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The anatomy, histology, and oxidative stress level of the liver in fruit bat (*Rousettus amplexicaudatus*)

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Abstract. *Dhamayanti Y, Suryadiningrat M, Mujiburrahman A, Firdausy LW, Maslamama ST, Purnama MTE. 2025. The anatomy, histology, and oxidative stress level of the liver in fruit bat (*Rousettus amplexicaudatus*). Biodiversitas 26: 102-110.* Among the wildlife species known for propagating zoonoses is the fruit bat (*Rousettus amplexicaudatus*). It is known that the liver organ serves as a detoxifying center in the only flying mammal. An examination of the liver's anatomy, histology, and oxidative stress level was essential to bridge the gap between earlier research and take into account the liver's critical function in bats. Seven bats—four males and three females—were enlisted to examine the liver's gross anatomy, histology, levels of glutathione peroxidase (GPx), superoxide dismutase (SOD), malondialdehyde (MDA), and catalase (CAT). The study's findings, which highlight the unique aspects of bat liver anatomy and the absence of gender differences in enzyme levels, have significant implications for our understanding of zoonotic diseases. As a result, the liver is covered by connective tissue, which also separates the liver parenchyma into three lobes: the cranial, middle, and caudal lobes. The liver of a bat lacked an additional lobe, in contrast to most mammals. According to histology, the liver lobule's hepatocytes are grouped radially from the center and terminate in the central vein. The portal vein, bile ductules, and hepatic artery branches were all visible in the triad. Furthermore, this study found no significant differences ($p<0.05$) between male and female bats in terms of MDA ($p = 0.463$), SOD ($p = 0.686$), GPx ($p = 0.455$), and CAT ($p = 0.443$). As a reference for typical circumstances, this study concluded the anatomy, histology, and antioxidant enzymes of fruit bats' livers. Furthermore, fruit bats with normal liver conditions might be compared to the antioxidant enzyme profiles we reported.

Keywords: Biological diversity, Chiroptera, histomorphology, liver

INTRODUCTION

Bat colonies, numbering in the millions, make them one of the most prevalent mammals and one of the organisms with the highest numbers on Earth (Hammerson et al. 2017). The two suborders of the order Chiroptera, Yinpterochiroptera and Yangochiroptera, encompass 1,300 species of bats (Teeling et al. 2016). Bats play a variety of crucial ecological functions, including pollination and seed distribution, which help restore degraded areas, preserve plant genetic diversity, and manage insect populations, including pests in cities and agriculture. It is important to appreciate the distribution of their variety across settings, which several reasons may influence. Many bat species worldwide are experiencing population declines due to a variety of factors, including habitat loss and fragmentation, climate change, air pollution, and illnesses like white-nose syndrome (Rodhouse et al. 2012).

According to Allocati et al. (2016), bats are among the wild animals that may transmit zoonoses. Bats, as flying mammals, have the potential to be the largest zoonotic reservoir when compared to other mammalian groups (Olival et al. 2017). It is crucial to understand that bats can carry several viruses, including Ebola (Caron et al. 2018), SARS-related coronaviruses (SARSr-CoVs) (Yu et al. 2019), Rabies (Begeman et al. 2020), Marburg (Wood et al. 2023), and Hendra and Nipah (Halpin and Rota 2023).

The liver is the largest gland in the body and an accessory organ of the digestive system. It is the largest gland and performs many essential responsibilities, such as synthesis and metabolism of proteins, carbohydrates, albumin, and different hormones; glycogen storage; detoxification of endogenous and exogenous substances; bile secretion; and control of inflammatory responses (Qin and Crawford 2018). The liver organ, which serves as a detoxification center, is found in the only flying animal. Producing protein, which is the building block for the immune system, is another job the liver does. The primary source of Reactive Oxygen Species (ROS) and an organ frequently subjected to the consequences of oxidative stress is the liver (Ramos-Tovar and Muriel 2020). Compared to other organs, the liver has the most cytochrome p450

isoenzymes and is involved in the metabolism of xenobiotics and foreign compounds (Esteves et al. 2021). In order to neutralize poisons, cytochrome p450 metabolism can generate ROS, which the liver might then store (Sukmanadi et al. 2021). Excessive free radical damage to the liver can result in oxidative stress and lipid peroxidation, which can lead to inflammation, necrosis, and morphological abnormalities (Al-Zuhroh et al. 2021). The number of Kupffer cells in the liver increases significantly when it is exposed to viruses and other infectious agents. The liver is full of macrophages called Kupffer cells. Kupffer cells are innate immunity-related cells that control the immune response and inflammatory process (Purnama et al. 2021). This study examined the anatomy, histology, and oxidative stress levels of the liver. It was essential in bridging the gap between previous investigations and explaining the critical role of the liver in bats.

MATERIALS AND METHODS

Ethical approval

The Research Ethics Commission has approved this study, Animal Care and Use Committee, Universitas Airlangga with approval No.390/HRECC.FODM/VII/2021. An ethical declaration was submitted to protect the animals from injury and unnecessary stress throughout the investigation. From July to December 2021, this study was carried out in the Veterinary Anatomy Laboratory, Faculty of Health, Medicine, and Life Sciences, Universitas Airlangga, Banyuwangi, East Java, Indonesia.

Animals

A total of 7 fruit bats, *Rousettus amplexicaudatus* (E.Geoffroy, 1810) (4 males and 3 females) were caught randomly, implementing an accidental sampling technique using a 60 cm diameter net trap installed at a height of 8 meters in the Djawatan forest area, Banyuwangi. The bats were then reared in a dark cage measuring 75×45×110 cm, fed with fruits, drinking water ad-libitum, and maintained a natural photoperiod. For 7 days, the bats were acclimatized, and their sex was determined based on the presence of the uropatagial gland in males.

Gross anatomical evaluation procedures on bat liver

Euthanasia was performed by intramuscular injection of Xylazine (2 mg/kg BW) and Ketamine (10 mg/kg BW), and death was indicated by the anus relaxing and the pupillary light reflex ceasing. After that, each bat was placed dorsally recumbent (Figure 1) for the purpose of dissecting their abdominal cavity and weighed using a portable balance (OHAUS CL 201T®, Canada).

The abdominal region's cutaneous, muscular, and skin layers were identified as the site of dissection in a longitudinal orientation from the sternum's caudal side. The diaphragmatic layer on the cranial side served as a barrier to the peritoneal layer and all digestive organs, which were discovered during abdominal dissection. Next, using a portable balance, the stomach's liver was removed from the surrounding serosal tissue and weighed. A camera (Fujifilm X-A3®, Japan) was used to record the liver's full gross anatomical structure at a distance of 25 cm from the object after the liver's length, width, and thickness were measured with a vernier caliper (Figure 2).

Histological processing of bat liver

Following their division into many parts, the livers were promptly fixed in a solution containing 10% neutral buffered formalin and Bouin's fluid for 24 hours. After 24 hours, tissue samples were dehydrated in a sequence of increasing ethyl alcohol solutions and then cleaned with xylene until transparent and embedded in paraffin. Next, 5 µm histological sections were stained with hematoxylin and eosin (H&E), and histological slides were mounted using Canada balsam. Every preparation was inspected using a binocular microscope (Olympus CX-23®, Japan).

Figure 1. Fruit bat *Rousettus amplexicaudatus* with dorsal recumbency

Oxidative stress level quantification procedure in bat liver

The levels of glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA) were measured in four sections of liver samples collected from each bat individual. After weighing each liver, the weight was noted in milligrams. The MDA Assay Kit (Cat#E-BC-K025-S, Elabscience® Biotechnology Inc., USA) was utilized to quantify the levels of MDA. Thiobarbituric acid (TBA) and MDA can combine to form a crimson mixture that can be absorbed in 532 nm. Utilizing the SOD Assay Kit (Cat#SKT-214-SOD®, Stress Marq, Canada), SOD levels were quantified. SOD activity was measured in units/mL, where one unit of SOD is the quantity of enzyme that, at 25°C and pH 7.8, inhibits the reduction of 1.5 Mm Nitro blue tetrazolium in riboflavin by half. CAT levels were measured with the CAT Assay Kit (CAT#E-BC-K031-S, Elabscience® Biotechnology Inc., USA), and GPx levels were measured with the GPx Assay Kit (Calbiochem®, Germany).

Data analysis

Based on observations of histology and gross anatomy, the liver that was removed from each bat was assessed and described. Meanwhile, each bat's body weight, liver morphometry, and antioxidant enzyme profiles were recorded and examined using a 95% confidence level independent T-test. SPSS v.26 (IBM, USA) was used to support the statistical analysis.

RESULTS AND DISCUSSION

Gross anatomy of bat liver

This study found a significant difference (p = 0.017) between the weight of male bats (63.05±2.63) and female bats (55.43±3.10) based on the initial assessment of the bats' body weight (Table 1). According to this study, the bat liver was located in the abdominal cavity's cranial dexter and was bounded by the anterior side diaphragm. The ventral side of the liver contains the stomach, duodenum, jejunum, ileum, large intestine, and uterus (Figure 2). A thin capsule of connective tissue, known as the Glisson capsule, encloses the liver and includes tiny blood arteries. The cranial, medial, and caudal lobes are the three lobes of the liver parenchyma that are separated by the connective tissue that covers the liver. The liver of a bat lacked an accessory lobe, in contrast to other mammals (Figure 3). Following the separation of the hepatic blood vessels and serous tissue, measurements of the bat liver were performed. Male bats (2.50±0.21) and female bats (1.83±0.30) had significantly different relative liver weights (p = 0.019), according to the current study (Table 2). Furthermore, a significant connection (y = 0.0415x-0.268) was found between bat body weight and relative liver weight; this study emphasized the notion that relative liver weight increases when body weight increases. It is evident that the liver weight rises with body weight, albeit more slowly. If one compares the exponential equation of each, the relationship between bats' body weight and liver weight becomes more linear. Thus, the constant logarithm of the mean liver weight was plotted as the ordinate, and the logarithm of the body weight as the abscissa (Figure 4).

Table 1. Body weight of male and female bats

Sample no	Gender	Body weight (g)	Mean±SD	p-value
1	Male	62.9	63.05±2.63	0.017*
2		60.9		
3		61.6		
4		66.8		
5	Female	55.5	55.43±3.10	
6		52.3		
7		58.5		

Note: Significant at * p<0.05; ** p<0.01; *** p<0.001

Table 2. Liver morphometry of male and female bats

Parameter	Male (n: 4)	Female (n: 3)	p-value
Relative liver weight (g)	2.50±0.21	1.83±0.30	0.019*
Length (mm)	30.85±0.64	31.7±0.50	0.117
Width (mm)	16.00±1.99	14.43±1.75	0.329
Liver thickness (mm)	6.23±1.79	5.28±0.90	0.443

Note: Superscripts * p<0.05; ** p<0.01; *** p<0.001 represent significance levels in the same row

Figure 2. Liver location in the abdominal cavity of bats. (X) liver: a. Stomach; b. Duodenum; c. Jejunum; d. Ileum; e. Large intestine; f. Uterus

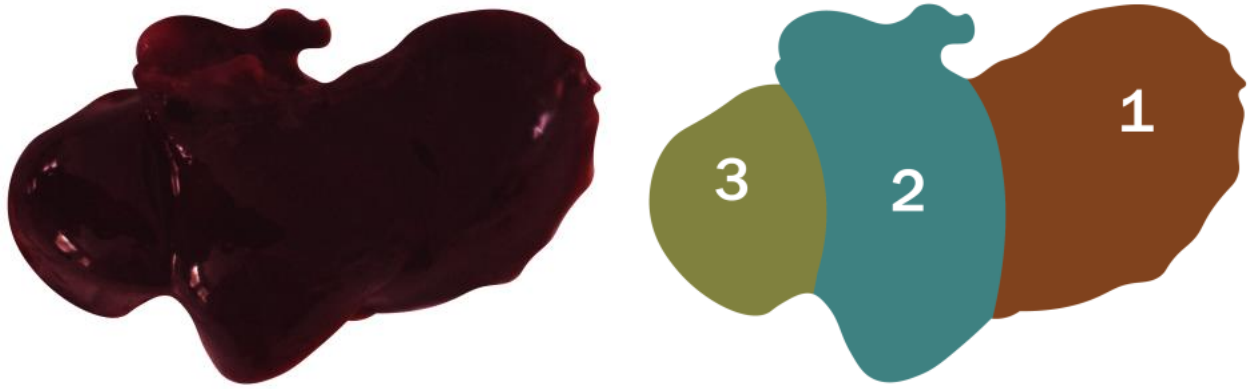

Figure 3. Gross anatomy of the bat liver. 1. Cranial lobe; 2. Medial lobe; 3. Caudal lobe

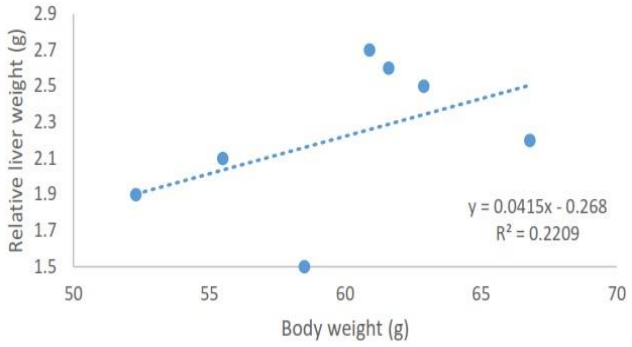

Figure 4. Correlation of body weight to relative liver weight

Histological evaluation of bat liver

The primary component of the bat liver identified in this study's histological analysis was the hepatocytes. These cells, each with one or two spherical nuclei and one or more nucleoli, form a layer known as hepatocytes. The liver lobule, a structural unit composed of interconnected groups of hepatocytes, was also identified. The hepatocytes are arranged radially from the center and terminate in the central vein. The hepatic plates, lined with simple layered hepatocytes, have a central vein as their axis. Columns of hepatic cells are oriented radially from the central vein to the lobule's perimeter and a small portion of the portal triad on the plates. The portal vein, bile ductules, and hepatic artery branches were all visible in the triad. Notably, the sinusoidal capillaries were found to be irregularly dilated and composed of a discontinuous layer of fenestrated endothelial cells (Figure 5). This meticulous study provides a comprehensive understanding of the bat liver's histology, instilling confidence in the research.

Oxidative stress level in bat liver

The current study found no significant differences ($p>0.05$) between male and female bats in terms of MDA ($p = 0.463$), SOD ($p = 0.686$), GPx ($p = 0.455$), and CAT ($p = 0.443$), according to the evaluation of antioxidant enzymes (Table 3). A favorable association between the liver's relative weight and MDA ($y = 10.443x-1.695$), SOD ($y = 0.3684x+2.568$), GPx ($y = 0.2398x+2.6931$), and CAT ($y = 180.03x+1498.7$) was also revealed by the current investigation (Figure 6). The current results highlight the fact that antioxidant enzyme levels in male and female bats do not differ significantly, and it is presumed that normal levels of antioxidant enzymes can be attributed to the current investigation.

Table 3. Antioxidant levels in bat livers

Parameter	Male (n: 4)	Female (n: 3)	p-value
MDA (µM)	23.97±11.36	18.04±6.74	0.463
SOD (µ/mL)	3.16±1.30	3.67±1.97	0.686
GPx (µ/L)	2071.42±574.80	1665.34±763.74	0.455
CAT (µ/L)	2.96±1.17	3.58±0.58	0.443

Note: Malondialdehyde (MDA); superoxide dismutase (SOD); glutathione peroxidase (GPx); catalase (CAT). Superscripts * $p<0.05$; ** $p<0.01$; *** $p<0.001$ represent significance levels in the same row

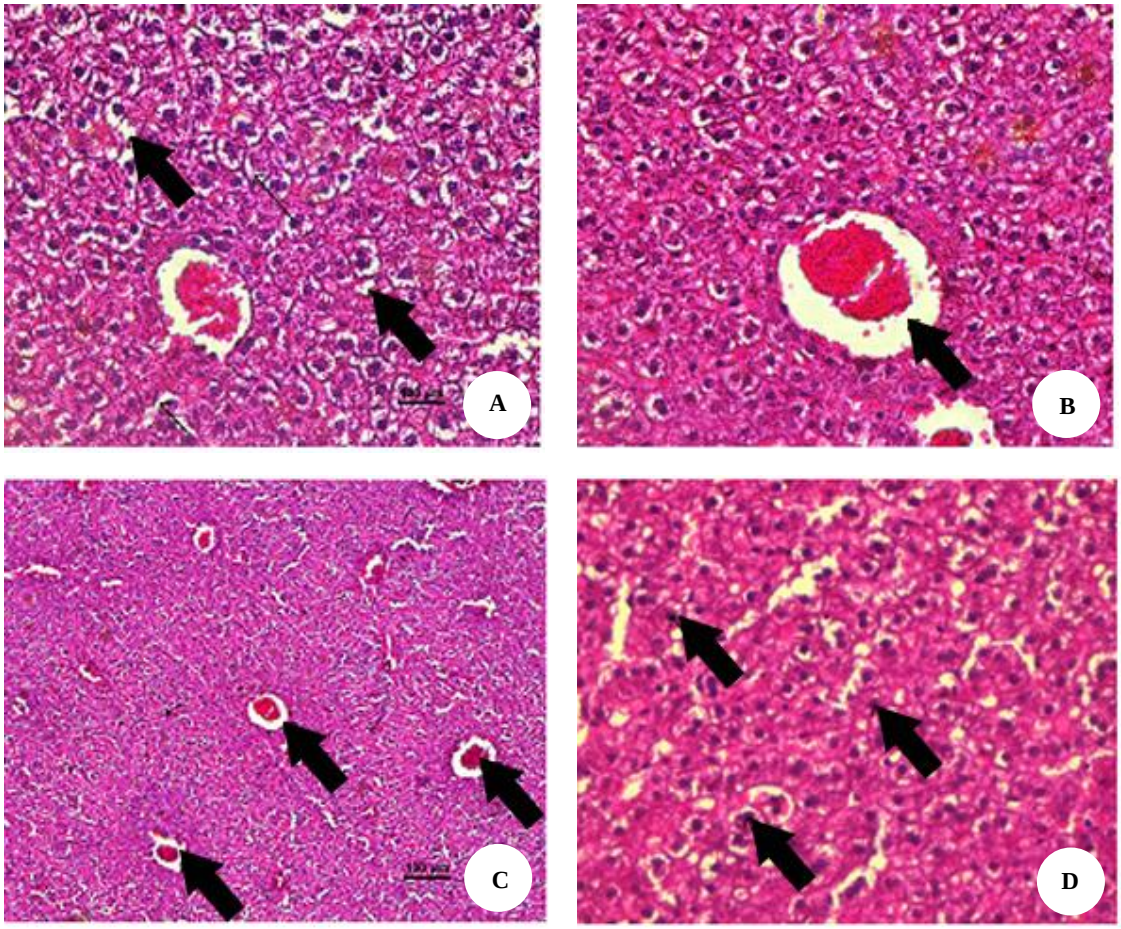

Figure 5. Bat liver histology is represented by arrows on: A. Sinusoids (H&E, 100 magnification); B. Central vein (H&E, 100 magnification); C. Central vein (H&E, 40 magnification); D. Hepatocytes (H&E, 100× magnification). Hepatic plates or hexagonal lobules extending outward from a core vein are visible in this liver segment; the central veins are very noticeable. Hepatocytes displayed endothelial-lined blood sinusoids, conspicuous envelopes, properly distributed chromatin, and uniformly granulated cytoplasm with peripherally placed nuclei

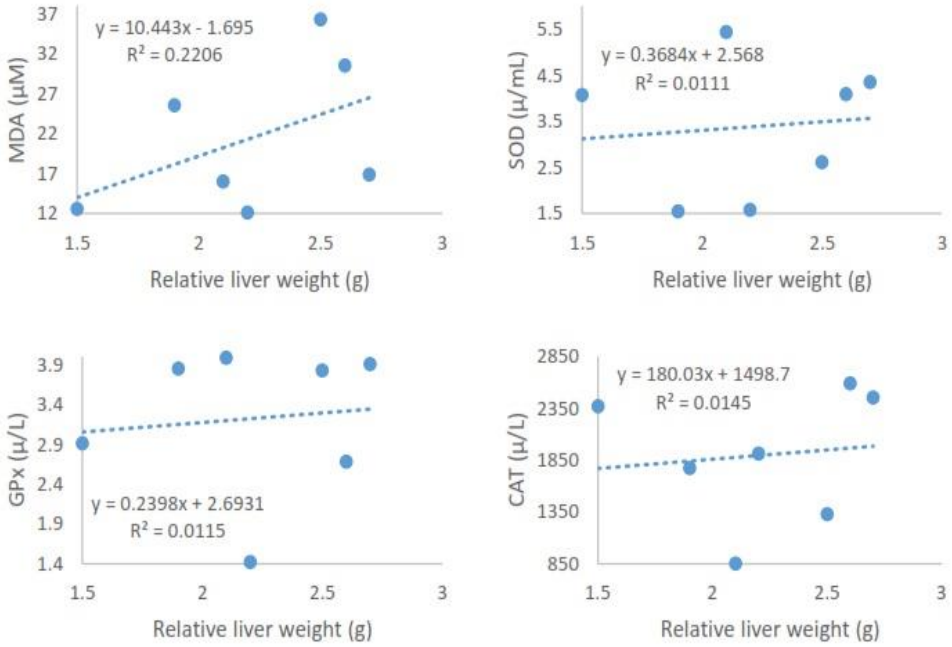

Figure 6. Correlation of relative liver weight to the respective oxidative stress level

Discussion

The bat species found in this investigation was *Rousettus* sp.. Fruit-eating bats, medium-sized, short-tailed, hairless, cone-shaped earlobes, powerful, prominent snouts, prominent nostrils divided by a philtrum, and large, dark brown eyeballs are the morphological traits of *Rousettus* sp.. The lingual mucosa, lingual aponeurosis, and lingual muscle make up the three layers that make up the tongue. Bats can handle their food more easily since the lingua is a stretchy organ (Gunawan et al. 2020). Male bats in this study were found to weigh considerably more on average than female bats ($p<0.05$). In order to locate females during the mating season, male bats engage in polygynous mating behavior, which is bolstered by elevated testosterone levels (Ruff et al. 2017). Male bats often weigh more because testosterone, an anabolic steroid androgen, can increase muscular mass. The inverse link between testosterone and obesity, which is influenced by a bidirectional mechanism, has been conclusively demonstrated. Testosterone is changed into oestradiol by adipose tissue, particularly visceral fat. Homeostatic processes initially accelerate the rate of testosterone release, but as body fat increases, a threshold is reached where insulin resistance and adipocytokines suppress synthesis and levels decrease. Low testosterone levels promote the growth of fat depots, especially visceral fat in the abdomen, and may affect the liver, muscles, and arteries (Kelly and Jones 2015).

According to earlier research on Indian short-nosed fruit bats, females forage less than males, and there is a positive relationship between body weight and foraging behavior. Furthermore, when the size of the female group increases, males devote more time to social grooming, tent upkeep, and tent guarding (Mahandran et al. 2018). Another study found that female bats would be more nimble and able to adapt to the increase in weight during pregnancy if they had lower wing loading and a lower dimension ratio than males of the identical foraging guild. These studies confirmed that pregnant female bats had more wing loading than nonpregnant bats, while nonpregnant females also had higher wing loading than male bats. This indicates that the species in those studies exhibited comparable patterns to those reported in the literature, with pregnant females having higher wing loading than males but being less mobile prior to gaining mass during pregnancy. This immediately contradicts our last prediction by implying that pregnant female bats would become even less maneuverable. Pregnancy may make it more challenging for bats to forage in a congested area if they need to fly with agility. Pregnancy would only make this decreased maneuverability worse (Maucieri et al. 2021).

Histological analyses of bat livers showed variations from those of other mammals. As endocrine and exocrine glands, hepatocyte cells generate and accumulate some chemicals while detoxifying others (Sugahara et al. 2021). Hepatocytes with larger mitochondria manifest eosinophilic once stained using hematoxylin and eosin. Hepatocyte cells have a big nucleus (Calleri et al. 2021). From the central vein, hepatocytes branch out towards the liver lobule's margin, forming walls that are aligned in a circle. Capillary blood channels called hepatic sinusoids fill the lobules and transport blood from the interlobular veins and arteries into the sinusoids and then to the central vein. In the liver sinusoids, the endothelium lacks a basal lamina. The disse or perisinusoidal gap is the space between sinusoids and hepatocyte cells. The exchange of nutrients among cells and plasma may occur at the Disse gap (Liu et al. 2019). Meanwhile, the hepatic sinusoids are capillary regions that transport blood to the central veins from the lobules' periphery via the hepatic artery and portal vein branches. Recto endothelial cells, also known as Kupffer cells, and a thin discontinuous endothelium with a discontinuous basal lamina, frequently absent, line the hepatic sinusoids (Arráez-Aybar et al. 2018).

Interestingly, Kupffer cells, which are involved in the immunological response, were not seen in additional liver histology studies. These results show that the bats examined in this study were free of pathogens. Kupffer cells were seen to have many pseudopods and sporadic cup-like indentations in a prior investigation. The cells have big nuclei that range in form from triangular to oval. Electron-dense lysosomes were seen, and cytoplasmic membranes had noticeable pseudopodia that projected into the sinusoidal lumen. Erythrophagocytosis was noted, and the cytoplasm typically contained a large number of lysosomes that were dispersed at random and varied in size and internal composition. The black, oval, elongated nuclei of lymphocytes were irregularly shaped, with fenestrated nuclear membranes and a small amount of cytoplasm in a cytoplasmic process. Seldom seen in the perisinusoidal region were found in the sinusoidal lumen. According to El-Nahass and Elwan (2023), a lymphocyte in the perisinusoidal region was probably undergoing amoeboid migration to the sinusoidal lumen. In their homeostatic function, host cells effectively protect the immune response by expressing sophisticated surveillance mechanisms on scavenges and macrophages through lysosomal degradation and phagocytosis. The mechanism of Kupffer-cell uptake is either erythrophagocytosis or pinocytosis, depending on the particle size. In the liver, Kupffer cells are the main cells that phagocytose big particles. Although periportal Kupffer cells often exhibit higher levels of phagocytic activity than those found in other liver areas, not all Kupffer cells are actively phagocytic (Taban et al. 2022). An important function of Kupffer cells is to process and present antigens to T lymphocytes. In a study conducted on squirrel monkeys, the presence of these cells in the liver parenchyma was linked to infectious diseases, specifically yellow fever (Ferreira et al. 2023).

The main functional and structural component of the bat liver is the acinus. A portal triad, located in the portal gaps among hepatic lobules with a portal canal at each corner, and interconnected hexagonal hepatic lobules with hepatocytes and sinusoidal structures make up the organ. Collagen fibers make up the majority of the dense connective tissues that envelop the liver, whereas fibroblasts make up the majority of the small cells. A layer of peritonitis mesothelium sits on top of these connective tissues; the collagen fibers and mesothelium combine to produce what is known as Glasson's capsule. A branch of

the vena cava, the central vein, is situated in the middle of each hepatic lobule and radiates outward from there. The cell sheets extend sharply or resemble bands as they approach the lobules' margins. Except for the one located in the Portal area, which contains the portal vein, hepatic artery, bile duct, and lymphatic arteries, it is unknown which connective tissues split apart among the lobules (Delgado-Coello 2021). In line with those who found comparable outcomes in the avian liver, collagen fibers protected hepatocytes and sinusoids in our study. Elastic fibres were also seen in the connective tissue sheaths surrounding polygonal hepatic lobules. In the current investigation, perisinusoidal gaps were discovered between sinusoidal endothelial cells and hepatocytes. The hepatic cells were isolated from the vascular channel wall and covered in a large number of long microvilli. Slender bile canaliculi created by the apical faces of neighboring hepatic cords flowed centrifugally to the neighboring portal canal. In contrast to those who revealed multiple layered plates of hepatocytes detached by narrow and curly, complex sinusoids in fish liver, hepatic plates were made up of simple layered hepatocytes, also known as one-cell-thick plates. These findings were in line with those of researchers who examined the livers of mammals and snakes. Hepatocytes had uniform surfaces and polygonal, spherical, or oval peripheral nuclei. For the *Mustela nivalis* (Linnaeus, 1766) liver, somewhat comparable findings were noted. Similar to *Acanthodactylus boskianus* (Daudin, 1802), the cytoplasm included several vacuoles of different sizes, which caused the hepatocyte nuclei to move to the periphery (Alshamy et al. 2019). On the other hand, binuclear hepatocytes are more common in mammals. The current investigation revealed vesicular nuclei with noticeable scattered chromatin granules and one or more nucleoli, as well as hepatocyte nuclei with nucleoli and chromatin scattered throughout the nucleoplasm. Similar findings reported in the livers of chickens, mice, hamsters, and bats were confirmed by the discovery of a large number of rough endoplasmic reticulum and closely packed, spherical, oval, and elongated mitochondria. Thermogenic capability in birds is associated with modifications to the mitochondrial machinery of the muscles or liver. Increases in oxidative phosphorylation and proton leak were not the only factors contributing to the thermogenic capacity increase. It may, therefore, possibly be the consequence of increased ATP synthesis in muscle fibers (Milbergue et al. 2022).

The portal triad structure, which represents the interlobular artery, interlobular vein, and interlobular duct sections, was not clearly visible in the bat liver used in this investigation. The hepatic veins carry the portal venous blood back to the inferior vena cava. These are big and distributed differently than the portal triad. The right, left, and central hepatic veins are the three main veins. These drain into the inferior vena cava at the postero-superior aspect of the liver after passing through the liver material in a postero-superior manner. Although the configuration varies, the center vein—the smallest of the three—typically opens into the left vein shortly before it ends. Furthermore, a variable number of accessory veins always extend straight from the liver to open along the inferior vena cava, which is located distal to the main vein openings (Ellis 2011).

The mitochondrial electron transport chain generates ROS in addition to ATP during aerobic metabolism. While low to moderate ROS concentrations aid in cellular physiological functions, high ROS concentrations harm lipids, proteins, and DNA, among other components. In this study, MDA levels were used as an indication to analyze ROS in the bats' livers. Male and female bats' MDA levels did not differ significantly, according to the results ($p>0.05$). A prior investigation in rats found that increased SOD activity was accompanied by either unchanged or reduced catalase activity. Acute psychological stress may cause even more severe oxidative damage than acute physical stress because it raises corticosterone and lipid peroxidation levels. Furthermore, there is compelling evidence that counter-regulatory mechanisms are triggered to stop additional hepatocyte damage due to the development of cell protection in the hepatic tissue of rats under chronic stress (Jafari et al. 2014). Bats' rapid metabolic rate causes them to produce free radicals, or ROS, which are a reservoir of viruses. Both foreign chemicals and regular intracellular metabolism can generate ROS. Both endogenous and external sources can produce ROS, which are pleiotropic molecules. The endoplasmic reticulum, mitochondria, peroxisomes, lysosomes, oxidases such as xanthine oxidase and nicotinamide adenine dinucleotide phosphate oxidase, cytochrome p450, and lipoxygenase are examples of endogenous or cellular sources (Soundararajan et al. 2024).

Lipid peroxidation is a chain reaction that can result in the formation of MDA when hydroxyl free radicals, like ROS, interact with fatty acid components of the cell membrane. Proteins, cell membranes, and nucleic acids can conjugate with lipid oxidation carbonyls to form genotoxic advanced lipid oxidation end products, which are also potentially lethal fatty acid degradation products. Antioxidant concentration, prooxidants, and the degree and type of fatty acid unsaturation all affect the stability of lipids and the lipid oxidation products that develop (Vieira et al. 2017). Under natural circumstances, vertebrate animals generate antioxidants to counteract free radicals and preserve the body's equilibrium. SOD and oxygen have a unique and close association since SOD depends on oxygen to survive. SODs consistently show the initial line of defense against disease or damage caused by ROS. Superoxide anions are either a result of metabolic processes such as mitochondrial respiration or signaling enzymes. After being catalyzed by SOD, these ROS are transformed into molecular oxygen and H_2O_2, which are then utilized in other procedures. It was the metal ions at the SOD active site that catalyzed the ROS conversion (Saxena et al. 2022). The dismutase reaction that breaks down superoxide anions (O_2^-) into oxygen molecules (O_2) and hydrogen peroxide (H_2O_2) is catalyzed by SOD. In this study, the antioxidant activity of fruit bat liver was assessed by analyzing the levels of SOD in the liver. The SOD levels of male and female bats did not differ significantly ($p>0.05$), suggesting that when oxidative stress arises, the cells in

male and female bats make the same proactive attempts to prevent cellular damage by generating oxidants and re-establishing redox balance. The activity of glutathione peroxidase and catalase is linked to the physiological role of SOD (Li et al. 2015).

The antioxidant enzymes SOD, GPx, and CAT are crucial for biological systems' ability to fend off free radical damage. The cytoplasm and mitochondria include the enzyme GPx, which is crucial for hydrogen peroxide detoxification. In addition to protecting cells from hydrogen peroxide, GPx also helps shield cells against organic peroxides, including those that cause fatty acid and cholesterol oxidation (Oliveira et al. 2017). Selenium is essential for GPx because it allows this enzyme to operate normally as an antioxidant. According to Mansourian et al. (2018), GPx readily releases protons from muscles, tissues, and red blood cells. GPx is present in high concentrations in erythrocytes and the liver. GPx catalyzes the conversion of reduced glutathione into oxidized glutathione in an acidic environment with the coenzyme NADP. Male and female CAT levels in bat liver did not differ significantly, according to the results ($p>0.05$). The quantity of hydrogen peroxide, measured in moles, that CAT can reduce in a minute is one unit of CAT activation. Peroxisomes, cytoplasm, and red blood cells are the common locations for CAT (Dutta et al. 2021). The liver contains high levels of CAT (Mansourian et al. 2018). As a unique peroxidase, CAT's primary job is to convert or get rid of hydrogen peroxide in the body. According to Oliveira et al. (2017), CAT and GPx are both crucial for hydrogen peroxide detoxification. Five different bat species—*Tadarida brasiliensis* (I.Geoffroy, 1824), *Anoura caudifer* (E.Geoffroy, 1818), *Myotis nigricans* (Schinz, 1821), *Artibeus lituratus* (Olfers, 1818), and *Sturnira lillium* (E.Geoffroy, 1810)—were used in earlier research. These investigations found that SOD and CAT are significant antioxidants in the bat's body when it is infected with a virus (Filho et al. 2007). The bat's function as a viral reservoir is strongly supported by fluctuating levels of ROS and antioxidants in its body. The bat's liver produces antioxidants, including SOD, GPx, and CAT, that help the body survive in its natural state of wellness. Our perspective suggests that this study's limitation is that it did not fully investigate whether bats have a history of subclinical infectious diseases and whether they could serve as natural reservoirs for other vertebrates. However, our findings provide a solid foundation for future investigations into bats associated with certain diseases, guiding their research and potentially leading to comparable findings.

In conclusion, the cranial, caudal, and caudate lobes represented the liver of fruit bats, according to macroscopic observations. In histological sections, hepatic lobules formed by plates of hepatocytes, interspersed with sinusoidal capillaries and the centrolobular vein, can be observed. MDA, SOD, GPx, and CAT levels in male and female bats did not differ significantly ($p>0.05$) according to oxidative stress level quantification. Furthermore, our results might be used as a baseline for fruit bats' typical levels of antioxidant enzymes.

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