

Comparative anatomy the heart and lungs of the Rat (*Rattus norvegicus*), Bat (*Manis tricuspis*), and Pangolin (*Eidolon helvum*)

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Abstract

Introduction: The difference in the physical activities of these three animals necessitated this study, (bat being a flying animal, rat terrestrial, pangolin terrestrial and slow or sluggish animal). **Aim:** This study compared the heart and lungs of rat, bat and pangolin in relation to their physical activities, feeding pattern, morphology and behavior and function. **Methods:** The study was carried out using fifteen (15) animals, five rats, five bats and five pangolins of both sexes. The pangolins were sourced from Asejire, a village in the North West of Osun state, Nigeria. The bats were procure from a local hunter in Oke-Baale Osogbo Osun state, Nigeria. While the rat where obtain from the animal holdings Osun State University Osogbo, Nigeria. The animals were sacrifice under chloroform inhalation and the heart and lungs were obtained after longitudinal incision was made through the mid-thoracic and mid-abdominal wall of the animals. The heart and lungs were fixed in 10% formol saline for 24 hours and prepared for histological analysis. **Results:** The result shown that the cross striation in rat heart is more compare to that of bat and pangolin suggested a highly organized arrays of cellular parallel arrangement of actin and myosin filaments in the sarcomeres unlike the smooth muscle in which their myofibrils are not arrange in the sarcomeres. There are more numerous centrally located nuclei in rat heart. The nuclei are slender and more elongated in rat compare to that of pangolin and bat which are broad and oval in shape. The larger and dominant alveolar macrophages in the wall of some bronchiole in rat lungs suggested that could need more cellular defense than flying animal (bat). The interalveolar septa is thinner in bat lungs compare to that in rat and thinnest in pangolin. The thicker interalveolar septa has indicated that there are more alveolar type I (type I pneumocyte) cells in rat lungs compare to bat and pangolin. Alveolar type I cells (type I pneumocytes) is found more in rat compare to that of bat and very scanty in pangolin while the alveolar type II cells (type II pneumocytes) is found more in pangolin compare to bat and rat. There are numerous alveoli in bat lungs compare to rat and pangolin suggested that bat is more exposed to higher oxygen and will need adequate alveoli for its gaseous exchange. **Conclusion:** The histological study of the comparative anatomy of heart and lungs in rat, bat and pangolin revealed major differences and similarities in the micro-architecture of the heart and lungs of these mammals in relation their physical activities.

Keywords: Physical activities, Heart, Lungs rat, Bat, Pangolin.

INTRODUCTION

The heart and lungs are vital organs of the body system. The heart is a cone-shaped muscular organ in humans and other animals, which pumps blood through the blood vessels of the circulatory system [1]. Blood provides the body with oxygen and nutrients, and also assists in the removal of metabolic

wastes [2]. The heart is located in the middle compartment of the mediastinum in the chest [3]. An adult heart has a mass of 250–350 grams. The heart is typically the size of a fist, 12 cm in length, 8 cm wide, and 6 cm in thickness [4]. The lungs are the essential organs of respiration. Each lung is attached by its root and pulmonary ligament to the heart and trachea but is otherwise free in the thoracic cavity [5]. The lungs are light, soft, spongy, and elastic. Because they contain air, they float in water.

The surface of an adult lung is usually mottled, and it presents dark gray or bluish patches caused by inhalation of atmospheric dust. The right lung, which is heavier than the left, is also shorter (the right dome of the diaphragm being higher) and wider (the heart bulging more to the left) [5].

Each lung has an apex, three surfaces (costal, medial, and diaphragmatic), and three borders (anterior, inferior, and posterior). The right lung is divided into upper, middle, and lower lobes by oblique and horizontal fissures, whereas the left lung has usually only upper and lower lobes, separated by an oblique fissure [5].

Pangolin, bat and rat are mammals with different activities, characteristics and feeding patterns. Pangolin (Figure 1b) being special categories of mammals under the order Pholidota is also known as scaly ant eater or trenggiling. The body of the pangolin remains covered with hard keratinized scales which provide it a distinct and unique characteristic from the others [6]. The mammal inhabits the regions of Asia and Africa [7]. The scale covering present on the body of the pangolin are hardened plate like which hardens up as the animal matures in age. Initially, at birth the scales remain soft. The body of the pangolin is so flexible that it curls up like a ball when threatened from external agencies. The physical appearance of a pangolin is marked by large, hardened, plate-like scales. The overlapping scales act as armor for its body and the face remains covered under the scale. The scales are also blade like edges and sharp for defense. The fore paws of the animal have extraordinarily long claws which make it difficult to walk on ground, so generally it rolls its fore paws to protect the claws. For defense purposes, the pangolins emit obnoxious smelling acid from its anal glands. The sharp claws in the short forelegs of the pangolin are also used for burrowing and climbing trees. They are insectivores and feed on ants and provided with long a tongue which is so long in

size that it can extend to its thorax and abdominal cavity [6].

Bat (Figure 1a) the order Chiroptera, is the second most diverse among mammalian orders, which exhibits great physiological and ecological diversity, aided by ability to fly and have specialized life-history strategies [8]. With over 1,300 they form one of the largest nonhuman aggregations and the most abundant groups of mammals when measured in numbers of individuals [9]. They evolved before 52 million years ago and diversified into more than 1,232 extant species [10]. They are small, with adult masses ranging from 2 g to 1 kg; although most living bats weigh less than 50 g as adults [11]. They have evolved into an incredibly rich diversity of roosting and feeding habits. Many species of bats roost during the day time in foliage, caves, rock crevices, hollows of trees, beneath exfoliating bark, and different man-made structures [9]. During night, they become active and forage on diverse food items like insects, nectar, fruits, seeds, frogs, fish, small mammals, and even blood [10]. Bats have long been postulated to play important ecological roles in prey and predator, arthropod suppression, seed dispersal, pollination, material and nutrient distribution, and recycle [10].

Bat bones tend to be light and slender (which accounts in part for the sparse fossil record of bats). As in birds, bats have some reduced bones; the ulna and fibula are shortened and thin. Bats, like birds, also have fused cranial bones for additional lightness.

Rat (Figure 1c), *Rattus norvegicus* is a rather large member of the mouse family. On average, these rats reach nearly 400 mm nose-to-tail, and weigh 140 to 500g. Males are usually larger than females. The ears and tail are bald. The length of the tail is shorter than the length of the body [12]. Molars are lophodont and the dentary is 1/1-0/0-0/0-3/3. The ears are typically shorter than those of related species, and do not cover up the eyes when pulled down [13].



Figure 1: Photograph of the three mammals showing (a) Bat, *Eidolon helvum*, (b) Pangolin, *Manis tricuspids*, (c) Rat, *Rattus norvegicus*.

Rats are nocturnal animals with most activity occurring at night and in the early morning [14]. Changing the light cycle permits rats and investigators to share peak activity periods. This 12-hour shift will require a 2-week accommodation period for the rat. Although there are strain differences, rats are typically non-aggressive, inquisitive, and easily trainable. Frequent

handling encourages their non-aggressive nature as they adapt to new surroundings or experimental situations. Improper handling, nutritional deficiencies, and vocalizations from other rats can result in undesired behavior. Males are usually more aggressive than females and when striking, bite once. Rats feel most comfortable in small, dark, confined spaces; a behavior

investigator may use as a reward. When designing experiments, it is important to understand the rat's coprophagic behavior and its potential impact on metabolic, drug, and other studies. Male rats, unlike mice, are unlikely to fight when housed together. Rats also differ from mice in their willingness and acceptance of single housing. Like most mammals, rats use a variety of communication avenues. They are vocal, and also use visual cues such as body postures when communicating [15].

Chloroform, cotton wool, weighing balance, distilled water, specimen bottles, formalin, disposable gloves, dissecting kit and board, slides and cover slips, binocular microscope with digital camera, and cages were used for this study.

CARE AND MANAGEMENT OF ANIMALS

For this research, a total number of fifteen animals, rat (n=5), bat (n=5) and pangolin (n=5) of both sexes were used for this comparative study. The rats weighing between 259g-281g were procured 24 hours prior to sacrifice from the animal holdings Osun State University Osogbo. They were fed with pellets and given water. The animals were carefully accessed and confirmed to be in a normal state of health. The pangolins weighing between 1500g-3000g were obtained from Asejire, a village in the North West of Osun state, Nigeria. The bats were sourced from a local hunter in Oke-Baale Osogbo Osun state. Prior to sacrifice they were fed with ripe bananas liberally.

SURGICAL PROCEDURE

The animals were anaesthetized under chloroform inhalation and a longitudinal incision was made through the mid-thoracic and mid-abdominal wall of the animals to obtain the heart and the lungs. Both organs were excised and weighed using the weighing balance to obtain their absolute weights.

HISTOLOGICAL PROCEDURE

TISSUE PROCESSING FOR HEMATOXYLIN AND EOSIN:

The tissues were observed and cut into small pieces of not more than 4mm thick into pre-labeled cassettes. These were further immersed in 10% formal saline for 24 hours to fix. This is done automatically using automatic tissue processor (Leica tp1020). the tissue was allowed to pass through various reagents including; station one containing 10% formal saline, station 3 to station 7; alcohol (70%, 80%, 90%, 95%, absolute 1 and absolute 11) for the purpose of dehydration. The tissues continued to pass through station 8 and station 9 containing two changes of xylene for the purpose of clearing and finally transferred into three wax baths for infiltration/impregnation. The machine has been programmed to run for 12 hours, tissues stayed in each station for 1hour. Each processed tissue was given a solid support medium (paraffin wax) and this is done using a semi-automatic tissue embedding center. The molten paraffin wax was dispensed into a metal mold and

the tissue was buried and oriented in it, a pre-labeled cassette was placed on this and are transferred to a cold plate to solidified. The tissue block formed was separated from the mold. The blocks were trimmed to expose the tissue surface using a rotary microtome at 6micrometer. The surfaces were allowed to on ice before sectioning. The tissues were sectioned at 4 micrometers (ribbon section). The sections were floated on water bath set at 55 °C and these were picked using clean slides. The slides were labeled. The slides are dried on a hotplate (raymon lamb) set at 60 °C for 1 hour.

RESULTS

Heamatoxylin and Eosin Stain of Rat Heart

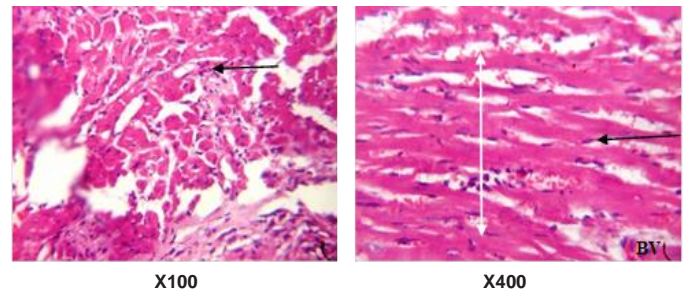


Plate 1: Photomicrographs of the rat heart showing numerous slender centrally located nuclei. (black arrow). The myocardial layer (white arrow) of this rat section are well compacted and robust and exhibit dominant cross striations with presence of moderate blood vessels (BV).

Heamatoxylin and Eosin Stain of Pangolin Heart

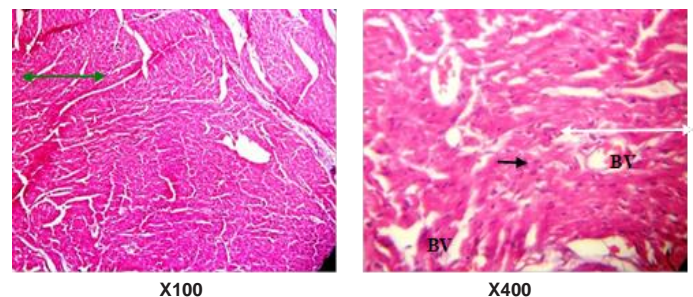


Plate 2: Photomicrograph of Pangolin heart showing presence of nuclei (black arrow) but not as much in rat (presence of moderate nuclei). The myocardial layer (white arrow) is thicker, robust and also well compacted than that of rat. The layer shows fine muscle fibers. Striations are faintly visible in pangolin. The epicardial layer (green arrow) of this pangolin section is much thicker than that of rat heart section. There is presence of blood vessels (BV) and they appear normal.

Heamatoxylin and Eosin Stain of Bat Heart

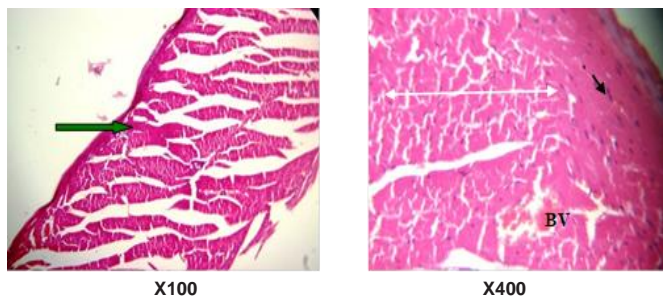


Plate 3: Photomicrograph of bat heart showing presence of few nuclei (black arrow). The myocardial layer (white arrow) is not well compacted but spaces, it exhibits more cross striations than that of pangolin but rat being the most striated. The epicardial layer (green arrow) is also thicker than that of rat but not as thick as the one in the pangolin. Pangolin being the thickest of all. This layer also consists of a simple squamous mesothelium with moderate blood vessels.

Heamatoxylin and Eosin Stain of Rat Lung

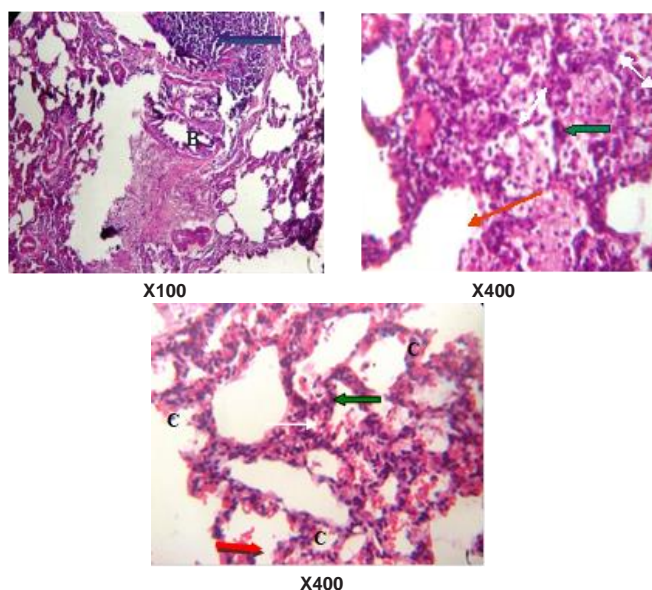


Plate 4: Photomicrograph of rat lungs showing aggregation of alveolar macrophages (blue arrow) in the walls some bronchioles (B) larger and are more dominant compare to those found in bat while less of the alveolar macrophages is present in pangolin. There are numerous flattened alveolar type I cells (type I pneumocytes) (white arrow) which line the alveolar surface interspersed among them is the irregular (sometime cuboidal) shaped alveolar type II cells (type II pneumocytes) (green arrow) they form small bulges on the alveolar wall. There are moderate presence of capillaries (C).

Heamatoxylin and Eosin Stain of Pangolin Lung

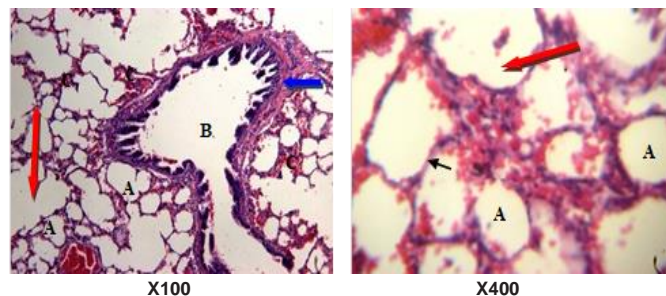


Plate 5: Photomicrograph of pangolin lungs showing. The alveolar duct (red arrow) which open into two or more alveolar sacs is thinner in bat compare to that of rat and the thinnest in this section pangolin. The capillaries (A) in interalveolar septa (black arrow) are less in pangolin compare to bat and rat. The interalveolar septa (black arrow) is thinner in bat compare to that in rat and thinnest in pangolin. The bronchioles (B) is larger and wider surrounded by a thin layer of smooth muscles (blue arrow).

Heamatoxylin and Eosin Stain of Bat Lung

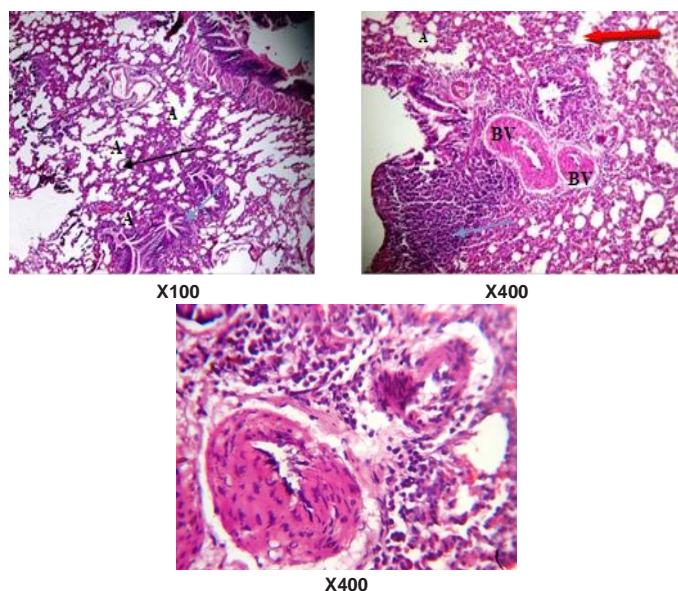


Plate 6: Photomicrograph of bat lungs shows numerous alveoli present in bat compare to that of rat and pangolin which could be as a result of higher metabolic activity in bat. The alveolar duct (red arrow) appear slightly thinner compare to that of rat. The blood vessels (BV) shows moderately thickened walls, while the alveolar macrophages (blue arrow) are mildly present.

DISCUSSION

The microscopic observation has conferred some certain degree in the comparative anatomy of bat (only mammals of sustainable flight), rat a running animal and pangolin (slow or sluggish animal) [16]. The rat heart shows numerous centrally located nuclei. The nuclei are slender and more elongated in rat compare to that of pangolin and bat which are broad and oval in shape. The myocardial layer in rat is well compacted and robust with the cardiac muscle fibers therefore cannot be easily distinguished from other cardiac layers. This myocardial layer of

the rat heart section exhibits dominant cross striations suggested their muscle fibers contain many myofibrils, the contractile unit of muscle [17]. The myofibrils run from one end of the cell to another and alternating bundles of thin filaments, comprising of primarily actin and the thick filament comprising of primarily protein myosin. There more striated appearance could be as result of the structural orientation of sarcomeres or because of the cellular parallel arrangement of actin and myosin filaments in the sarcomeres unlike the smooth muscle in which their myofibrils are not arrange in the sarcomeres [17]. Also, the epicardial layer of rat section consists of a simple squamous mesothelium with moderate blood vessels.

The histological heart section of pangolin illustrates the presence of nuclei but not as much in rat (presence of moderate nuclei). The myocardial layer is thicker, robust and also well compacted than that of rat suggested the pangolin will need a lot of pressure to pump blood into the aorta and throughout the systemic circulation. This layer shows fine muscle fibers. Striations are faintly visible in pangolin even though less nuclei are presence compare to that of rat but more that those presence in the bat. The seemingly thicker myocardium in pangolins than that of rats suggest a positive correlation between animal size and myocardial fibers. The supposed functional adaptation for this is that, a larger heart will require a higher conduction velocity as well as greater force of contraction which is met in relatively thicker myocardium [18]. The capillaries are also evident in this pangolin heart section. The epicardial layer in pangolin section is much thicker than that of rat heart. There is presence of blood vessels which appears normal.

The histological section in bat heart shows less nuclei (presence of few nuclei). The myocardial layer is not well compacted but spaces, the structural characteristics of more cross striations in bat than that of pangolin but rat being the most striated may also be as a result of the fact stated above. The less compatibility of the bat myocardium compare to that of rat makes it to be easily distinguish from other cardiac layers. The epicardial layer is also thicker than that of rat but not has thick as the one in the pangolin. Pangolin being the thickest of all. This layer also consists of a simple squamous mesothelium with blood vessels. Both rat, bat and pangolin have moderate and normal blood vessels.

The histological section of rat lungs has shown that there is aggregation of alveolar macrophages in the walls some bronchioles found in rat, they are larger and more dominant compare to those found in bat while less of the alveolar macrophages is present in pangolin these are easily identified because of their accumulated dense foreign particles. This suggested that, rat as a running animal could need more cellular defense than flying animal (bat), especially, when animal is faced with larger number of infectious particles of more virulent microbes [19]. The flattened alveolar type I cells (type I pneumocytes) which line the alveolar surface is found more in rat compare to that of bat and very scanty in pangolin

[16]. While the irregular (sometime cuboidal) shaped alveolar type II cells (type II pneumocytes) interspersed among the type I pneumocytes is found more in pangolin compare to bat and rat [16]. This may be as a result of occasional flying activity of bat, which is said to be responsible for the production of surfactant and also confer certain degree of defense mechanism [20]. The cuboidal shape of the type II alveolar cells (type II pneumocytes) and small bulges on the alveolar wall make it to be easily distinguish from the type II alveolar cells.

The alveolar duct which open into two or more alveolar sacs is slightly thinner in bat compare to that of rat and thinnest in the pangolin section [16]. The pulmonary capillaries in inter alveolar septa are found more in rat compare to bat and pangolin. The inter alveolar septa is thinner in bat compare to that in rat and thinnest in pangolin. The thicker inter alveolar septa has indicate that there are more alveolar type I (type I pneumocyte) cells in rat compare to bat and pangolin and may suggest that there are higher content of elastic fiber, smooth muscle cells, mast cells, lymphocyte and also monocyte in rat [21]. The bronchioles are surrounded by a thin layer of smooth muscles thereby allowing air flow regulation by altering the diameter of the bronchioles.

The photomicrograph in bat lungs indicate that there are numerous alveoli present in bat compare to that of rat and pangolin suggested that bat as a flying mammal has higher metabolic activity when compared to rat and pangolin as a result of their differences in their modes of locomotion, which appears to be the most responsive to the energetic requirements of the higher oxygen flows [22]. This may also suggest that bat is more exposed to higher oxygen when in air and will need more alveoli which are responsible for the transfer of gasses. The blood vessels show moderately thickened walls.

CONCLUSION

The histological study of the comparative anatomy of heart and lungs in rat, bat and pangolin has revealed major differences and similarities in the micro-architecture of the heart and lungs of these mammals in relation their physical activities. The larger and dominant alveolar macrophages in the wall of some bronchiole in rat a running animal could need more cellular defense than flying animal (bat), especially, when animal is faced with larger number of infectious particles of more virulent microbes. Seemingly, the myocardium of rat heart exhibit more cross striation than and bat pangolin suggested a highly organized arrays of cellular parallel arrangement of actin and myosin filaments in the sarcomeres unlike the smooth muscle in which their myofibrils are not arrange in the sarcomeres. This characteristic may confer high level of cellular contraction of actin and myosin filament in their muscle cell relative to muscle contraction. The numerous alveoli found in bat compare to pangolin and rat suggested bat as a flying mammal is more exposed to higher oxygen and will need more alveoli to gaseous exchange. The above fact will establish a baseline data in the vertebrate comparative anatomy of the heart and lungs of rat (*Rattus norvegicus*), bat (*Manis tricuspis*) and pangolin (*Eidolon*

helvum) in relation to their physical activities and also this result is expected to guide a further research on respiratory system and cardiovascular system of these nocturnal animals.

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