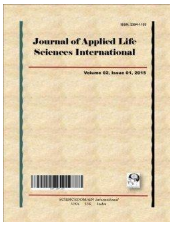

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Oesophageal and Gastric Morphology of the African Rope Squirrel *Funisciurus anerythrus* (Thomas, 1890)

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Authors' contributions

This work was carried out in collaboration between the two authors. Author OIC designed the study, wrote the protocol and wrote the first draft of the manuscript. Author SJO managed the literature searches and the experimental process. Author OIC identified the species of animal used with the help of a Zoologist. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study is to evaluate the gross, histological and histochemical features of the oesophagus and stomach of the African rope squirrel (*Funisciurus anerythrus*) were studied.
Study Design: Experimental morphological study was carried out.
Methodology: Gross dissection, routine histological technique and histochemistry using PAS and AB stains were conducted.
Results: Grossly the oesophagus was a simple musculo-membranous short tube weighing 0.17±0.02 g and measured 8.5±0.1 cm in length and the stomach was visibly uncompartmentalized, it weighed 4.98±0.05 g and measured 4.32±0.3 cm in length. Histologically, the oesophagus showed a non-keratinized stratified squamous epithelium with longitudinal folds in the cervical part. The laminae muscularis mucosa was prominent with a thick smooth muscle layer in the thoracic part. Submucosal glands were absent. The muscularis externa were typical consisting of inner circular and outer longitudinal smooth muscle layers as in many rodents. The internal lining mucous cells were PAS- positive and AB- negative. The gastric mucosa exhibited typical gastric pits,

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throughout the three regions of the stomach (cardia, fundus, and pylorus) which lead to gastric glands. Well developed gastric glands were more abundant in the fundic part than in other regions and these glands showed mucoid cells lining the upper neck regions and distinct chief and parietal cells. The surface mucosal lining was PAS-positive and weakly AB –positive. The pyloric glands were weakly PAS-positive and strongly AB-negative.

Conclusions: These findings indicated similarities to most omnivorous rodent species.

Keywords: Tree squirrel; oesophagus; stomach; gross anatomy; histology; histochemistry.

1. INTRODUCTION

Squirrels are mammals which belong to the order *Rodentia*. There are about 50 genera and 273 species known in the family *Sciuridae* [1-2]. The *species Funiscirus anaerythrus* belongs to the genus *Funiscirus* (rope squirrels), which is under the tribe of *Proxerini* (African Tree Squirrels), which make up the subfamily of *Xerinae* and family *Sciuridae* [2-3]. This species is found mainly in the West-African rain forests, where it is hunted widely for meat. Their smoked carcasses are often displayed in rural markets in Southern regions of Nigeria. Therefore extensive hunting, habitat fragmentation, deforestation and bush burning are major threats to the current population of this species.

There are several reports on musculo-skeletal anatomy of some species of squirrels [2-5]. The spectral mechanisms in the tree squirrel) (*Sciurus carolinensis*) retina was studied by [6]. Also the feeding ecology and anatomy of the gastrointestinal tract (GIT) of other exotic species such as the red squirrel [7-9] and Persian squirrel [10] have been studied. The spleen morphology of the African palm squirrels (*Epixerus ebii*) have been investigated recently [11]. To our knowledge, there is no information on the anatomical features of the gastrointestinal tract and other morphological aspect of *Funiscirus anaerythrus*, which is native to West African rain forests, especially southern parts of Nigeria. In an attempt to understand the biology of this species, the knowledge of the anatomy of the digestive system is essential for maintaining it in captivity; for biomedical research through feeding, diagnosis and treatment of digestive disorders.

The present study will provide gross, histological and histochemical information on the oesophagus and stomach of *F. anareythrus*. These data will provide base line anatomical information on the digestive system that will certainly be necessary in feeding and clinical management of this rodent in captivity for biomedical research. The study will also be of comparative anatomical importance to other rodents.

2. MATERIALS AND METHODS

Nine adult rope squirrels *(F. anaerythrus*) that included 5 males and 4 females were captured alive through rope-trapping in forests around the university town of Nsukka, Nigeria during the dry season. The average weight was 410.5 g. Captives were maintained for two weeks in cages of the Animal House of the Department of Veterinary Anatomy, University of Nigeria. They were fed various fruits, including palm fruits mixed with standard laboratory animal feed during the acclimatization period of two weeks. In addition water was given *ad libitum* and their physical health status was ascertained through careful restraint and physical examination. Thereafter, live weight of each animal was determined after sedating with Thiopental sodium (20 mg/kg, Rotexmedica, Trittau, Germany) and subsequently euthanized with lethal dose of same drug following approved procedures. Following death, they were placed in dorsal recumbency and an incision was done from the first cervical vertebrae to the pelvic region to expose and harvest the gastrointestinal tract (GIT). Two fixed intact animals were available for topographical study. The topographical anatomical study of the oesophagus and stomach was carried out and photographed with a Samsung 5X digital camera (Samsung, China). The GIT was carefully dissected and the entire length from proximal oesophagus to the rectum were placed on a tray and washed of contents with normal saline. Each compartment was separated, placed without stretching in a straight line. The length (cm) and weight (g) measurements of the oesophagus and stomach were done with vernier caliper, metre rule and Ohaus weighing balance (Germany). For histometry, the ocular micrometer gauge calibrated with stage micrometer was used to measure the thickness of the oesophagus and stomach using X10 objective magnification. The recorded weight and length and histometrical values were expressed as mean and standard error of mean (SEM) using the statistical Package for Social Science (SPSS) Windows Version 16.

For histological study, slices of oesophagus and different compartments of the stomach (cardiac, fundic and pyloric) were fixed in 10% neutral buffered formalin for 24 hours. The fixed tissues were dehydrated through a series of ascending ethanol (70%, 80%, 90% and 100%), cleared in xylene and embedded in paraffin according to [12]. Sections of 5-6μm were cut, dewaxed in xylene, in series of descending ethanol concentration and routinely stained with haematoxylin and eosin. Histochemical studies were carried out with dewaxed 5-6 μm sections stained with periodic acid- Schiff (PAS) for identification of neutral mucin and Alcian blue (AB) at pH of 2.5 for acidic mucin identification and Fast green was used as counterstain in place of Eosin [13]. Sections with diastase digestion were used as negative controls. Stained sections were examined under light microscope and selected images were captured with digital camera (Moticam® 1000, 1.3M pixel, China Corporation) attached to a computer system.

3. RESULTS

3.1 Oesophagus

The African rope squirrel (Fig. 1) oesophagus was a short, simple, whitish, membranous tube which lay on the dorsal aspect of the trachea. It weighed 0.17±0.02 g and measured 6.5±0.1 cm in length. The oesophagus had cervical and thoracic portions (Fig. 2).

Histologically, it was a hollow organ with all layers (tunics) typical of the digestive tube. The structure of the oesophagus varied slightly in the thickness of the various layers that included mucosa (comprising the surface epithelium, lamina propria and muscularis muosa), submucosa, and tunica muscularis in the cervical and thoracic portions. Adventitial layer was present mainly in the cervical region, while a serosal lining was seen in some portions of the thoracic oesophagus. The mucosal (surface) epithelium was non-keratinized stratified squamous epithelium, which folded longitudinally in the cervical part (Fig. 3) more than in the thoracic part (Fig. 4).

The lamina propria was typical and consisted of loose connective tissue with some collagenous tissue. The laminae muscularis mucosa was a prominent and complete thick smooth muscle layer. It appeared thicker in the cervical part than in the thoracic part. In the cervical part the thickness of the lamina propria mucosa, muscularis mucosa, submucosa and muscularis externa were 86.4±0.02, 15.4±0.01, 21.2±0.01, 32.7±0.04 μm respectively. The submucosa was thin and consisted of loose fibrous connective tissue, blood vessels and nerves. Submucosal glands were not observed. The mucosal layer was moderately PAS-positive (Fig. 5) and AB-negative (Fig. 6). The tunica muscularis showed more striated skeletal muscle in the proximal cervical part which was gradually replaced by smooth muscles in the distal thoracic part. The inner and outer longitudinal layers were quite distinct. The tunica adventitia in the cervical part consisted of loose connective tissue with blood vessels and nerves. Adventitial layer (tunica adventitia) surrounded the thoracic part of oesophagus as it entered the cardiac part of the stomach.

3.2 Stomach

The stomach was a simple, C-shaped with angular incisures and non compartmentalized stomach. It weighed 4.98±0.05 g and measured 4.32±0.3 cm in length. The pylorus was distinct. Internally, longitudinal folds (rugae) were apparent in the fundic, corpus (body) and in the pyloric part. In the fundic region, the thickness of the glandular mucosal layer, muscularis mucosa, submucosa (tela submucosa) and tunica muscularis were 472.5±0.7μm, 42.8±0.8 μm, 267.5±0.3 μm and 64.2±0.2 μm respectively.

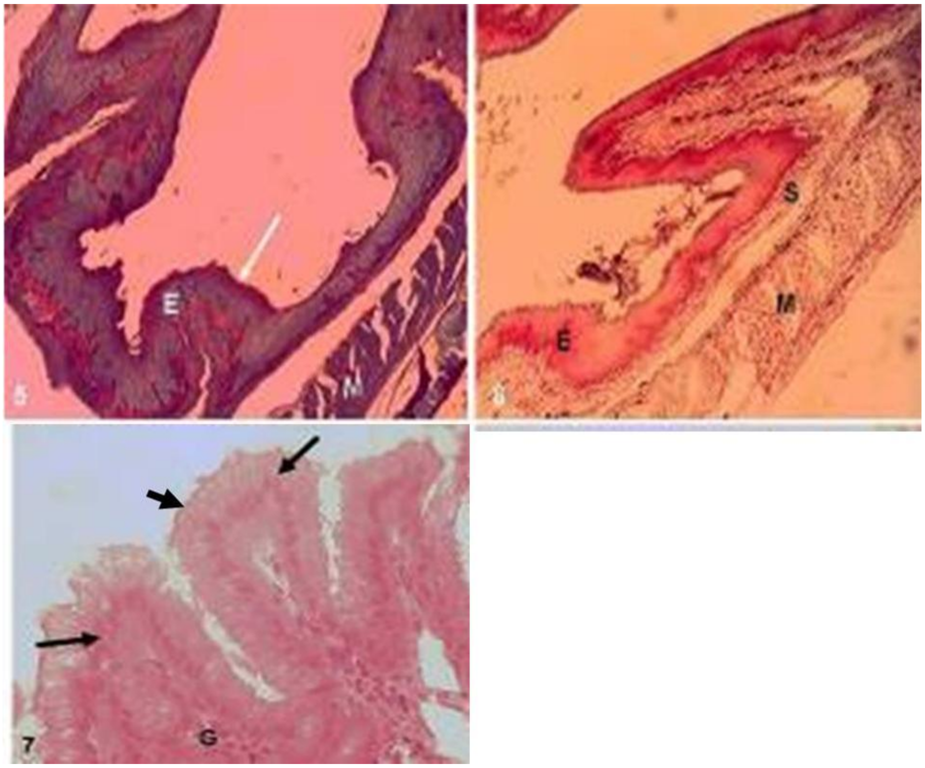
3.2.1 Tunica mucosa

Simple columnar epithelium lined the stomach (Fig. 7). The gastric glands and gastric pits were observed in all regions of the stomach, with thicker layer of gastric glands (fundic glands) being present in the fundic region (Fig. 8). In fundic and body (corpus) regions showed copious tubular gastric (fundic) glands. The epithelial cells in the neck of the gastric glands included mucous neck cells and parietal, while chief cells were present at the base of the glands (Fig. 9). The lamina muscularis mucosa was present as in the oesophagus. The submucosa was typical composed of loose connective tissue, nerves, few vessels, while tunica muscularis was made up of usual inner longitudinal and outer circular smooth muscle layer (Fig. 10). The cardiac region of stomach (Fig. 11), showed AB-positive and weakly PAS-positive but strong reaction were seen in the gastric pits and surface lining cells (Figs. 14 & 15 respectively). It also maintained the typical histologic gastric pits and the surface epithelial cells were strongly PAS-positive, while the inner gastric glands were weakly PAS-positive. The surface mucous lining cells and gastric pits lining cells were strongly PAS-positive (Fig. 12) and the deeper fundic glands were strongly AB-positive, with weak-AB positive staining of surface lining of the epithelium (Fig. 13). The pyloric part showed a typical glandular surface epithelium that was PAS-positive and AB-positive. The pyloric gastric glands were and showed similar features found in the fundic stomach. The tunica serosa was very thin.

4. DISCUSSION

The average weight of the African rope squirrel was 410.5 g. Grossly the oesophagus was a simple musculo-membranous short tube weighing 0.17±0.02 g and measured 8.5±0.1 cm in length and visibly uncompartmentalized, it weighed 4.98±0.05 g and measured 4.32±0.3 cm in length. There was no widening of the lumen before the oesophagus opened into the cardia part of the stomach unlike in some rodents Histologically, the oesophagus showed a non-keratinized stratified squamous epithelium with longitudinal folds in the cervical part. The stomach was uncompartmentalized (unilocular) structure lined exclusively by glandular epithelium. Submucosal glands were absent in the oesophagus. The internal lining mucous cells were PAS-positive and AB-negative. The surface mucosal lining of the stomach regions were PAS-positive and weakly AB –positive. The pyloric glands were weakly PAS-positive and strongly AB-negative.

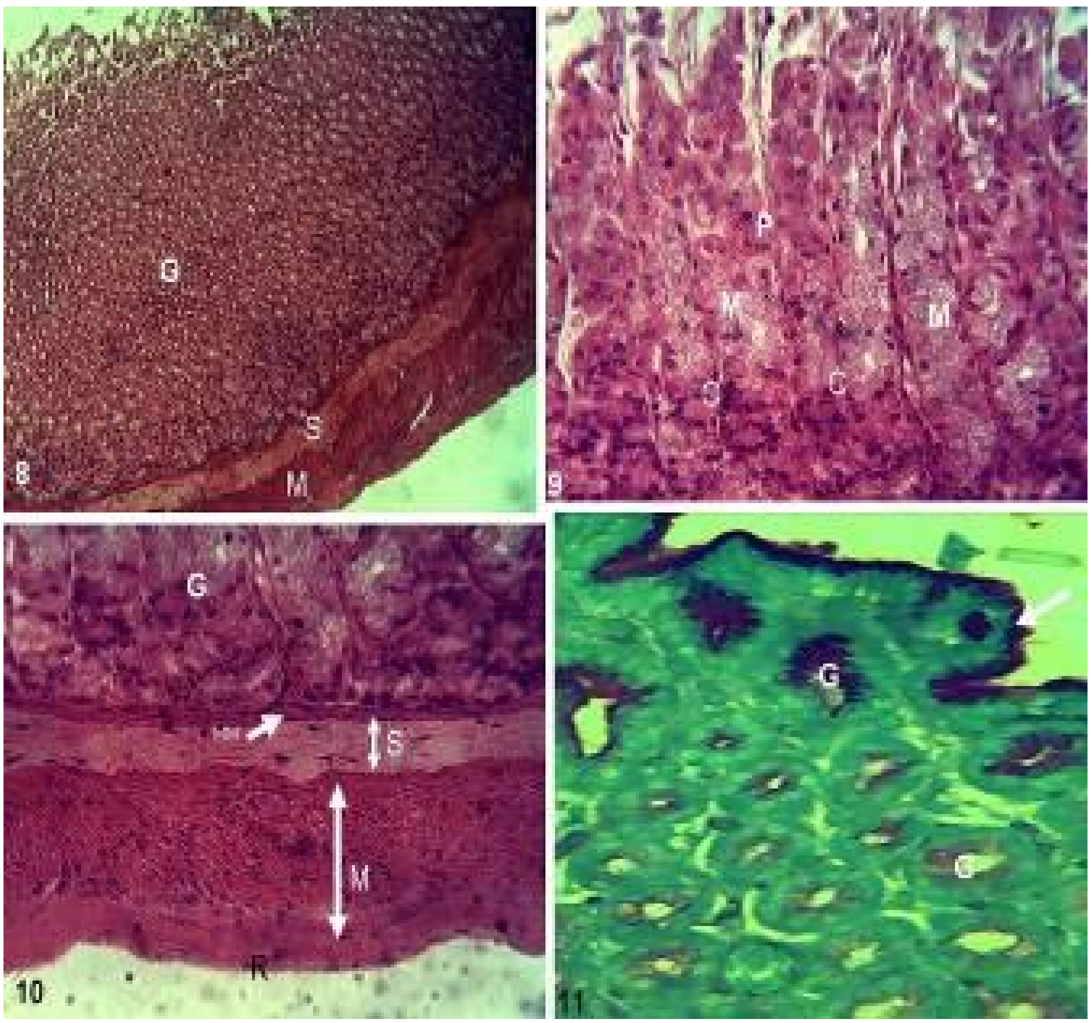
Figs. 1. Gross photograph showing adult African rope squirrel *(F.anaerythrus)*. (2): Gross photograph of the gastrointestinal part of African rope squirrel show oesophagus (E), stomach (S), duodenum (D), jejunum (J), ileum (L), caecum (C), rectum (R). (3): Histological section of the cranial (cervical) oesophagus showing folds of non-keratinized stratified squamous epithelium (E), muscularis (MM), submucosa (S) and tunica muscularis externa (M) X200 H & E. (4): Histological sections of the caudal (thoracic) part of the oesophagus showing reduced folding of the epithelium in places , stratified squamous epithelium (E), muscularis mucosa (MM), submucosa (S), tunica muscularis externa (M) X 200 H & E



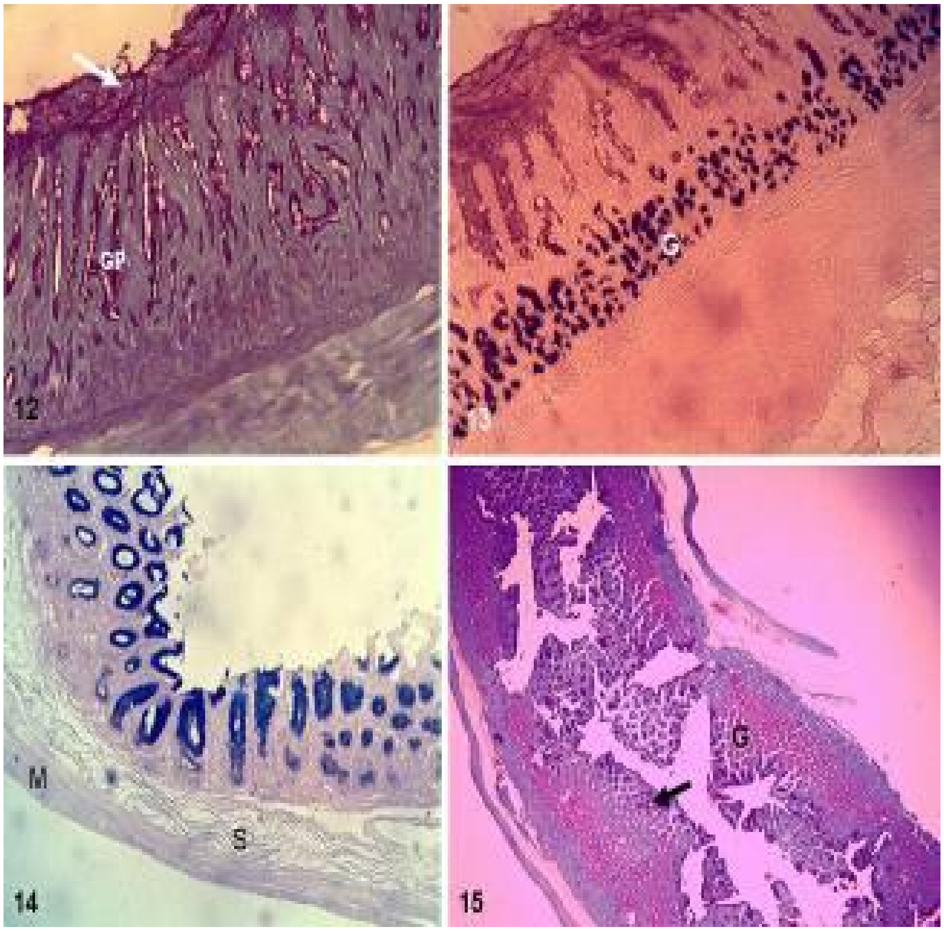
Figs. 5. Histological section of the cranial (cervical) oesophagus showing PAS-positive surface epithelial cells (arrow0, stratified squamous epithelium (E) PAS X200. (6): Histological section of cranial (cervical) oesophagus showing AB-negative reaction, stratified squamous epithelium (E), submucosa (S), muscularis externa (M). AB X200. (7): Histological section of the stomach showing surface mucous cells (simple columnar) (arrow head) with prominent nuclei (arrows), glands (G) X 400 H & E

The gross appearance and topographical relationship of the oesophagus and stomach of the African rope squirrel (*F. anerythrus*) did not vary from that of the Persian Squirrel (*Sciurus anomalus*) [10], African Giant rat [14] and from that of some insectivorous rodents [15]. However the shape of the stomach, which was simple, C-shaped with well developed angular incisures (*incisura angularis*) and uncompartmentalized stomach differed from that of African giant rat [16] and some small insectivorous rodents (*Amblyosomus hottentos and Crocidura cyanea*) which do not have well developed angular incisures, but was similar to that of Acomys spinosissimus [15] and Persian Squirrel (*Sciurus anomalus*) [10] with angular incisures and uncompartmentalization of the stomach. The study has shown that the size and shape of the stomach varies in the rodents and this may affects the digestive efficiency and the diet.

The average body weight of the squirrel in the present study (410.5 g) was higher than that of Persian squirrel (*S. anomalus*, 310.1 g) [10], but smaller than that of some squirrels such as *Sciurus aberti* (511.7 g) and *Sciurus niger* (532.2 g) [9]. It is likely that body size may play a significant role in food consumption and sorting in small rodents a have been suggested by [17]. The length of oesophagus (6.5 cm) and stomach (4.32 cm) and their weights (0.17 g and 4.98 g) respectively did not differ significantly from values of similar parameters obtained for Persian squirrel [10], despite the weight differences of these squirrel species. It means that body weights specifically may not have a significant relationship with the length of these organs, but may have with weights of various segments of the GIT. It is also widely known that food habits may be closely related to gut morphology and structure [18,19]. The length of the oesophagus (6.5 cm, body weight 410.5 g) of *F. anaerythrus* was lower than that of African giant rat (oesophageal length of 11.78±0.53 cm and body weight (1045±29.06 g). However the length of the stomach of *F. anaerythrus* studied was similar to some rodents with comparable lower body mass like *Cricetus triton* (body mass,120.2±9.6 g and stomach length 4.3±0.3 cm) and *Spermatophilus daurica* (body mass; 151.2±22.2 g and stomach length 4.6±0.5 cm), [20]. These species are known to be omnivores. It therefore suggests that the African rope squirrel (*F. anaerythrus*) studied is also an omnivore like some other species of squirrels. It appears that omnivores seem to have greater stomach length than herbivorous rodents. Omnivores and even granivores rodents species, because of their high quality and non –fibrous diets do not depend upon post-gastric fermentation chambers for storage of digesta and for nutrient extraction, they carry out this process in the foregut and the relatively large size of the stomach as storage chamber for food allows them feed intermittently. This enables them to meet their nutrient requirement [21]. Therefore the size and length of the stomach obtained in this study is consistent with that given in other omnivores [22].



Figs. 8. Histological section of the fundic part of the stomach showing copious fundic glands (G), muscularis mucosa (MM), submucosa (S), muscularis externa (M) and serosa (s). X200 H & E. (9): Histological section of glandular mucosa showing mucoid neck cells (M), parietal cell (P) and chief cells (C). X 400 H & E (10): Histological sections of the lower part of the wall organization showing mucosal glands (G), muscularis mucosa (arrow), submucosa (S), muscularis externa (M) and serosa (R). X400 H & E. (11): Section showing PAS-positive staining of the cardiac surface epithelial lining, gastric pits (arrow) and cardiac glands (G) X 400 PAS



Figs. 12. Fundic part showing PAS-positive surface lining cells (arrow) and gastric pits (GP). X 200 PAS. (13): Fundic glands (G) showing AB-positive reaction. X100 AB. (14) Pyloric part showing AB-positive (X 200 AB), and (15): PAS-positive reaction (X100 PAS)

Histologically, the oesophagus was observed to consist of non-keratinized stratified squamous epithelium. This epithelial lining is common in several omnivorous rodents studied [14,15,23,24]. The degree of keratinization varies with species. It is little or non-keratinized in carnivores, lightly in the pig and copiously keratninzed in ruminants. This present observation of lack of oesophageal glands is in agreement with observations in several rodents in including the African giant rat and some African insectivorous rodents (South African spiny mouse, musk shrew and golden mole) [15] and several domestic animals. Compound tubular oesophageal glands have been described in human [25], fowl, goose and wild duck oesophagus [26]. The lack of keratinization of the oesophagus of African tree squirrel may be because it does not consume enough abrasive diets unlike in the keratinized oesophagus of reptiles and lizards [27]. The squirrel studied is known to eat hard nuts along with fruits which are the primary component of the diet in addition to some arthropods, leaves, flowers and bird eggs. The non-keratinized stratified squamous epithelium of the oesophagus was PAS-positive because of large quantity of neutral glycogen in the cytoplasm of the flat surface prickle cells.

The histological features of mammalian stomach differ considerably across species, although it shows some basic structural similarities. The stomach morphology is highly influenced by adaptation, type of food, frequency of food intake, duration and need for food storage, body size and shapes. The extent of glandular and non-glandular partitioning of the stomach also varies in mammalian species, including rodents. A simple glandular epithelium was common to all regions of the stomach of squirrel studied. This is contrary to that seen in the mouse, rat, hamster, guinea pig and gerbil which have in addition, a non-glandular left compartment lined by keratinized stratified squamous epithelium in most cases [28].

In the present study, the cell types of the glandular stomach include the mucous neck cells, which secrete mucous, that provide a protective barrier against injury, toxins and pathogens, in addition to contributing to the innate defense system [29]. Parietal cells (oxyntic cells) were also observed as large polyhedral cells with central nucleus and they secrete hydrochloric acid. Also chief cells (peptic cells) were observed and they are known to secrete pepsinogen that is converted to pepsin by gastric acid [30]. The location of these glandular cells did not vary from that of other rodents. However enteroendocrine cells were not identifiable in this study, because it required specialized silver stains to localize it. The surface epithelium of the stomach clearly demonstrated PAS-positive reaction but was not AB-positive. It is likely that this layer is responsible for secretion of neutral mucosubstances. The gastric glands in the fundic, pyloric and cardiac regions showed weak PAS-reaction, with only few cells stained, showing that they also elaborate neutral mucosubstances similar to gastric glands of guinea pig [31]. The present work concerning the PAS- reaction in gastric mucous cells and gastric glands is similar to the report of [32] in 11 mammals including some rodents. Like the present report, neutral mucous substances were predominant on gastric surfaces, but in the three regions of the stomach, the gastric glands showed less PAS- positive reaction, indicating less neutral mucosubstances. The gastric glands however showed strong AB- positive reaction in all regions of the stomach indicating secretion of acidic mucosubstances from these glands.

5. CONCLUSION

In conlusion, the general morphology of stomach and oesophagus of the African rope squirrel did not differ significantly from that of other small rodents studied previously inspite of their dietary, environmental and climatic differences. Further ultrastructural and immunohistological evaluation of these compartments is warranted.

ETHICAL APPROVAL

The authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed. All experiments have been examined and followed the appropriate guidelines of Ethics and Research committee of University of Nigeria (2005 Revision).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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