blue, while neutral mucins and glycogen were stained magenta with PAS. Sections were washed in distilled water, stained with Alcian blue for 15 mints then washed well in running tap water for 2 mints before being rinsed in distilled water. Sections were then treated with periodic acid for 5 mints, washed well with distilled water, stained with Schiff's reagent for 10 mints and washed well in running tap water for 5 mints. Nuclei were stained with hematoxylin for 1 min, washed in running tap water for 2 mints, differentiated with acid alcohol, washed with Scott's tap water to color nuclei, washed in water, dehydrated, cleared, and mounted[24].

Trichrome stain

Extra-paraffin sections were stained with Masson's trichrome to distinguish collagen fibers by a blue color in sinusoids and central veins[25].

Transmission electron microscopic examination

Small portions of liver, 1-mm3 were quickly processed following the protocol of the Tanta University, Faculty of Medicine EM units

Tissues were fixed in formaldehyde/glutaraldehyde (4: 1) at pH 7.4, postfixed in 2.0 percent buffered osmic acid, and dehydrated in an ascending series of ethyl alcohol solutions. Fixed tissues were then infiltrated with propylene oxide:epon, initially 1:1, then 1:3 overnight, followed by embedding in freshly prepared araldite epon mixture and polymerized at 60°C. Embedded tissues were sectioned on a Reichert Jung ultramicrotome. Semithin sections were stained with toluidine blue for light microscopy.

Ultrathin 60-nm sections were collected on copper grids, stained with uranyl acetate and lead citrate, and examined using a Philips 400T transmission electron microscope with an accelerating voltage of 80 kV. (Tanta, Egypt). Photographs were taken on Kodak EM sheet films, which were processed, enlarged, printed, and examined.

RESULTS

Anatomical observations

The liver of Taphozous perforatus is small with a gall bladder. It consists of a small lobe to the left of the suspensory limit of the cystic lobe; a right lobe is also present, which is the site of initial processing of materials absorbed by intestinal capillaries and transported via hepatic portal vein tributaries (Figure 1).

Histological and histochemical observations by light microscopy

T. perforatus liver sections displayed a compound tubular structure with a thin layer of peritoneal epithelium composed of flattened cells resting on a basement membrane of fibrous connective tissue. This tissue was composed of collagenous fibers and relatively few cells. The majority of cells were fibroblasts forming the hepatic capsule (Figure 2a). This capsule helped divide the parenchyma into structural units known as polyhedral hepatic lobules; interlobular connective tissue was restricted to the portal canal at the angle of the lobules where central veins and portal triads were seen (Figure 2b). Hepatic plates were lined with simple layered hepatocytes and showed a central vein as its axis. Plates were made up of columns of hepatic cells arranged radially from the central vein to the periphery of the lobule and portal triad. The triad exhibited branches of the hepatic artery, portal vein, and bile ductules (Figure 2c). Hepatocytes were polygonal with a definite limiting membrane and a clump of finely granulated basophilic cytoplasm containing peripherally positioned spherical or ovoid nuclei with regular surfaces in a wide range of sizes. Hepatocyte columns were separated by a network of narrow, short, tortuous capillaries or blood sinusoids that ran in the same radial direction as the hepatocytes. Columns were lined with small, elongated endothelial cells with darkly stained nuclei and greatly attenuated cytoplasm, and Kupffer cells with large oval nuclei cytoplasmic arms (Figure 2d). Lymphatic aggregations were scattered throughout the liver parenchyma.

A moderate amount of connective tissue stroma, primarily collagenous fibers, was detected around the central vein and sinusoidal walls, particularly the walls of portal veins, hepatic arteries, and bile ductules. Each portal area was surrounded by a moderate amount of collagenous network that was continuous with scant interlobular collagenous fibers that stained blue with Masson's trichrome (Figures 2e,f). Glycogen granules were stained magenta with PAS using Alcian blue – PAS. These granules clumped together into enormous patches condensed primarily at one pole of the cell, producing the flight phenomenon (Figures 2g,h).

Electron microscope observations

T. perforatus hepatocytes were polyhedral, each being bounded by a distinct cell membrane. Each cell contained peripherally located spherical nuclei limited by a prominent nuclear membrane and surrounded by ground cytoplasm. A variety of cell organelles were discerned in the perinuclear region along with non-living inclusions. Mitochondria ranged from spherical or oval to elongate and rod-shaped and distributed throughout the cytoplasm. The highest concentrations of these organelles were perinuclear with many or closely packed cristae of rough endoplasmic reticulum.

Glycogen was a common component of liver cells and occurred as nano-sized (beta) particles scattered throughout the cytoplasm and in polyparticulate (alpha) glycogen rosettes formed by aggregation of beta particles. Hepatocytes displayed substantial granular or rough endoplasmic reticulum (ER) mostly around nuclei and mitochondria. ER appeared in parallel stacks with narrow lumens (Figures 3a,b).

Hepatocytes were organized radially around bile canaliculi. Cell membranes exhibited multiple alterations or specializations on different faces, resulting in several types of intercellular connections. Desmosomes, tight junctions, and intermediate junctions (adjacent occluding junctions) were identified between adjacent hepatocytes near these canaliculi. This region of the cell membrane was specialized for adhesion via junctional complexes, including desmosomes (Figure 3c).

Wide basal poles on cell surfaces were orientated near to liver sinusoidal blood spaces. Cells were delimited from the lining of the sinusoid by a narrow perisinusoidal gap, the space of Disse, and contained microvilli that projected into this space (Figure 3d). The canalicular domain was the origin of the liver bile drainage system. Canaliculi were an interstitial space between neighboring hepatocytes separated by junctional complexes with an irregular array of microvilli covering the canalicular surface. Hepatic stellate cells (Ito cells) or fat-storing cells were found in the perisinusoidal space (Disse space); they were irregularly in shape, ranging from oval to more or less elongated, had small round nuclei, and were rich in ER. These cells were smaller and had fewer mitochondria than hepatocytes and were frequently associated with the nuclear region of endothelial cells (Figure 3e).

The sinusoidal space represents hepatocyte vascular poles, allowing contact between hepatocytes and blood plasma. This region of the membrane allows exchange of substances between cytoplasm and circulating blood. This space was surrounded by endothelial cells that do not overlap and were spread out and fenestrated (Figure 3f). Stellate macrophages (Kupffer cells) were also observed with numerous pseudopods and occasional cup-like indentations. Cells were spindle-shaped with large triangular to oval-shaped nuclei. Cytoplasmic membranes showed conspicuous pseudopodia that protruded into the sinusoidal lumen, and electron-dense lysosomes were observed (Figure 3g). Erythrophagocytosis was observed, and cytoplasm usually contained numerous randomly distributed lysosomes which were variable in size and internal content. Lymphocytes had an irregular shape, with dark, oval elongated nuclei with fenestrated nuclear membranes and little cytoplasm in a cytoplasmic process. They were identified in the sinusoidal lumen and rarely in the perisinusoidal area. A lymphocyte in the perisinusoidal area was most likely in the process of amoeboid migration to the sinusoidal lumen (Figures 3h,i,j).

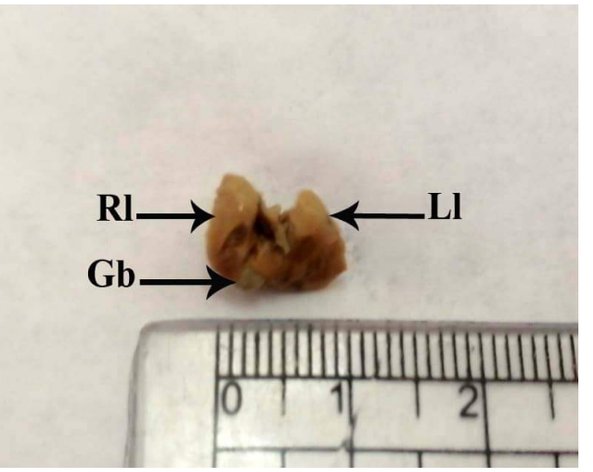

Fig. 1: Photograph of the liver of T. perforatus with a small gall bladder (Gb), and a small lobe to the left (Ll) of the suspensory limit of the cystic lobe; a right lobe (Lb) was also present.

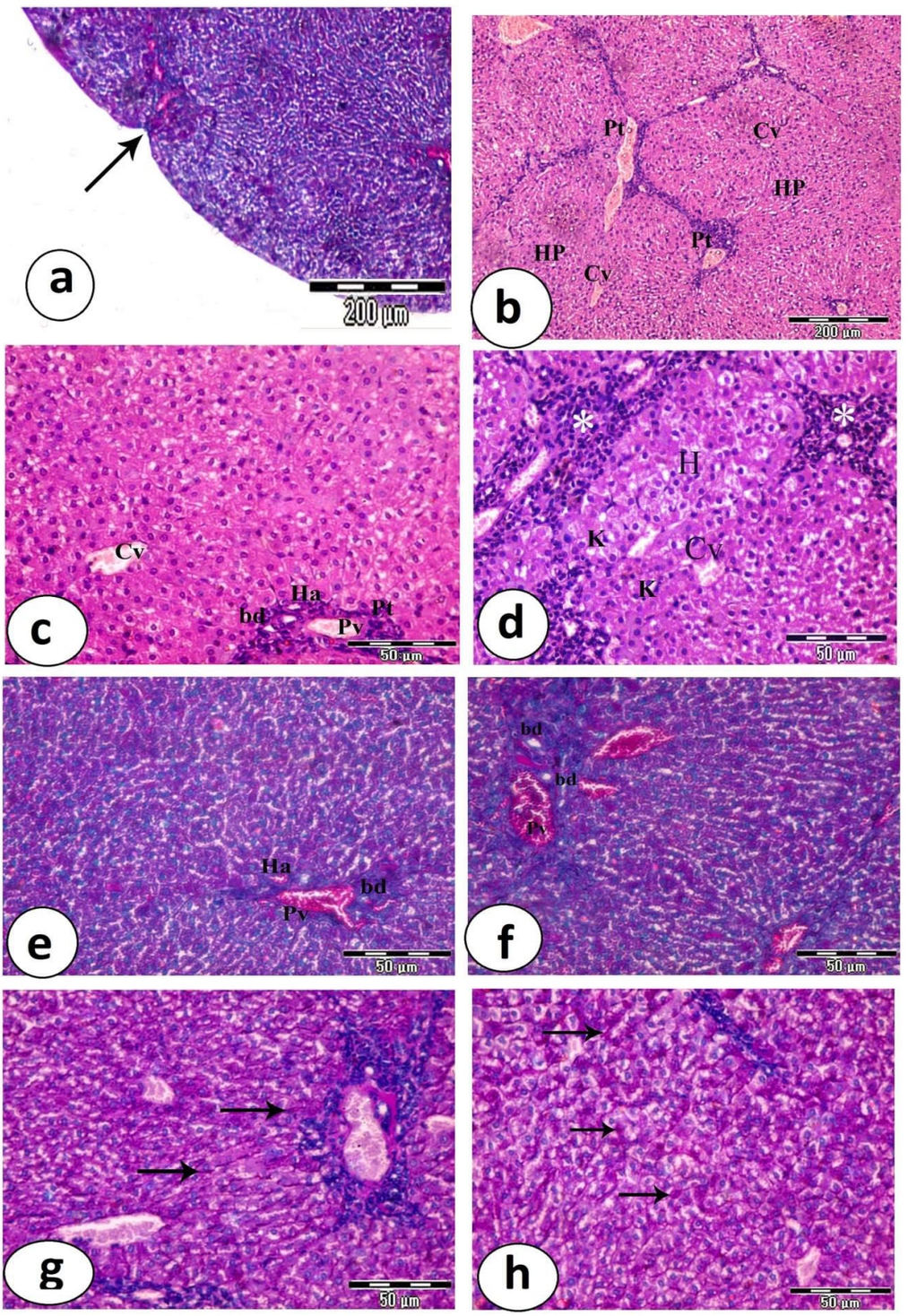

Fig. 2: (a-d): Photomicrographs of liver section from T. perforatus stained with haematoxylin and eosin. a) Liver section showing connective tissue septae invaginating from the capsule delineating hepatic lobules (arrow) (X 100). B) Liver section showing hexagonal hepatic lobules or hepatic plates (Hp) radiating outward from a central vein (Cv); central veins are quite prominent. At the vertices of the lobule are regularly distributed portal triads (Pt) (X 100). c) Liver section exhibiting the central vein (Cv) and portal tract (Pt) with branches of the portal vein (Pv), branch of hepatic artery (Ha) and bile ductules (bd) (X 400). d) Liver section showing central vein (Cv) and radiating polygonal hepatocytes (H). Hepatocytes showed homogenous granular cytoplasm with peripherally located nuclei, prominent envelops and normally distributed chromatin, blood sinusoids lined with endothelial and Kupffer cells (K) and aggregates of lymphocytes (*) were noticed (X 400). e,f) Liver sections stained with Masson trichrome showing minute collagen fibers in the portal tract especially in the walls of the portal vein, hepatic artery and bile ductules (X 400). g,h) Liver sections stained with PAS and Alcian blue showing PAS-positive granules representing glycogen. Granules accumulated mostly at one pole, reflecting the phenomenon of glycogen flight (X 400).

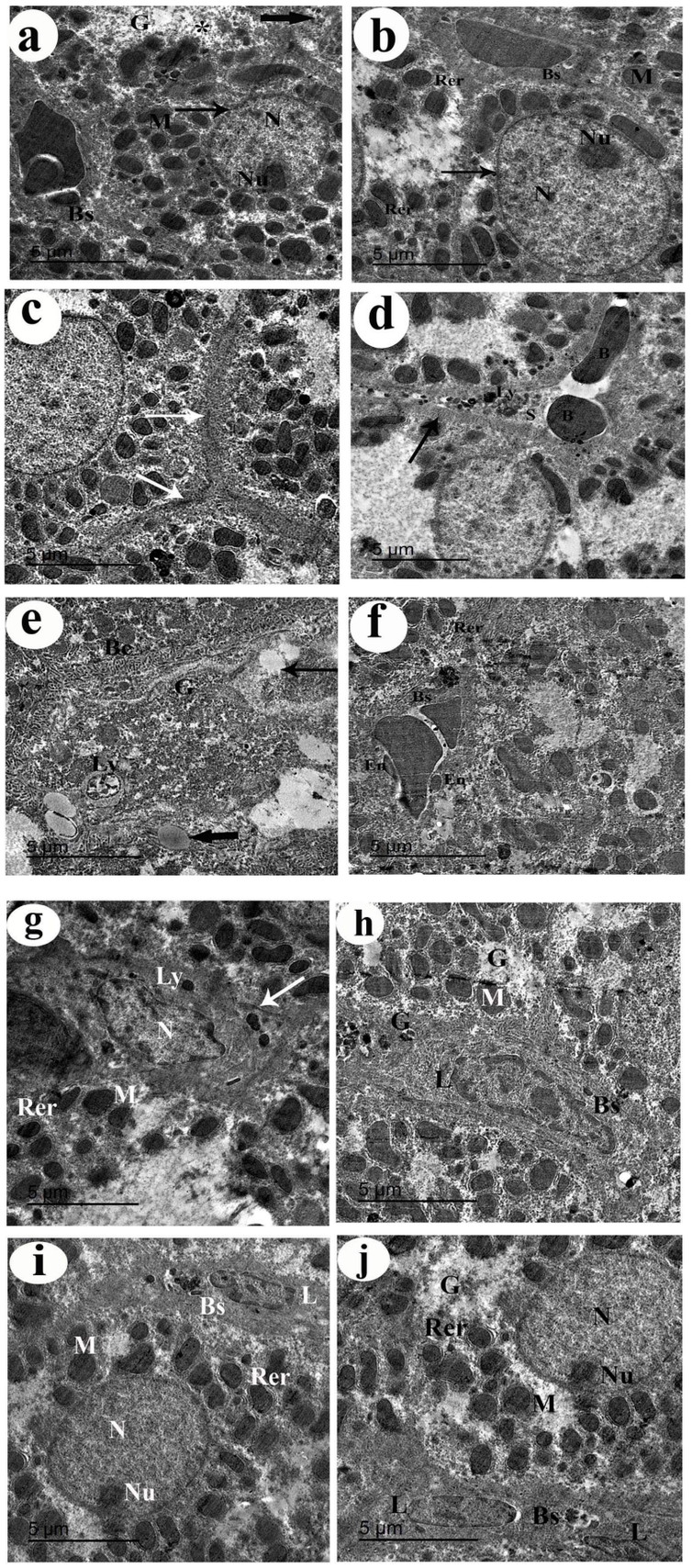

Fig. 3: (a-j) Electron micrographs of the liver section from T. perforatus exhibiting the fine structure of the hepatocyte cells.

Fig. 3: (a,b) Electron micrographs displaying normal hepatocyte architecture, normal nuclei (N) with normally distributed euchromatin, a prominent nucleoli (nu), a distinct nuclear envelop (arrow), many cytoplasmic glycogen granules (G) that distributed throughout the cytoplam, (β glycogen) (*) or aggregated together as rosette like granule (α glycogen) (thick arrow), parallel cisternae of rough endoplasmic reticulum (Rer), normal-sized mitochondria (M), and a blood sinusoid (Bs) with RBCs. c) High magnified electron micrograph illustrating adjacent occluding junctions and desmosomes between adjacent hepatocytes (arrows). d) Electron micrograph showing the space of Disse (S) with many short projections into its lumen (arrow), electron-dense lysosomes (Ly), and R.B.Cs (B). e) An electron micrograph showing fat storing cell with numerous saturated lipid droplets (thick arrow) and unsaturated (thin arrow) as well as bile canaliculi (Bc) was observed that is lined by protruding microvilli, hepatocyte cells with lysosomes (Ly) and aggregation of rosette like glycogen granules (G). f) An electron micrograph of hepatic sinusoids containing red blood cells and lined by fenestrated endothelial cells (En). g) An electron micrograph exhibiting Kupffer cells having many pseudopods (arrow), nucleus (N) and electron-dense lysosomes (Ly). (h,i,j): Electron micrographs exhibiting hepatocytes, blood sinusoids contain lymphocytes (L) with abundant cytoplasm and a large central nucleus with a fenestrated nuclear envelope.

DISCUSSION

Based on our histological, histochemical, and ultrastructural findings, we deduced some morphological and functional modifications in the liver of Taphozous perforatus. These conclusions indicated modest differences in feeding style and strategy. The liver is a small digestive gland with a gall bladder, enclosed by a thin tough capsule and plays a critical physiological role. On the other hand, the liver is the largest accessory gland of the digestive system in teleosts, aves, rodents and other mammals[26-28]. In snakes and some lizards, the organ is long and thin, but is thicker and more compact in other reptile species[29].

The acinus is the structural and functional unit of T. perforatus liver. The organ is comprised of interconnected hexagonal hepatic lobules with hepatocytes, sinusoidal structures, and a portal triad situated in the portal spaces between hepatic lobules with a portal canal at each corner. This finding is consistent with[30,31]. Hepatocyte lobules were less distinct than in Psammomys obesus, which displays prismatic hepatic lobules with a central vein forming the central axis, but comparable to liver architecture in Allactaga tetradactyla[32]. Vessels and ducts in portal triads were surrounded by a limited amount of connective tissue, similar to the liver of Acanthodactylus boskianus, and other lizards, Allactaga tetradactyla, M. hurrianae, T. indica[33-36]. Collagen fibers supported hepatocytes and sinusoids in our investigation, consistent with[28] who obtained similar results in avian liver;[18] observed elastic fibers in connective tissue sheaths around polygonal hepatic lobules. Perisinusoidal spaces in the current study were found between hepatocytes and sinusoidal endothelial cells and hepatic cells were covered by numerous long microvilli and were separated from the wall of the vascular channel. The apical face of adjacent hepatic cords formed slender bile canaliculi that drained centrifugally to the nearby portal canal. Similar results were obtained with chickens[28]. Hepatic plates were composed of simple layered hepatocytes, so-called one-cell-thick plates, consistent with the findings of[37] who studied the liver from mammals and snakes, but differed from[38] who reported multiple layered plates of hepatocytes separated by narrow and short, tortuous sinusoids in fish liver. Hepatocytes were polygonal with spherical or oval peripheral nuclei with a regular surface. Somewhat similar observations were recorded by[26] for the liver of Mustela nivalis. As in Acanthodactylus boskianus, the nuclei of hepatocytes shifted to the periphery due to the presence of numerous vacuoles of varying size in the cytoplasm[28]. In chicken, Phrynops geoffroanus and Tropidurus torquatus, however, some nuclei were found in the central region of hepatic cells, but the majority was found toward the periphery[34,35,28]. Conversely, in mammals, binuclear hepatocytes predominate[39].

T. perforatus hepatocyte nuclei appeared with nucleoli and chromatin dispersed throughout the nucleoplasm[19] observed vesicular nuclei with conspicuous dispersed chromatin granules and one or more nucleoli. Abundant rough ER and closely packed, large rounded, oval and elongated mitochondria were observed, confirming similar observations reported by[28] in chickens, mice, hamster and bat liver[40-43]. Such mitochondria were necessary for the production of large amounts of energy and the high oxygen consumption required for flying.

Small glycogen granules were found scattered throughout the cytoplasm or clustered together, similar findings in Trachomys scripta elegans[44,45], who reported that hepatic glycogen was the main energy reserve energy for prolonged fasting and flying. Ito cells were located in the space of Disse and containing lipid droplets which derived from the blood stream[46]. These fat- storing cells are reported in fish, amphibians, reptiles, birds, and mammals[38], including humans[47,48]. As documented by[49] in mammals, Kupffer cells in T. perforatus are sinusoidal macrophages and free radical scavengers. Lymphatic aggregations were seen throughout the liver parenchyma, with comparable findings in chicken[28] and turkey[50] liver, where both encapsulated and non-encapsulated lymphatic aggregations were found. However, these findings differ with[51] who found lymphoid aggregations in non-lymphoid organs like the pancreas, kidney, endocrine glands, gonads, brain, and spinal cord. Conversely, numerous melanomacrophages were detected in the liver and spleen of Acanthodactylus boskianus, as well as various fishes, anurans, and reptiles[52-57,36], but were less common among snakes[58,59]. The histology and ultrastructure of the liver of T. perforatus were found to be identical to those of other mammals, with minor changes based on lifestyle and energy demand during flight.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Altringham, JD, Mcowat, T and Hammond, L: Bats: Biology and Behaviour. Oxford University Press. (1996).
2. Wilson, DE and Reeder, DM: Mammalian Species of the World: A Taxonomic and Geographic Reference. Baltimore, Johns Hopkins University Press. (2005).
3. Thomas, SP: Metabolism during flight in two species of bats, Phyllostomus hastatus and Pteropus gouldii. The Journal of Experimental Biology. (1975) 63: 273–293.
4. Caviedes-Vidal, E, McWhorter, TJ, Lavin, SR, Chediack, JG, Tracy, CR and Karasov, WH: The digestive adaptation of flying vertebrates: High intestinal paracellular absorption compensates for smaller guts. Proceedings of the National Academy of Sciences. (2007) 104: 19132–19137.
5. Linscott, TM, Roche, E and Bonett, RM: The effects of diet, ecology, and physiology on the evolution of endothermic gastrointestinal tract lengths. In: Annual Meeting. Society For Integrative and Comparative Biology. Society for Integrative and Comparative Biology. TX, Austin. (2014) pp. 5–6.

6. Freeman, PW: Specialized insectivory: Beetle-eating and moth-eating molossid bats. Journal of Mammalogy. (1979) 60: 467–479.
7. Freeman, PW: Frugivorous and animalivorous bats (Microchiroptera) dental and cranial adaptations. Biological Journal of the Linnean Society. (1988) 33: 249–272.
8. Freeman, PW: Nectarivorous feeding mechanisms in bats. Biological Journal of the Linnean Society. (1995) 56: 439–463.
9. Forman, GL: Mammalogists comparative macro- and micro-anatomy of stomachs of macroglossine bats (megachiroptera:Pteropodidae). Journal of Mammalogy. (1990) 71: 555–565.
10. Dumont, ER and O'Neal, R: Food hardness and feeding behavior in Old World fruit bats (Pteropodidae). Journal of Mammalogy. (2004) 85: 8–14.
11. Nogueira, MR, Monteiro, LR, Peracchi, AL and Araújo, AFB: Ecomorphological analysis of the masticatory apparatus in the seed-eating bats, genus Chiroderma (Chiroptera: Phyllostomidae). Journal of Zoology. (2005) 266: 355–364.
12. Saint-Hilaire, GE: Description des mammaliferes qui se trouvent en Egypte. Descript. Egypte Histoire naturelle. (1818) 2: 119–128.
13. Monadjem, A, Taylor, PJ, Cotterillm, E and Schoeman, MC: Bats of Southern and Central Africa. Wits University Press. Johannesburg, South Africa. (2010)
14. Fenton, MB, Bell, GP and Thomas, DW: Echolocation and feeding behaviour of Taphozous mauritianus (Chiroptera: Emballonuridae). Canadian Journal of Zoology. (1980) 58: 1774–1777.
15. Weterings, R, Wardenaar, J, Dunn, S and Umponstira, C: Dietary analysis of five insectivorous bat species from Kamphaeng Phet, Thailand. The Raffles Bulletin of Zoology. (2015) 63: 91–96.
16. Martinez del Rio, C, Cork, SJ and Karasov, WH: Modelling gut function: An introduction. In: The Digestive System in Mammals – Food, Form and Function Chivers DJ & Langer P, editors. Cambridge, Cambridge University Press. (1994) pp. 25–53.
17. Julian, RJ: Production and growth related disorders and other metabolic diseases of poultry–a review. The Veterinary Journal. (2005) 169: 350–369. https://doi.org/10.1016/j.tvjl.2004.04.015 PMID: 15848778.
18. Leeson, TS, Leeson, CR and Paparo, AA: Text Atlas of Histology. USA, W. B. Sounders Co. (1988).
19. Novikoff, AB and Essner, E: The liver cell: Some new approaches to its study. The American Journal of Medicine. (1960) 29: 102–131.
20. Purohit, KG and Ghosh, PK: Histological and histochemical studies of tissues of two desert rodents. Meriones hurrianae and Tetra indica. Annals of Arid Zone. (1963) 2: 26–34.
21. Hodges, RD: The Histology of Fowl. 1st ed., University of London: Academic Press, London, New York. San Francisco. (1974) pp. 88–101.
22. Tanuma, Y and Ito, T: Electron microscope study on the hepatic sinusoidal wall and fat-storing cells in the bat. Archivum histologicum japonicum. (1978) 41: 1–39. https://doi.org/10.1679/aohc1950.41.1 PMID: 736751.
23. Bancroft, JD and Gamble, M: Theory and Practice of Histology Techniques. 6th ed. London, Churchill Livingstone. (2008) p. 725.
24. Mowry, RW. Journal of Histochemistry and Cytochemistry. (1956) 4: 407.
25. Masson, P: Some histological methods: Trichrome staining and their preliminary technique. Japanese Technical Methods. (1929) 12: 75–90.
26. El-Naggar, ER: Studies on the Weasel Mustela nivalis. subpalmate M.Sc. thesis. Deptartment of Zoology, Faculty of Science, Tanta University. Egypt. (1989).
27. Schaffner, F: The liver. In: Visceral Organs Gans C, editor. Philadelphia, Saunders. (1998) pp. 485–531.
28. Alshamy, Z, Richardson, KC, Harash, G, Hünigen, H, Röhe, I and Hafez, HM: Structure and age-dependent growth of the chicken liver together with liver fat quantification: A comparison between a dual-purpose and a broiler chicken line. PLOS ONE. (2019)14: e0226903. https://doi.org/10.1371/journal.pone.0226903.
29. Starck, JM and Beese, K: Structural flexibility of the small intestine and liver of garter snakes in response to feeding and fasting. The Journal of Experimental Biology. (2002) 205: 1377–1388.
30. Rappaport, AM: Anatomical considerations. In: Schiff's Diseases of the Liver. 10th ed Schiff ER, Sorrell MF & Maddrey WC, editors. Philadelphia, Lippincott Wilkins & Williams. (1963) pp. 1–46.
31. Motta, PM: The three-dimensional microanatomy of the liver. Archivum histologicum japonicum. (1984) 47: 1–30.
32. El-Salkh, AB, Zaki, TZ, Basuony, IM and Khidr, AH: Anatomical, histological and histochemical studies on some organs of true desert rodents in the Egyptian habitats. Egypt. The Egyptian Journal of Hospital Medicine. (2008) 33: 587–603.
33. Koca, YB, Gúrcú, B and Balcan, E: The histological investigation of liver tissues in Tritures karelinií and Tritures vulgaris (Salamandridae, Urodela). Russian Journal of Herpetology. (2004) 11: 223–229.

34. Moura, LR, Santos, ALQ, Belleti, ME, Vieira, LG, Orpinelli, SRT and De Simone, SBS: Morphological aspects of the liver of the freshwater turtle Phrynops geoffroanus Schweigger, 1812 (Testudines, Chelidae). Brazilian Journal Morphological Sciences. (2009) 26: 129–134.

35. Firmiano, EMS, Cardoso, NN, Vieira, DA, Sales, A, Santos, MAJ, Mendes, ALS and Nascimento: Histological study of the liver of the lizard Tropidurus torquatus Wied 1820, (Squamata: Tropiduridae). Journal of Morphological Sciences. (2011) 28: 165–170.

36. Nafady, AM and Awadalla, EA: Microscopically Studies on the Liver of Acanthodactylus boskianus lizard. Egyptian Academic Journal of Biological Sciences D. Histology & Histochemistry. (2019) 11(1): 94-62.

37. Jacobson, ER: Overview of reptile biology, anatomy and histology. In: Infectious Diseases and Pathology of Reptiles Jacobson ER, editor. London, UK, Taylor & Francis. (2007) pp. 1–2.

38. Akiyoshi, H and Inoue, A: Comparative histological study of teleost livers in relation to phylogeny. Zoological Science. (2004) 21: 841–850.

39. Kawanami, O: Electron microscopic study of mammalian liver with periodic acid methenamine silver stain—Basement membrane structure and fibrogenesis in space of Disse. Acta Pathologica Japonica. (1973) 23: 717–738. https://doi.org/10.1111/j.1440-1827.1973.tb02771.x PMID: 4131636.

40. Moreland, JE: Electron microscopic studies of mitochondria in cardiac and skeletal muscle from hibernated ground squirrels. Anatomical Record. (1962) 142: 155–167.

41. Horwitz, BA: The effect of cold exposure on liver mitochondrial and peroxisomal distribution in the rat, hamster and bat. Comparative Biochemistry and Physiology. (1976) 54a: 45–48.

42. Storch, V, Juário, JV and Pascual, FP: Early effects of nutritional stress on the liver in milkfish Chanos chanos (Forsskal) and on the hepatopancreas of the tiger praw Penaeus monodon (Fabricius). Aquaculture, (1984) 36: 229–236.

43. De Brito-Gitirana, L and Storch, V: Temperature induced alterations in the liver of wall lizard (Hemidactylus frenatus): Morphological and biochemical parameters. Micron. (2002) 33: 667–672.

44. Silva, DA, SM and Migliorini, RH: Effects of starvation and refeeding on energy-linked metabolic process in the turtle (Phrynops hilarii). Comparative Biochemistry and Physiology. (1990) 96: 415–419.

45. Marycz, K, KlećkowskA-nawrot, J, Maksymowicz, K and Wojciechowicz, E: Topographic and macroscopic characteristics of liver in red-eared turtle (Trachomys scripta elegans) after hibernation. Part I. Electronic Journal of Polish Agricultural Universities. (2009) 12 (2).

46. Ito, T: Cytological studies on stellate of Kupffer and fat storing cells in the capillary wall of the human liver (Japanese abstract). Acta Anar Nippon. (1950) 26:42

47. Barbara, Y, Geraldine, O and Phillip, T: Histology of the Digestive Tract: Wheater's Functional Histology. 11th ed. Philadelphia, Elsevier, Churchill Living Stone p. (2014) pp. 274–280.

48. Singh, I: Digestive System: Textbook of Human Histology. New Delhi, India: Jaypee Brother's Medical Publishers (P) Lt. (2014) p.249–57.

49. Scalia, M, Geremia, E, Corsaro, C, Santoro, C, Sciuto, S and Sichel, G: The extracutaneous pigmentary system: Evidence for the melanosynthesis in Amphibia and Reptilia liver. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry. (1988) 89: 715–717.

50. Hünigen, H, Mainzer, K, Hirschberg, RM, Custodis, P, Gemeinhardt, O and Al Masri, S, *et al*.: Structure and age-dependent development of the turkey liver: A comparative study of a highly selected meat-type and a wild-type turkey line. Poultry Science. (2016) 95: 901–911. https://doi.org/10.3382/ps/pev358 PMID: 26908884.

51. Oláh, I, Nagy, N and Vervelde, L: Structure of the avian lymphoid system. Avian Immunology. Elsevier (2014) p. 11–44.

52. Manera, M, Sera, R, Isani, G and Carpenea, E: Macrophage aggregates in gilthead sea bream fed copper, iron and zinc enriched diets. Journal of Fish Biology. (2000) 57: 457–465.

53. Roberts, RJ: Fish Pathology. 3rd ed. London, W. B. Saunders, 489p. (2001)

54. Sichel, G, Scalia, M and Corsaro, C: Amphipia Kupffer cells. Microscopy Research and Technique. (2002) 57: 477–490.

55. Agius, C and Roberts, RJ: Melano-macrophage centres and their role in fish pathology. Journal of fish Diseases. (2003) 26: 499–509.

56. Kardong, KV: Comparative Anatomy of Associated Glands of Digestion, Vertebrates, Comparative Anatomy. 4th ed. New York, Hata, McGraw-Hill. (2005) p.13.

57. Zieri, R, Taboga, SR and de Oliveira, C: Melanocytes in the testes of Eupemphixnattereri (Anura, Leiuperidae): Histological, stereological and ultrastructural aspects. Anatomical Record. (Hoboken) (2007) 290: 795–800.

58. Lamers, CHJ and De Haas, MJ: Antigen localisation in the lymphoid organs of carp (Cyprinus carpio). Cell and Tissue Research. (1985) 242: 491–498.

59. Guida, G, Zanna, P, Gallone, A, Argenzio, E and Cicero, R: Melanogenic Response of the Kupffer cells of Rana esculenta L. to melanocyte stimulating hormone. Pigment Cell Research. (2004) 17: 128–134.

الملخص العربى

دراسة نسيجية ، كيميائية نسيجية ودقيقة باستخدام الميكروسكوب الالكتروني لتركيب الكبد في خفاش المقابر المصري (تافوزوس بيرفوراتس)

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المقدمة: تظهر الخفافيش اسلوب حياة مميز. هذه الثدييات قد تكون اكلة للفاكهة او الحشرات او ماصة للدماء اعتمادا علي الصفات المورفولوجية والبيولوجية والتشريحية. الخفافيش المصرية Taphozous perforatus هي آكلة للحشرات.

الهدف من الدراسه: بحثت هذه الدراسة في الأنسجة والبنية التحتية الدقيقة لكبد T. perforatus لتقييم تكيفها مع نظامها الغذائي وطريقة حياتها.

المواد وطرق البحث: تم تجميع عشرة من T. perforatus البالغة من كل جنس من أبورواش ، محافظة الجيزة ، مصر. تم تثبيت عينات الكبد من أجل الفحص المجهري الضوئي والإلكتروني لفحص البنية الكبدية. كما تم استخدام الصبغات الهستوكيميائية والخاصة.

النتائج: أظهرت المقاطع المصبوغة بالهيماتوكسيلين والأيوزين أن خلايا الكبد مرتبة في اشرطة او الواح كبدية. الترتيب الشعاعي للاشرطة الكبدية حول الأوردة المركزية غير واضح بشكل عام ، ويتم فصل الخلايا الكبدية عن طريق أشباه الجيوب الدموية. أظهرت الدراسات النسيجية الكيميائية وجود ألياف الكولاجين حول المنطقة البابية بين الجيوب الكبدية وحبيبات الجليكوجين المنتشرة في جميع أنحاء سيتوبلازم بعض خلايا الكبد. الجليكوجين المتراكم في اطراف خلايا الكبد الأخرى يشير إلى ظاهرة طيران الجليكوجين. تشبه الخلايا الكبدية T. perforatus تحت TEM الخلايا من الطيور والثدييات ، ولكن مع نوى محيطية طرفية ، والعديد من الميتوكوندريا ، وعدد كبير من تجمعات الخلايا الليمفاوية غير المنتظمة ، ونواة داكنة وبيضاوية ممدودة مع أغشية نووية مثقبة وقليل جدًا من السيتوبلازم.

الخلاصة: يمكن استنتاج انه تتطابق السمات النسيجية والكيميائية النسيجية والبنية التحتية لكبد الخفافيش T. perforatus مع سمات الثدييات الأخرى ، مع استثناءات قليلة تعتمد على نمط الحياة واحتياج الطاقة أثناء الطيران.