HISTOLOGICAL AND HISTOCHEMICAL STUDIES ON THE GASTROINTESTINAL TRACT AND ACCESSORY DIGESTIVE GLANDS OF THE GRASSCUTTER (*Thryonomys swinderianus*)

BY

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DECLARATION

I declare that the work in this dissertation, entitled "HISTOLOGICAL AND HISTOCHEMICAL STUDIES OF THE GASTROINTESTINAL TRACT AND ACCESSORY DIGESTIVE GLANDS OF THE GRASSCUTTER (*Thryonomys swinderianus*)" has been carried out by me in the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria.

The information derived from literature has been duly acknowledged in the text and in the reference list provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

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Name of Student	Signature	Date

CERTIFICATION

This Dissertation entitled "HISTOLOGICAL AND HISTOCHEMICAL STUDIES OF THE GASTROINTESTINAL TRACT AND ACCESSORY DIGESTIVE GLANDS OF THE GRASSCUTTER (*Thryonomys swinderianus*)" by Kevin Kas BARNABAS, meets the regulations governing the award of the Master of Science (M.Sc.) degree at the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this work to God Almighty, the reason for everything.

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ABSTRACT

The gastrointestinal (GIT) morphology and the distribution of the different types of mucin secreting cells were investigated in the grasscutter (*Thryonomys swinderianus)*. Previous research studies carried out on the GIT have only focused on the gross morphology and histology, The aim of the study was to provide a comprehensive morphological assessment of the GIT, some accessory digestive glands, identify and characterise the distribution of mucin (neutral and acidic) in the GIT of this rodent. Seven (7), apparently, healthy grasscutters were purchased from a farm in Zaria. The animals were anaesthesised and sacrificed, with the stomach, small and large intestines, liver, pancreas and gall bladder dissected. The weight, shape and length of the accessory digestive glands, stomach, small and large intestines were taken. The organs were fixed in neutral buffered formalin and routinely processed for histological and histochemical studies. Histomorphological characteristics of the different parts of the GIT and accessory digestive glands were studied using Hematoxylin and Eosin staining technique. Histochemical staining methods were used to detect and distinguish between neutral and acidic mucins using Alcian Blue (AB), Periodic Acid Schiff (PAS) and Alcian Blue and Periodic Acid Schiff combined (AB-PAS) techniques while PAS with diastase control technique was used to study the histochemistry of the liver. The result of the macroscopic observations in the stomach revealed three distinct parts; the cardia, fundus and pylorus, while the small intestine also revealed three regions, namely; the duodenum, the jejunum and the ileum. The large intestine revealed three distinct regions; the caecum, the colon and the rectum. The result of the morphometric studies showed sexual dimorphism with male values higher in most of the parameters measured than in the females. The body weight of 1582.25 ± 207.95g was recorded in the males and 1089.67 ± 276.93g in the females. The mean GIT length in the males was 253.13 ± 10.68cm and 243.63 ± 6.73cm in

the females. The mean liver weight of 45.65 ± 3.20g and 37.43 ± 5.84g were recorded in the males and the females respectively. The result of the microscopic observations of the GIT and the gall bladder of the grasscutter revealed simple columnar epithelial cells across all the regions while intestinal glands were observed for the secretion of mucus and in the stomach, glands were observed for the secretion of mucus and gastric juice. The liver cells (hepatocytes) were closely packed and arranged from a diffuse to radial pattern. The pancreas was made up of lightly stained pancreatic islands (islets of Langerhans) and darkly stained serous acinar cells. The result of the histochemical studies revealed the presence of acid and neutral mucins across the segments of the gastrointestinal tract with the stomach positive for only neutral mucins while the small and large intestines were positive for both acid and neutral mucins. The acid mucins were dominant across the regions of the intestines. Histochemical studies of the liver revealed PAS positive hepatocytes which suggested the presence of glycogen deposits within their cytoplasm. In conclusion, the grasscutter showed a unique pattern in the distribution of acid and neutral mucins across the GIT which could be as a result of difference in the quality of biofilm required in the various segments of the GIT. This not too far from what is seen in the Wistar rats except for the total absence of acidic mucins in the stomach.

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LIST OF ABBREVIATIONS

AB - Alcian Blue

AB/PAS- Alcian Blue and Periodic Acid Schiff combine

AGR- African Giant Rat

CHO-CHO- Dialdehyde

CHOH-CHOH- 1, 2- glycol groups

ER- Endoplasmic Reticulum

GIT- Gastrointestinal Tract

H and **E-** Haematoxylin and Eosin

Hcl- Hydrochloric Acid

kDA- Kilo Dalton

NBF- 10% Neutral Buffered Formal

NY- New York

PAS- Periodic Acid Schiff

PASD- Periodic Acid Schiff with Diastase

SEM- Standard Error of Mean

UK- United Kingdom

USA- United States of America

CHAPTER ONE

INTRODUCTION

The Grasscutter (*Thryonomys swinderianus*) is common in Africa, South of the Sahara ranging from Gambia to Southern Sudan and from South to North Namibia and South Africa (Fitzinger, 1997). It belongs to the family *Thryonomyidae* and order *Rodentia.* It is found only in Africa (Baptist and Mensah, 1986). Some common names by which it is known include; Cutting grass, Cane rat or Greater cane rat. Locally, they are called Jauji in Hausa, Nchi in Igbo and Õyà in Yoruba.

Thryonomys swinderianus (T. swinderianus) are herbivores and their natural diet is mainly grasses and cane. Sometimes they also eat bark of trees, fallen fruits, nuts and many different kinds of cultivated crops. Some of the cultivated crop fields that *T. swinderianus* invade include sugar cane, maize, millet, cassava, round nuts, sweet potatoes, and pumpkins (Fitzinger, 1997). Greater cane rats' favorite food is elephant grass and sweet potatoes (NRC, 1991). They prefer plants with lots of moisture and soluble carbohydrates (Agbelusi, 1997). *T. swinderianus* cut the grasses and other foods with their incisors, producing a chattering sound that is relatively loud and very distinguishable (Mills and Hes, 1997).

The Grasscutter can be found in areas with tall grasses such as in the Guinea-Gulf savannah especially areas with abunadant supply of elephant and guinea grasses which they eat as food (Ankrah, 2005). They also inhabit cleared forest areas. Several reports have revealed that the skin and fur of the the rodent as well as its limbs and tails are

easily torn out when handled (Kingdom, 1974). This makes the animal very difficult to catch and even more difficult to handle after capture.

The Grasscutter has been reported to be a fast runner also possessing good swimming skill, despite the blunt snout. It has relatively poor vision, basing its communication majorly on hearing and also using its well developed sense of smell. The rodent can stay in captivity for up to four years with careful handling (Jori and Chardonnet, 2001).

Despite the features of this rat that have been studied, the digestive system is yet to be fully investigated. Macroscopic and microscopic studies have been done on the gastrointestinal tract of the African Grasscutter (Byanet *et al*., 2008), Gross and microscopic studies have been done on the anatomy of thyroid gland of the wild African Grasscutter (Igbokwe, 2010), Morphometric observations of the brain of the African Grasscutter have been done (Byanet *et al*., 2008) and sex differences in the cerebellum and its correlation with some body traits in African Grasscutter have been studied (Byanet, 2012)

The morphology of the digestive tract of a given animal species is related to the nature of food, feeding habits, body size and shape (Smith, 1989). Parts of the digestive system of vertebrates may show a wide range of structural and functional diversities, both within and among the class of the vertebrate. A proper understanding of the system's adaptation to diet and feeding habit is of great importance for the proper care of domesticated animals and preservation of endangered species (Finegan and Stevens, 2008).

1.1 STATEMENT OF THE RESEARCH PROBLEM

Research works done on the gastrointestinal tract (GIT) of the rodent have focused on the gross morphology and histology, little or no work has described the mucin histochemistry of the digestive tract of the Grasscutter.

1.2 JUSTIFICATION OF THE STUDY

The present study may be useful in the understanding of the feeding patterns and give an insight into the efficiency of the digestive system in the Grasscutter, the knowledge of which may be employed in the breeding programmes, or as animal models for feed formulation and nutrient trials.

1.3 SIGNIFICANCE OF THE STUDY

- The findings of this study could be used in comparative anatomical studies and establishment of evolutionary trends with other species of the *Murideae* family and higher orders like humans.
- The results of the present study may also provide base-line data on the histological and histochemical features of the GIT and accessory digestive glands of the Grasscutter.

1.4 AIM AND OBJECTIVES OF THE STUDY

1.4.1 Aim of the Study

The aim of the present study was to shed light upon the anatomy and characterize the mucin characterize the mucin contents of the GIT and the accessory digestive glands of the Grasscutter.

1.4.2 Objectives of the Study

The objectives of the study were:

i. To determine the morphometric and gross morphological features of the GIT and accessory glands (liver, pancreas and gall bladder) of the Grasscutter.

ii. To determine the histomorphologic features of the GIT and accessory glands of the Grasscutter using Hematoxylin and Eosin (H and E).

iii. To identify and characteristise the distribution of mucins using Alcian Blue (AB), Periodic Acid Schiff (PAS) and Alcian Blue and Periodic Acid Schiff combined (AB-PAS) techniques in the GIT and gall bladder.

iv. To demonstrate glycogen distribution in the liver using Periodic Acid Schiff with Diastase control.

CHAPTER TWO

LITERATURE REVIEW

2.1 THE GRASSCUTTER

The Grasscutter (*Thryonomys swinderianus*) also known as the greater cane rat belongs to the kingdom *Animalia*, phylum; *Chordata*, subphylum; *Vertebrata*, class; *Mammalia*, order; *Rodentia*, family; *Thryonomyidae* and genus; *Thryonomys*. Grasscutter is found naturally near marshes and river banks (Mills and Hes, 1997). Populations can also reach very high densities in plantations of cultivated crops (Merwe, 2000).

The body length of *T. swinderianus* is usually about 35-61 cm, and their tail reaches between 6.5- 26 cm in length (Fitzinger, 1997). Greater cane rats have an average weight in males of 4.5 kg and 3.5 kg in females (Merwe, 2000). They have a rounded nose, short ears, and incisors that grow continuously (Mills and Hes, 1997). The pelage is coarse, with flattened bristle like hairs that grow in groups of five or six. The upper parts are a yellowish brown color and the underside is a much lighter gray. Greater cane rats have no under fur (Fitzinger, 1997). The forefeet are smaller than the hind feet and have three well developed middle digits with the first and fifth digits greatly reduced. The hind feet have no first digit and all digits have heavy claws (Fitzinger, 1997). The dental formula for *T. swinderianus* is $I\ ^{1}/_{1}$, $C\ ^{0}/_{0}$, $PM\ ^{1}/_{1}$, $M\ ^{3}/_{3}$ where I,C,PM and M are incisors, canines, premolars and molars respectively (Merwe, 2000).

T. swinderianus are usually found in groups composed of one male, several females and young from more than one generation (NRC, 1991). They are nocturnal and create trails through grass and reeds that lead from shelter to feeding and water sites. They pound down tall grass to make nests and also make shallow burrows for shelter. They are good

swimmers and divers (Fitzinger, 1997). Some of the features off the Grasscutter as described above can be seen in Figure 2.1.



Figure 2.1: The Grasscutter (CIRAD, 2003)

2.2 THE GASTROINTESTINAL TRACT (GIT)

The gastrointestinal tract, GI tract, or GIT is an organ system responsible for consuming and digesting foodstuffs, absorbing nutrients, and expelling waste. The tract consists of the stomach and intestines (DMD, 2009). However, a broader definition of the GI tract includes all structures between the mouth and anus. It is divided into the upper and lower gastrointestinal tracts. The GIT conforms to a general structure that is noticeable from the oesophagus to the anus (Young *et al.*, 2006). Essentially, it is a muscular tube lined by a

mucous membrane. In the different regions of the GIT, minor variations are evident in the muscular component, but most strikingly is the underlying changes in structure and function of the mucosa in the different regions. The GIT has four functionally distinguishable layers, namely: mucosa, submucosa, muscularis externa and adventitia. The mucosa consists of an epithelial lining, an underlying lamina propria of vascularised loose connective tissue, and a thin smooth muscle layer (the muscularis mucosae) (Young *et al.,* 2006). Furthermore, the mucosa undergoes sudden changes during the transition from one region of the GIT to another. This occurs at the gastro-oesophageal junction, the gastro-duodenal junction, the ileo-caecal junction, and also at the recto-anal junction. The submucosa supports the mucosa and consists of loose fibrous connective tissue, blood vessels, lymphatics and nerves (Young *et al.,* 2006). The muscularis propria, usually consisting of smooth muscle, is generally arranged as an inner circular- and outer longitudinal layer, which is responsible for peristaltic contraction (Young *et al.,* 2006). Only in the stomach is there a third muscle layer, namely the inner oblique muscle layer. The adventitia is an outer layer of loose supporting tissue and it conducts major blood vessels, nerves and adipose tissue. Where the GIT lies within the peritoneal cavity, the adventitia (outermost connective tissue layer) is referred to as the serosa and it is lined by mesothelium (Young *et al.,* 2006). The different segments, layers and contents of gastrointestinal tract are shown in Figure 2.2

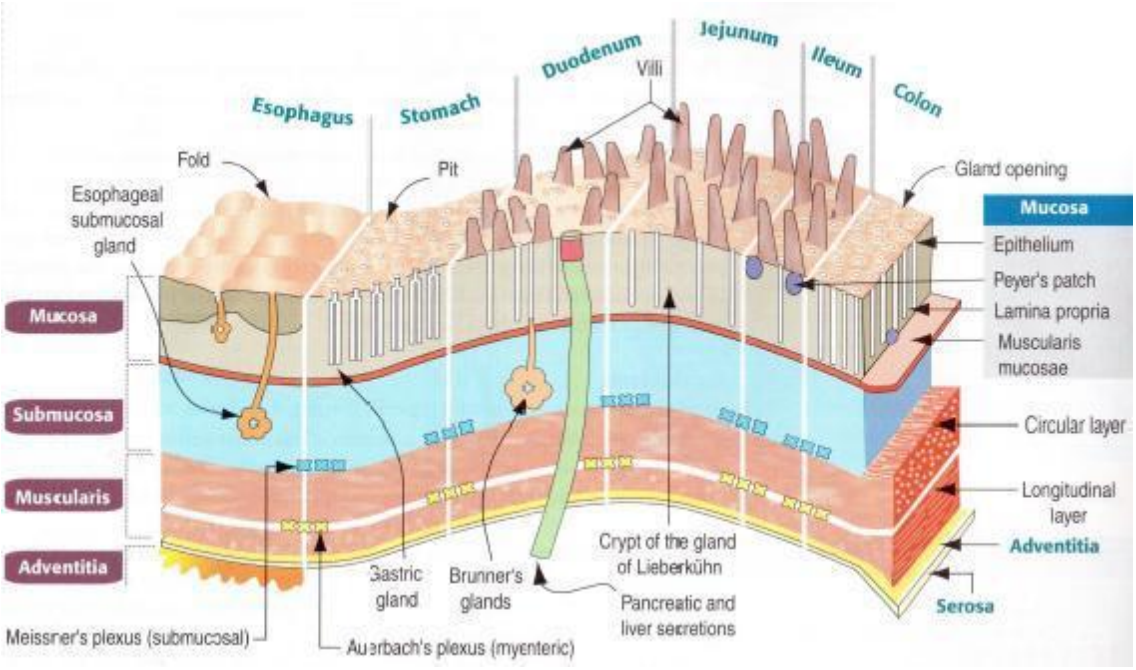


Figure 2.2: Histological representation of the different segments, layers and contents of gastrointestinal tract (Kierszenbaum, 2002).

2.3 GIT MORPHOLOGY AND FEEDING HABITS IN RODENTS

Most rodents feed exclusively on plant material such as seeds, stems, leaves, flowers and roots of trees (strict herbivores). Some are omnivorous, feeding on plant and some invertebrates, a few are predators (Waggoner, 2000). Wilczyńska (1999) suggested that feeding habit may be closely related to gut morphology and structure, the different feeding habits in rodent species may be the reason for the differences in the morphology of the GIT. In some rodent species, the estimated volume and surface area of the caecum and colon were greater in the herbivorous species than that in the omnivorous species Strict herbivores have been reported to have larger colon and caecum, and the tracts of omnivorous species varied to different extent depending on the proportions of seed, vegetative and animal foods in their diets (Wang *et al*., 2003) which suggests that the hind gut is more important for herbivorous than for omnivorous rodents and could be a

relative reliable indicator for food habits. Schieck and Millar (1985) compared the digestive tract morphology for 35 species of rodents and found that the masses and lengths were greater in herbivores than in omnivores; however, small intestine is not a good indicator for food habits as the small intestine length did not reflect any differences among the rodent species compared. The stomach lengths in omnivorous rodents were not larger than herbivores (Schieck and Millar, 1985).

2.4 THE GIT OF HERBIVORES

A high body temperature and high rates of microbial activity contributes largely to the success of the mammalian herbivores. The majority of the mammalian orders consist of herbivorous species (Stevens and Hume, 1995). The diet of herbivores consists largely of the fibrous portions of plants (leaves, petioles and stems). Most of the mammalian herbivores obtain a large portion of their nutrients via retention and microbial fermentation of plant materials in a voluminous caecum, colon or fore-stomach (Stevens and Hume, 1995; 1998).

A characteristic feature in small herbivorous animals is a big caecum that serves as the main site of microbial fermentation (Stevens and Hume, 1995). Large herbivorous mammals such as elephants, orangutans, gorillas etc. have enlarged colon which serve as the principal site for digesta retention and microbial fermentation. Digesta are retained with the help of haustra, as well as compartmentalisation in horses and elephants. For the remainder of the large herbivores a large compartmentalised or haustrated stomach is the main site for microbial fermentation (Stevens and Hume, 1995).

2.5 HINDGUT FERMENTERS

The hindgut or large intestine functions as the final site for storage of digesta and retrieval of endogenous electrolytes and water (Stevens and Hume, 1995). It is also the main site of microbial fermentation in herbivorous reptiles, most herbivorous birds and herbivorous mammals as it contains a large number of diverse microbes capable of digesting different plant materials. Cell walls containing cellulose and lignin in plant materials are difficult to digest because they are complex organic polymers which can only be digested by enzymes specific to them, such as lignases and cellulases found in some fungi and bacteria (Vaughan *et al.,* 2000; Martone *et al.,* 2009). Micro-organisms in the digestive tract can synthesise enzymes which can break down plant material, although the process of microbial fermentation is slow. Hindgut fermenters masticate food as they eat, initiating digestion with salivary enzymes (White, 2007). Digestion occurs by enzymatic activity within the simple stomach. Hindgut fermenters do not regurgitate food. Food passes from the small intestine into the caecum. Large food particles move through to the large intestine (White, 2007). Micro-organisms ferment the ingested cellulose in the caecum and large intestine. Mass-specific energy requirements of homeothermic animals are high and related to body mass, i.e. the smaller the animal the greater its energy need per unit of body mass (Björnhag, 1994). Thus, small animals that feed on plant material with low energy density cannot only rely on microbial fermentation because the process is too slow to produce sufficient amounts of energy. Small herbivorous animals combine auto enzymatic digestion in the foregut with microbial fermentation in the large intestine. These small herbivorous animals are hindgut fermenters. The Grasscutter (*Thryonomys swinderianus*) falls into this group of

hindgut fermenters and as such combine both enzymatic digestion and microbial fermentation.

2.6 THE STOMACH

2.6.1 Macroscopic Features

The Rabbit stomach holds approximately 15% of the volume of the entire gastrointestinal tract (Harcourt-Brown, 2002). The stomach of the Grasscutter was reported to account for about 3% of the entire GIT length (Byanet *et al.,* 2008). Investigations carried out on the morphometry of the stomach of the African Giant Rat (AGR), showed that the stomach accounted for about 1.86% of the total weight of the animal (Nzalak, 2010), with maximum stomach width of between 3.13 ± 0.31 and 3.75 ± 0.28 cm (Ali *et al.,* 2008). Dissection of the stomach in AGR revealed the internal surface mucous membrane of the gastric wall of the cardiac region of the glandular stomach to be continuous with the esophagus. The gastric wall of the internal diverticulum's region has two distinct thick mucosal folds. Most of the glandular mucosa is occupied by the fundic glands while non-glandular mucosa presented a brownish coloration with numerous soft towel–like papillae or projections (Ali *et al.,* 2008).

The stomach of Guinea Pig is U-shaped and consists of two distinct parts: the left non-glandular part (proventriculus or fore stomach) which is greyish, thin walled and slightly transparent, this section receives the esophagus and serves as a holding chamber for food and the right glandular or ventricular part which is white and thick walled (Berghes *et al.,* 2011). The stomach of the Rabbit is thin-walled, J-shaped, lying to the left of the midline and divided into a cardiac, a fundic and a pyloric region (O'Malley, 2005). The

Grasscutter has a simple stomach which takes the shape of an inverted J with a thin wall and relatively distended when full (Byanet *et al.,* 2008). Viewed from the exterior, the stomachs of the Rabbit and grascutter are divided into the cardia (entrance), fundus and pylorus (terminus), the cardia and pylorus being sphincters controlling the passage of feed through the stomach (O'Malley, 2005; Byanet *et al.,* 2008).

2.6.2 Histological Features

According to Berghes *et al.* **(**2011), the mucosa of the glandular (right) part of the stomach in guinea pig is lined by simple columnar epithelium while the non-glandular (left) part of the stomach is lined by keratinized, stratified squamous epithelium. Byanet *et al.,* (2011) and Eman and Haider (2012) carried out studies on the stomach of the Grasscutter and Rabbit, reporting that the stomach wall had same structural layers (the mucosa, the sub mucosa, muscularis externa and serosa) within the three regions with the entire surface of gastric mucosa lined by simple tall columnar epithelium with lightly stained cytoplasm which forms the surface mucous lining cells that invaginate the lamina propria at varying depths according to the region of stomach. Simple tubular, gastric glands occupy the lamina propria in guinea pigs and these glands are discernible into three distinct zones. The chief cells occupy the lower third region; the parietal cells are predominantly present in the upper half of the glandular tubule and a few of them appear to be mixed with the chief cells in the lower third of the gland. The neck cells can be seen near the openings of the tubular portion of the gland at the gastric pits (Berghes *et al.,* 2011).

In the Grasscutter, the cardiac region surrounding the point of entry of the esophagus contained simple/branched tubular cardiac glands in its lamina propria which extended deep into the mucosa. The pyloric glands were observed to be similar to those of the cardiac glands and were characterized by deep, large or open gastric pits and short coils pyloric glands. The fundic region had the most numerous types of gastric glands. The region had shallow gastric pits, long branched tubular glands that contain several cell types like parietal cells and chief cells (Byanet *et al.,* 2011). The mucosa of stomach in Rabbits is divided into three regions according to the types of glands which it contains, the cardiac, fundic and pyloric glands region. The Rabbit's well-developed cardiac sphincter is lined with non glandular stratified squamous epithelium. The fundus contains parietal cells that secrete acid and intrinsic factor as well as chief cells that secrete pepsinogen (O'Malley, 2005).

2.7 THE SMALL INTESTINE

2.7.1 Macroscopic Features

Studies done on the small intestine of AGR recorded a mean length of 129 ± 3.27 cm making it the longest segment of the GIT accounting for approximately 56% of the total GIT length (Ali *et al.,* 2008). The small intestine in the Rabbit is approximately 12% of the gastrointestinal volume (O'Malley, 2005). The small intestine of the Grasscutter is approximately 2m long begins from the pylorus and ends in the caecum. The small intestine of the AGR, Rabbit and Grasscutter were subdivided into the duodenum,

jejunum and ileum as reported by Ali *et al.* (2008), Bob *et al.* (2012) and Byanet *et al.* (2008) respectively.

In the Rabbit, the duodenum is supported by extensive peritoneal folds that form the mesoduodenum, the duodenum is divided into three parts: a descending, a transverse and an ascending segment. The concavity formed by the three duodenal segments houses the pancreas with a diffuse appearance. The accessory pancreatic duct opens into the duodenum, at about the passage from the descending segment of the duodenum to its transverse segment (Bob *et al.,* 2012). Byanet *et al.* (2008) reported that the Grasscutter duodenum forms a loop with the pancreas in the middle. The duodenum is smooth and measures from the pylorus to the origin of the jejunum. The duodenum in the AGR appeared smooth and was seen to lie between the pylorus and the jejunum (Ali *et al.,* 2008).

The jejunum of the both AGR and grassscutter occupy the abdominal floor between the stomach cranially and the urinary bladder caudally. It is very long, convoluted or coiled, but gradually stops forming coils near its end (Ali *et al.,* 2008; Byanet *et al.,* 2008). The ileum in AGR was smooth, slightly straight and curved only where it joined the large intestine at the ileocecal junction (Ali *et al.,* 2008). The internal surface (mucous membrane) of the small intestine has a soft and smooth appearance like the glandular part of the stomach (Ali *et al.,* 2008). The ileum in Grasscutter is smooth and curved only where it joins the large intestine. This is the main absorption site, and contains a series of finger-like projections, the villi, which greatly increase the surface area available for absorption of nutrients (Schrage and Yewadan, 1999).

The ileum of the South African spiny mouse as reported by Boonzaier (2012) were remarkably short with the number of goblet cells in the crypts also numerous, than in the duodenum and the middle small intestine.

2.7.2 Histology

The histology of the small intestine of the AGR showed three different segments, the duodenum, jejunum and ileum (Nzalak, 2010). Histological Studies on the small intestine of the Grasscutter showed similar arrangement as that seen in the AGR (Byanet *et al.*, 2011).

The shape of the villi in the duodenum of the AGR was leaf-like, lined by simple columnar epithelial cells and having Brunner's glands visible in the submucosa. The jejunum has ridge-like villi also lined by simple columnar epithelial cells with abundance of intestinal glands (crypts of Lieberkühn) in the lamina propria (Nzalak, 2010). The duodenal segment in the Grasscutter was observed to have the intestinal villi as an outgrowth of the mucosa projecting into the lumen. The goblet cells and duodenal glands (Brunner's glands) in the sub-mucosa were also noted as the major distinguishing features observed in the duodenum (Byanet *et al.*, 2011).

The jejunum of the Grasscutter had numerous goblet cells and long leaf-like villi. Between these villi were the openings of the simple tubular glands called, the intestinal glands (crypts or glands of *Liberkuhn*). Each villus of the jejunum was observed to be lined by simple columnar epithelial cells. The striated border formed by microvilli present on the surface of the cell was clearly visible (Byanet *et al.*, 2011). In the AGR, the jejunum had a similar arrangement with that of the Grasscutter with the villi surface observed to be more extensive compared to the duodenum and ileum (Nzalak, 2010).

2.8 THE LARGE INTESTINE

2.8.1 Macroscopic Features

The large intestine in the Rabbit has three segments: caecum, colon and rectum. The caecum of the Rabbit is very voluminous, taking almost the entire abdominal cavity, has no muscular strips, containing 55% of dry matter of the large intestine (Stan *et al.*, 2014). Ascending colon of Rabbit is very coiled with four segments: proximal one (ampula coli), 10 cm length, with three powerful muscular bands which separates rows of multiple haustra; the second portion has only one muscular band and fewer, smaller haustra; the third part (fusus coli) is short, about 4 cm in length but highly vascularized and innervated; the fourth portion is very short and opens into the descending colon. Transverse colon is very short. Rabbit descending colon is long and floating, show relative flexuous pattern with a dilated portion as a sigmoid colon. The rectum is short, straight and dilated (Stan *et al.*, 2014).

The large intestine of the Grasscutter consists of caecum, colon and rectum (Figure 2.2). The caecum is longer than the stomach and it is the largest organ in the abdominal cavity (Schrage and Yewadan, 1999; Byanet *et al.*, 2011). The caecum of the Grasscutter occupies about 60% of the abdominal cavity and harbours microbial organisms for efficient fermentation and utilization of fibrous diets. The caecum is a coma-shaped, blind ended sac situated at the ileo-caecal junction. Longitudinal bands of smooth muscle with intervening sacculations of haustra are common features of the caecum (Byanet *et al.*, 2011). The colon is the longest segment of the gastrointestinal tract with a wide

lumen and it contains the faecal balls. The rectum is relatively short and straight and terminates at an enlarged region, the anal orifice (Schrage and Yewadan, 1999).

The large intestine in the chinchilla has three segments: caecum, colon and rectum, in which the volume of the caecum is about 20% of dry matter of large intestine. In chinchillas, ascending colon has three parts: proximal loop with two distinct areas, simple intermediate portion, and a long distal portion extending to the right colic flexure (Stan *et al.*, 2014). The last segment consists of two parallel parts joined by an apical flexure. The transverse colon continues from the left flexure with the descending colon (Stan *et al.,* 2014). The descending colon was seen to have a convoluted pattern which was continuous with the rectum, having relatively reduced size in all examined chinchillas and their descending colon forms numerous convolutions (Stan *et al.,* 2014).

Studies carried out on the large intestine of the AGR recorded a mean length of 88.2 ± 2.73cm and a mean width of 0.55 ± 0.02cm also it was subdivided into three segments: the caecum, colon and rectum (Ali *et al.,* 2008). The caecum was the largest segment of the large intestine. It was very large, coma-shaped blind ended sac situated at the ileo-cecal junction. The colon was long with a wide lumen and contained fecal balls. The rectum was short and straight with a narrow lumen which terminates at the slightly enlarged area, the anal canal (Ali *et al.,* 2008). The internal surface (mucous membrane) of the large intestine has a soft and smooth appearance like the glandular part of the stomach (Ali *et al.,* 2008).

2.8.2 Histology

Byanet *et al.* (2011) and Nzalak (2010) both observed that the large intestinal segments (the caecum, colon and rectum) of the Grasscutter and African giant rat had four intestinal wall tunics similar to that observed in the small intestine. The segment is lined by simple columnar epithelium with long irregular microvilli (Nzalak, 2010). The glands of the large intestine are longer than that of the small intestine and the goblet cells are more numerous than in the small intestine. Nzalak (2010) observed that the large intestine was characterized by the absence of villi and an increase in the number of goblet cells from caecum to rectum. Other prominent features in the caecum are the external longitudinal muscle coat which has three bands, the taeniae coli, these longitudinal muscles push the "excess" mucosa to form pouches called the haustra (Byanet *et al.*, 2011). The colon has a wider lumen than any segment of the small intestine and the mucosal surface (Byanet *et al.*, 2011). Crypts of Lieberkühn, with abundant goblet cells were also observed in the colon. The muscularis externa layer as in caecum was observed to have thick longitudinal bands called taeniae coli (Byanet *et al.*, 2011). The rectum is short, straight and does not have taeniae coli (Drake *et al.*, 2005). It has numerous goblet cells in the crypts (Stan *et al*, 2014). An overview of the gastrointestinal tract of the Grasscutter is shown in Figure 2.3.

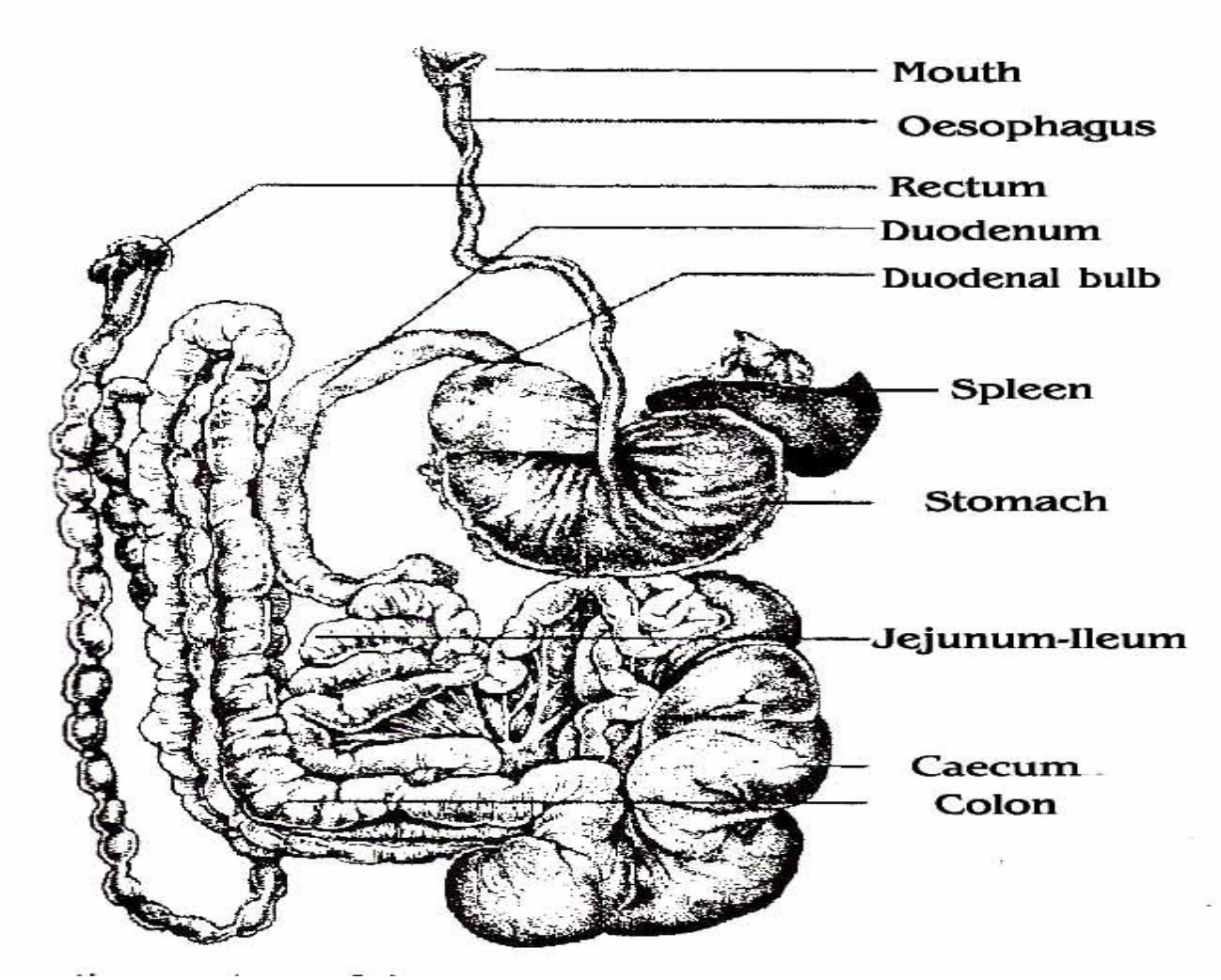


Figure 2.3: Digestive System of the Grasscutter (Schrage and Yewadan, 1999)

2.9 MUCOSAL SURFACES AND MUCOUS SECRETING CELLS OF THE GIT

Mucosal surfaces of the gastrointestinal, respiratory and urogenital tracts are areas where absorption and excretion of substances occur (Pearson and Brownlee, 2005). The movement of substances across these surfaces exposes them to the potentially harmful external environment, but the cells in the mucosa, along with their mucous secretions, create a protective barrier to maintain a pathogen-free internal environment of the body. Mucosal surfaces are the primary target areas of micro-organisms. The mucosal surfaces,

in response to microbes, secrete many defensive compounds into the mucus layer. These include compounds such as: antibodies, mucins, protegrins, defensins, collectins, cathlecidins, histatins, lysozyme, and nitric oxide (Linden *et al.*, 2008).

The mucosal surface of the intestinal tract is covered with a viscoelastic and lubricant layer of mucus (Pavelka and Roth, 2010). This mucus layer being a constantly changing mixture of many secretions and scraped-off epithelial cells, the main determinants of the physiological and physical properties of mucous secretions are highly glycosylated, high molecular weight proteins, known as mucins. Mucin granules are synthesised and secreted by specialised epithelial cells (goblet cells) that are located on the mucosal surface and also in the invaginated epithelial lining of the crypts of Lieberkühn in the GIT. Mucus has a number of functions in the GIT (Young *et al.*, 2006). It lubricates the oral cavity, the epithelial surface of the oesophagus, protects the epithelial lining of the stomach from auto-digestion, and in the distal part it lubricates for easy passage of faeces. The mucus layer of the GIT also protects the underlying cells from mechanical damage and prevents bacterial invasion (Montagne *et al.*, 2004; Pavelka and Roth, 2010).

2.10 MUCINS

Mucins are proteins bound to carbohydrates (Brockhausen *et al.*, 2009). Mucins are highly O-glycosylated, glycoproteins with a high molecular weight (larger than 200 kDA) (Devine and McKenzie, 1992). Some mucins are small with only one hundred amino acid residues, yet others can contain more than a thousand residues (Perez-Villar and Hill, 1999). Generally, mucins can be divided into two main categories; the membrane associated and secreted mucins (Montagne *et al.*, 2004). The secreted mucins

characteristically have a very high molecular weight and size with many O-linked oligosaccharides to form viscoelastic gels. Membrane-associated mucins have similar structural properties as the secreted mucins, but they have different functional properties because they are active membrane-bound components (Montagne *et al.,* 2004).

2.10.1 Functions of Mucins

Mucins display the tendency to aggregate and form gels (Taylor *et al.,* 2003). Therefore, the secreted gel-forming mucins of the respiratory, gastrointestinal, and genitourinary tracts, as well as the eyes, are protected by the ability of the O-GalNAc glycans of the mucus glycoproteins to lubricate and protect their epithelial surfaces (Brockhausen *et al.,* 2009). These O-glycans are usually negatively charged and hydrophilic, which allows for the binding of water and salt (Brockhausen *et al.,* 2009). In addition, these characteristics contribute to the viscosity and adhesiveness of mucus, forming the physical barrier between the external environment and the epithelium. An important physiological process is the removal of particles and micro-organisms that are trapped in mucus via peristaltic movements. Secreted and cell surface mucins express many oligosaccharide structures that are found on the cell surface and can therefore probably function as decoys for adhesins that have been evolved by pathogens to attach to the cell surface (Linden *et al.,* 2008). Some mucins can effectively clump viral agents together and exogenous mucins can inhibit viral infection. Viruses such as influenza, reo-, adeno-, and entero-virusses, bind to the sialic acid residues on mucins and can be removed from the GIT when the mucus layer is sloughed off.

Mucins have direct and indirect roles in defence from infections (Linden *et al.*, 2008). The mucin oligosaccharides can bind microbes and, in some cases, they either have direct antimicrobial activity or carry other antimicrobial molecules. For example, a mucin oligosaccharide expressed by gastric mucins, directly interferes with the synthesis of *H. pylori* cell wall components (Kawakubo, 2004). Cell-surface membrane associated mucins initiate intracellular signalling in response to bacteria, which suggests that they have both a barrier and reporting function on the apical surface of all mucosal epithelial cells. It is hypothesized that one of the main functions of cell-surface mucins is to act.

2.10.2 Histological Techniques Used for the Detection of Mucins

Histochemical studies on the morphological aspects of mucins are very informative (Walsh and Jass, 2000). The latter studies are able to show the relationship between the structural characteristics of the mucins at the site of synthesis and secretion. Two principal matters need to be considered in order to increase the potential value of morphologically based methods:

i. The methods used to fix tissue influences the staining of mucin (Walsh and Jass, 2000). When using light microscopy, tissues are generally fixed in formalin, which fails to preserve the mucus layer that lines the epithelial surface of the GIT.

ii. ii. For the interpretation and assessment of mucin stains, specific measures have to be in place in order to make correct conclusions. For example, know the restrictions of the staining methods used and make sure to use a fixed classification system to identify the different types of mucins.

2.10.3 Alcian Blue Technique

The AB-stain can be used on its own or in combination with other stains (PAS, Aldehyde Fuchsin, and High Iron Diamine) to detect acid mucin AB dye is positively charged and binds to the acid groups found on mucopolysaccharides and stains them blue. At a pH of 2.5, AB reacts with the sulfated (sulfomucins) and carboxylated (sialomucins) mucopolysaccharides. However, at a pH of 1, it specifically reacts with sulfated mucopolysaccharides only (Bancroft and Gamble, 2008).

2.10.4 Periodic acid Schiff (PAS) technique

Periodic acid-Schiff (PAS) is the essential mucin histochemical technique. Periodic acid (HIO_4) is an oxidizing agent used to detect mucosubstances. Periodic acid breaks (oxidizes) the C-C bonds in various structures, converting 1:2-glycol groups (CHOH-CHOH) into dialdehydes (CHO-CHO). Consequently, these oxidized dialdehyde groups cannot be further oxidized by Periodic acid and this allows for the binding of Schiff‟ s reagent to the molecules and give it a red colour. The O-C bond of the aldehyde groups oxidizes when it attaches to Schiff's reagent. The binding of Schiff's reagent to aldehyde groups produces a red/magenta colour, which is intensified by washing in running tap water. The PAS positive mucins will stain a deep magenta colour and will represent neutral mucins (Bancroft and Gamble, 2008).

2.10.5 Combined Alcian Blue-Periodic Acid Schiff (PAS) technique

AB is also used in combination with PAS. The combined stain of AB with PAS clearly separates the acid and neutral mucins from one another. To begin, the tissue sections are

stained with AB to detect all the acid mucins and mainly to prevent the acid mucins, which are also PAS positive, to react with the PAS subsequently. This left only the neutral mucins to react with the PAS solution (Bancroft and Gamble, 2008).

2.11 ACCESSORY DIGESTIVE GLANDS

2.11.1 The Liver

The liver is a reddish brown triangular organ with four lobes of unequal size and shape (Cotran *et al.*, 2005). The external surface is smooth and weighs approximately 1400 g in females and 1800 g in males which is about 2% of body weight in the adult human. The liver receives its blood supply from two sources: 80% is delivered by the portal vein, which drains the spleen and intestines; the remaining 20%, the oxygenated blood, is delivered by the hepatic artery (Sibulesky, 2013). It is both the largest internal organ and the largest gland in the human body. Located in the right upper quadrant of the abdominal cavity, it rests just below the diaphragm, to the right of the stomach and overlying the gallbladder. It is connected to two large blood vessels, the hepatic artery and the portal vein. The hepatic artery carries oxygen-rich blood from the aorta, whereas the portal vein carries blood rich in digested nutrients from the entire gastrointestinal tract and also from the spleen and pancreas. These blood vessels subdivide into small capillaries known as liver sinusoids, which then lead to a lobule. Lobules are the functional units of the liver and each lobule is made up of millions of hepatic cells (hepatocytes) which are the basic metabolic cells.

Microscopic anatomy shows two major types of cells of the liver: parenchymal and non-parenchymal cells. 80% of the liver volume is occupied by parenchymal cells commonly

referred to as hepatocytes. Non-parenchymal cells constitute 40% of the total number of liver cells but only 6.5% of its volume. The liver sinusoids are lined with two types of cells, sinusoidal endothelial cells and phagocytic cells (Pocock, 2006). Hepatic stellate cells are some of the non-parenchymal cells which are external to the sinusoids in the space of Disse (Kmieć, 2001). Each of the lobes is seen to be made up of hepatic lobules; a vein goes from the centre, which then joins to the hepatic vein to carry blood out from the liver. On the surface of the lobules, there are ducts, veins and arteries that carry fluids to and from them. A distinctive component of a lobule is the portal triad. The rat liver is made up of four lobes (left, middle, right, and caudate). The left and middle lobes form a single lobe but the middle lobe has a deep notch to which the round ligament is attached. The right lobe is split into two sub-lobes and the caudate lobe is divided into the paracaval portion and the Spiegel lobe, which is split into two sub-lobes (Kogure *et al.*, 1999). The Rabbit liver is divided into left and right regions by a deep cleft with the right and left lobes further divided into anterior and posterior lobules (Meredith and Rayment, 2000). It has a quadrate lobe, which is behind the gallbladder.

2.11.2 The Pancreas

The pancreas is an important mixed gland associated with the gastrointestinal system. It lies retroperitoneal and comprises the head, body and tail regions in the mammals (Khan, 2014). The head rests within the concavity of the duodenum, a body which lies behind the stomach, and a tail in contact with the spleen. The neck of the pancreas lies between the body and head, and is in front of the superior mesenteric artery and vein (Drake *et al.*, 2005). The pancreas is composed of exocrine and endocrine compartment; the exocrine tissue, which secretes pancreatic digestive juice, and the endocrine tissue, which secretes

the hormones insulin, glucagons, somatostatin and pancreatic polypeptide for the control of the carbohydrate metabolism (Ku *et al.,* 2000). The exocrine pancreas is formed by acinar cells and excretory ducts with different diameter. The cytoplasm of the acinar cells is rich in rough endoplasmic reticulum (ER), mitochondria of the crista type, free ribosomes, and secretory zymogen granules（Simsek and Alabay, 2008). It has two main ducts, the main pancreatic duct, and the accessory pancreatic duct. This releases enzymes through the ampulla of Vater into the duodenum (Young *et al.,* 2006).

Histologically, the pancreas contains endocrine and exocrine tissues, and this division is also visible when the pancreas is viewed under a microscope (Young *et al.,* 2006).The tissues with an endocrine role appear under H and E staining as lightly-stained clusters of cells, called Islets Of Langerhans (Young *et al.,* 2006). Darker-staining cells form clusters called acini, which are arranged in lobes separated by a thin fibrous barrier. The secretory cells of each acinus surround a small intercalated duct. Due to their secretory functions, these cells have many small granules of zymogens that are visible. The intercalated ducts drain into larger ducts within the lobule (intralobular), these empty into interlobular ducts and finally the excretory ducts. The ducts are lined by a single layer of columnar cells. With increasing diameter, several layers of columnar cells may be seen (Young *et al.,* 2006).

2.11.3 The Gallbladder

The gall bladder is a muscular sac situated on the visceral surface of the liver. It has a capacity of about 100 mL in humans (Young *et al.,* 2006). In adult humans, the gallbladder measures approximately 8 centimetres in length and 4 centimetres in diameter

when fully distended (Meilstrup, 1994). Grossly, it is divided into the fundus, body, and neck (Drake *et al.,* 2005).

Histologically, the epithelial lining of the gall bladder is seen to consist of very tall columnar cells with basally located nuclei. At high magnification, numerous short irregular microvilli are present. A relatively loose submucosa rich in elastic fibres, blood vessels and lymphatics is present, which drains water reabsorbed from bile during the concentration process. The the gallbladder does not have a muscularis mucosae, muscle fibres are arranged in longitudinal, transverse and oblique orientations but do not form distinct layers. Externally, there is a thick collagenous adventitial (serosa) coat which conveys the larger blood and lymphatic vessels (Young *et al.,* 2006).

In the neck of the gall bladder and in the extrahepatic biliary tree, mucous glands are found in the submucosa; mucus may provide a protective surface film for the biliary tract (Young *et al.*, 2006).

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Experimental Animals

Seven (7) adult Grasscutters of both sexes i.e. four (4) males and three (3) females were purchased locally from Farmers in Zaria and kept for observation and acclimatization in the Animal House, Department of Human Anatomy, Ahmadu Bello University, Zaria. The animals were fed with cassava (given in its raw state) and water.

3.1.2 Reagents

Alcian blue (AB), Periodic Acid Schiff (PAS), Haematoxylin and Eosin stain (H and E), 10% Neutral buffered formalin (NBF), normal saline, anesthesia (chloroform), graded alcohol, xylene, and Diastase (NATE-0190, Creative Enzymes, Shirley, NY, USA).

3.1.3 Instruments

Digital weighing balance, dissecting set, tissue containers, thread, metre rule, vernier caliper tissue bath, microtome, glass slides. Sensitive electronic balance (Mettler balance P1210, Mettler Instrument AG, Switzerland; sensitivity: 0.001).

3.2 METHODOLOGY

3.2.1 Dissection

The body weight of each rat was obtained using a digital weighing balance. The dissection was carried out as described by Byanet *et al.* (2008). All animals were anaesthetized with

chloroform. An incision was made from the first cervical region up to the pelvic region with the rat on a dorsal recumbency, to expose the alimentary canal and accessory digestive glands. Photographs of the organs were taken *in situ*.

The specimens studied include the liver, gall bladder, pancreas, stomach, small and large intestines. These were dissected and the various measurements of length and weight were taken. Sections were immediately fixed in neutral buffered formalin for further investigation.

3.2.2 Morphologic and Morphometric Studies

Seven samples, comprising both male and females were used for gross and morphometric analysis. The liver, gall bladder, and entire GIT were weighed. The liver, gall bladder, pancreas, stomach, small and large intestines were examined gross anatomically for the following: shape, size, surfaces, borders and angles. The lengths of the stomach, small and large intestines were measured using a meter rule. Definitions of gross anatomical structures were based on standard information on rodent anatomy.

3.3 HISTOLOGY

3.3.1 Tissue Processing

Tissue sections from the liver, gall bladder, pancreas, stomach, small and large intestines were fixed in Neutral buffered formalin (NBF).

Following fixation, the tissue *samples* were dehydrated using progressive grades of alcohol hourly, they were then cleared in xylene and infiltrated in molten paraffin wax at 62ºC. Then the tissues were embedded in paraffin wax. The blocks of tissues obtained were trimmed and sectioned at 5 microns using a rotary microtome. The ribbons of sections were floated out on a water bath at 55ºC. The tissue sections from the various tissue types were picked on slides

by immersing the slide lightly smeared with adhesive vertically in water bath to three quarters of its length and making the section come in contact with the slide. On lifting the slide vertically, from the water, the section was flattened onto the slide and labeled accordingly. The Sections were correctly positioned on the slides and drained in the vertical position for 60 minutes and then dried in oven at 37ºC overnight.

3.3.2 Staining

i. Haematoxylin and Eosin (H and E)

The H and E staining was done to show the general morphology of the liver, gall bladder, pancreas, stomach, small intestine (duodenum, jejunum and ileum) and large intestine (caecum, colon and rectum). All the tissue samples were deparaffinised in xylene and brought to water. Tissue sections were stained in Mayer's hematoxylin for 10 minutes and washed well in running tap water for 7 minutes until sections 'blue'. The tissue samples were then differentiated in 1% acid alcohol (1% HCl in 70% alcohol) for 10 seconds and washed well in tap water for 10 minutes until sections 'blue again and then followed by another 5-min tap water wash. Samples were stained in 1% eosin Y for 10 min and washed in running tap water for 5minutes. Samples were dehydrated through alcohol, cleared, and mounted (Bancroft and Gamble, 2008).

ii. Alcian Blue (AB) technique

The tissues were stained with Alcian blue to identify and characterize the distribution of acidic mucins, the mucin granules stained dark blue while the nuclei stained red. Sections from the stomach, gall bladder, small and large intestines were deparaffinised in xylene and brought to water. The tissues were then stained in Alcian Blue solution (pH 2.5) for 30 minutes. The tissue sections were rinsed in running tap water for 5 minutes. The tissue

sections were then counterstained in nuclear fast red solution for 10 minutes and washed in running tap water for 1 minute. The sections were dehydrated in graded alcohol. Sections were cleared in xylene and mounted in DPX (Bancroft and Gamble, 2008).

iii. Periodic Acid Schiff (PAS) technique

The tissues were stained with Periodic Acid Schiff to identify and characterize the distribution of neutral mucins, these mucin granules stained magenta while the nuclei stained blue. Sections from the stomach, gall bladder, small and large intestines were de-waxed and brought to water. The sections were oxidized with periodic acid solution for 5 minutes and subsequently rinsed several changes of distilled water. The sections were placed in Schiff reagent for 15 minutes and washed in tap water for 10 minutes. Tissue sections were then counterstained in hematoxylin for 1 minute and washed in tap water for 5 minutes. Sections were dehydrated in graded alcohol, cleared with xylene and mounted in DPX (Bancroft and Gamble, 2008).

iv. Combined Alcian Blue-Periodic Acid Schiff (PAS) technique

To begin with, the tissue sections were stained with AB to detect all the acid mucins and mainly to prevent the acid mucins, which are also PAS positive, from reacting with the subsequent PAS. This left only the neutral mucins to react with the PAS solution. Sections from the stomach, gall bladder, small and large intestines were deparaffinised in xylene and brought to water. The tissues were then stained in Alcian Blue solution (pH 2.5) for 30 minutes. The tissue sections were rinsed in running tap water for 5 minutes. The sections were oxidized with periodic acid solution for 5 minutes and subsequently rinsed several changes of distilled water. The sections were placed in Schiff reagent for 15 minutes and washed in tap water for 10 minutes. Tissue sections were then counterstained in hematoxylin

for 1 minute and washed in tap water for 5 minutes. Sections were dehydrated in graded alcohol, cleared with xylene and mounted in DPX (Bancroft and Gamble, 2008).

v. Periodic Acid Schiff with Diastase control (PASD) technique

The liver tissue was stained with Periodic Acid Schiff (PAS) after diastase treatment to demonstrate the presence of glycogen. Sections taken from the liver were de-waxed and brought to water. The sections were treated with diastase (amylase) solution for 1 hour and washed well in running tap water. The sections were oxidized in periodic acid solution for 5 minutes and subsequently rinsed in distilled water. The sections were placed in Schiff reagent for 15 minutes and washed in tap water for 5 minutes. Tissue sections were then counterstained in hematoxylin for 1 minute and washed in tap water for 5 minutes. Section were dehydrated in graded alcohol, cleared in xylene and mounted in DPX (Bancroft and Gamble, 2008).

3.3 STATISTICAL ANALYSIS

Data obtained from weights and lengths were subjected to statistical analysis using independent sample t-test and expressed as mean ± standard error of mean (Mean ± SEM) using SigmaStat3.5. Pearson's correlation analysis was done for the different parameters. Independent sample t-test was used to compare the organ weight to body weight ratios in male and female Grasscutters. Values of $p \leq 0.05$ were considered significant.

CHAPTER FOUR

RESULTS

In the present study, the results of morphometry, morphology, histology and mucin histochemistry of the GIT and some accessory digestive glands of the Grasscutter are described as follows.

4.1 MORPHOMETRIC STUDIES

Morphometric analyses revealed that the mean body weight was 1582.25 ± 207.95g in males and 1089.67 ± 276.93g in females. The mean GIT length in males was 253.13 ± 10.68cm while in females the mean GIT length was 243.63 ± 6.73cm. The segmental morphometry demonstrated longer small and large intestines in males (115. 43 ± 5.64cm and 132.20 ± 4.83cm) than in females (109.60 ± 5.64cm and 130.63 ± 3.69cm) respectively. The GIT weights in males and females were 133.60 ± 10.01g and 119.14 ± 13.20g while the liver weights were 45.65 ± 3.20g and 37.43 ± 5.84g respectively, as shown in Table 4.1. Pearson's correlation analysis revealed a positive linear relationship between the different parameters studied which were significant while a strong positive relationship was observed between body and liver weights, gall bladder and liver weights in both male and female Grasscutters as shown in Tables 4.2 and 4.3 respectively. Results for the organ weight to body weight ratios analysis between the male and female Grasscutters revealed relatively close mean values which were not statistically significant as shown in Table 4.4.

Table 4.1: Morphometric features of the gastrointestinal tract in male and female Grasscutters.

Variable	**Male (n=4)**	**Female (n=3)**	**p**
	Mean ± SEM	Mean ± SEM	
Body weight (g)	1582.25 ± 207.95	1089.67 ± 276.93	0.21
Stomach length (cm)	10.35 ± 0.62	10.00 ± 0.10	0.96
Duodenum length (cm)	19.00 ± 1.19	17.70 ± 2.56	0.63
Jejunum length (cm)	68.58 ± 3.75	66.40 ± 3.15	0.69
Ileum length (cm)	27.85 ± 1.69	25.50 ± 1.35	0.35
Caecum length (cm)	30.40 ± 1.74	30.23 ± 0.59	0.94
Colon length (cm)	100.80 ± 3.96	98.37 ± 3.02	0.67
Rectum length (cm)	2.00 ± 0.10	2.03 ± 0.09	0.83
SI length (cm)	115. 43 ± 5.64	109.60 ± 5.64	0.51
LI length (cm)	132.20 ± 4.83	130.63 ± 3.69	0.82
GIT length (cm)	253.13 ± 10.68	243.63 ± 6.73	0.52
GIT weight (g)	133.60 ± 10.01	119.14 ± 13.20	0.23
Liver weight (g)	45.65 ± 3.20	37.43 ± 5.84	0.24
Gall bladder weight (g)	0.50 ± 0.11	0.34 ± 0.05	0.31

Analysis by student's t-test between male and female. Data expressed as mean ± standard error of the mean (± SEM) of the values collected. SI= small intestine, LI= large intestine,

Table 4.2: Pearson's correlation analysis of parameters in male Grasscutter.

	Body wt	Sto lt	Duod lt	Jej lt	Ile lt	Cec lt	Col lt	Rec lt	Liv wt	Gall b wt
Body w**t**	1									
Sto lt	.840	1								
Duod lt	.229	-.118	1							
Jej l**t**	.896	.584	.633	1						
Ileum l**t**	.819	.563	-.091	.641	1					
Cec l**t**	.560	.545	.692	.717	-.001	1				
Col l**t**	.978[b]	.911	.229	.865	.697	.674	1			
Rec l**t**	.818	.898	-.369	.477	.803	.164	.810	1		
Liv w**t**	.970[a]	.773	.449	.966[a]	.691	.722	.965[a]	.665	1	
Gall b w**t**	.914	.852	.416	.890	.518	.839	.967[a]	.648	.962[a]	1

Where $p< 0.05^{a}$, $p< 0.02^{b}$, Weight=wt., Length=lt., Stomach= Sto, Duodenum= Duo, jejunum=jej, ileum=ile, Caecun=Cec, Colon= Col, Rectum= Rec, Liver =Liv and Gall bladder= Gall b.

Table 4.3: Pearson's correlation analysis of parameters in female Grasscutter.

	Body wt	Sto lt	Duod lt	Jej lt	Ile lt	Cec lt	Col lt	Rec lt	Liv wt	Gall b wt
Body wt	1									
Sto lt	.760	1								
Duod lt	.809	.997	1							
Jej lt	.686	.048	.126	1						
Ileum lt	.936	.481	.549	.898	1					
Cec lt	.916	.956	.976	.338	.717	1				
Col lt	.937	.939	.963	.389	.754	.998[a]	1			
Rec lt	.975	.756	.805	.690	.938	.914	.935	1		
Liv wt	.994[b]	.996	.705	.131	.553	.978	.964	.808	1	
Gall b wt	.674	.992	.980	-.075	.370	.913	.889	.970	.979[b]	1

Where $p< 0.05$[a], $p< 0.02$[b], Weight=wt., Length=lt., Stomach= Sto, Duodenum= Duo, jejunum=jej, ileum=ile, Caecun=Cec, Colon= Col, Rectum= Rec, Liver =Liv and Gall bladder= Gall b.

Table 4.4: Results for independent samples t-test analysis of organ weight to body weight ratios in male and female Grasscutters.

Organ	**Male** (n=4) Mean ± SEM	**Female** (n=3) Mean ± SEM	**p**
Stomach	0.73 ± 0.05	0.71 ± 0.12	0.21
Small intestine	2.79 ± 0.11	2.51 ± 0.13	0.15
Large intestine	3.64 ± 0.12	3.29 ± 0.15	0.10
Liver	2.98 ± 0.26	3.01 ± 0.19	0.42
Gall bladder	0.03 ± 0.01	0.03 ± 0.01	0.75

Data expressed as mean ± standard error of mean (SEM), n= number of animals.

4.2 MORPHOLOGICAL ASSESSMENTS

The incision made through the anterior abdominal wall of the Grasscutter revealed the abdominal intestinal topography, which was noted and photographed as shown in Plate I. The GIT was dissected and photographed to show the different segments as shown in Plate II.

4.2.1 Stomach

Gross examination of the stomach in this study externally revealed it to be simple and has the shape of an inverted J as shown in Plate III. Externally, the stomach did not show any vivid segmentation as it was smooth as shown in Plate III. However, internally it showed three distinct parts; the cardia, fundus and pylorus. On the internal surface a smooth cardiac region was separated from the oesophagus by a constriction (cardiac sphincter), the fundus was seen to be thrown into folds (rugae) as shown in Plate IV and the smooth pylorus was separated from the duodenum by a constriction (pyloric sphincter).

4.2.2 Small Intestine

Gross examination of the small intestine revealed three regions; duodenum, jejunum and ileum. The duodenum was in contact with the pyloric region of the stomach proximally (gastro-duodenal junction) and continues with the jejunum distally, while the jejunum continues with ileum distally. The ileum was seen to terminate where it joins the caecum at the Ileo-caecal junction as shown in Plate II.

4.2.3 Large Intestine

Results for the gross examination of the large intestine revealed three distinct regions; caecum, colon and rectum. The caecum was observed to be a large, coma-shaped blind ended sac with teniae and sacculations (haustra) on its entire length. The colon was the longest

portion of the intestine and had fecal balls, particularly at its distal part. The rectum was short, straight and was dilated around its terminal portion of the GIT as shown in Plate II.

4.2.4 Liver

Morphological examination of the liver showed that it was located below the diaphragm in the superior-medial aspect of the abdominal cavity. It laid on top of the stomach coming in contact with some viscera some part of the small intestine and spleen. It had a parietal and a visceral surface and was observed to be dark brown in colour with a smooth and glossy surface throughout. It presented a multi-lobular appearance with up to four lobes present on each of the liver examined which had a left lobe, two intermediate lobes, and three right lobes as shown in Plate V.

4.2.5 Pancreas

In this study, morphological examination revealed a diffuse pancreas located within the u-shaped duodenal curve and spreads from the duodenum down to the stomach. It was pale pink in colour, soft, flexible and friable to touch. The pancreas was covered in adipose tissue in some areas, making it difficult to distinguish between the two. It had a thread-like appearance, interconnected to form a matrix with a rough ridge-like surface.

4.2.6 Gall Bladder

In this study, morphological examination of the gall bladder revealed a sac-like appearance with a light yellowish-green colouration. It had a surface that was soft and smooth to touch and had a very thin papery wall. It was almost entirely covered by the visceral surface of the liver as shown in Plate V.

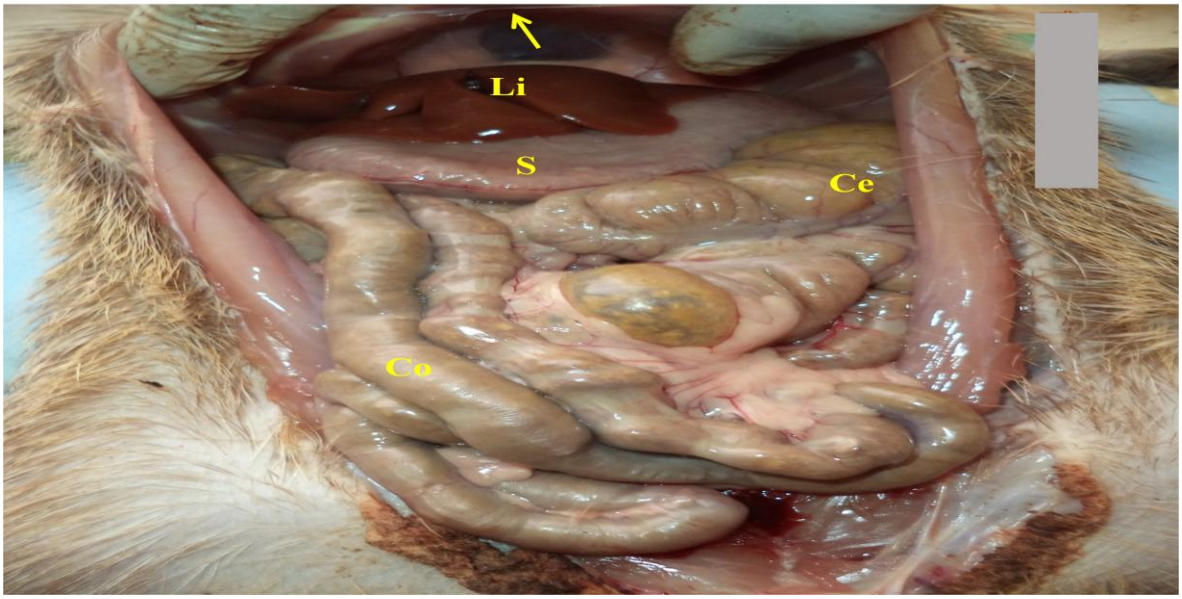


Plate I: Gastrointestinal tract of the Grasscutter *in situ*, the diaphragm (pointer), liver (Li), stomach (S), caecum (Ce) and colon (Co).

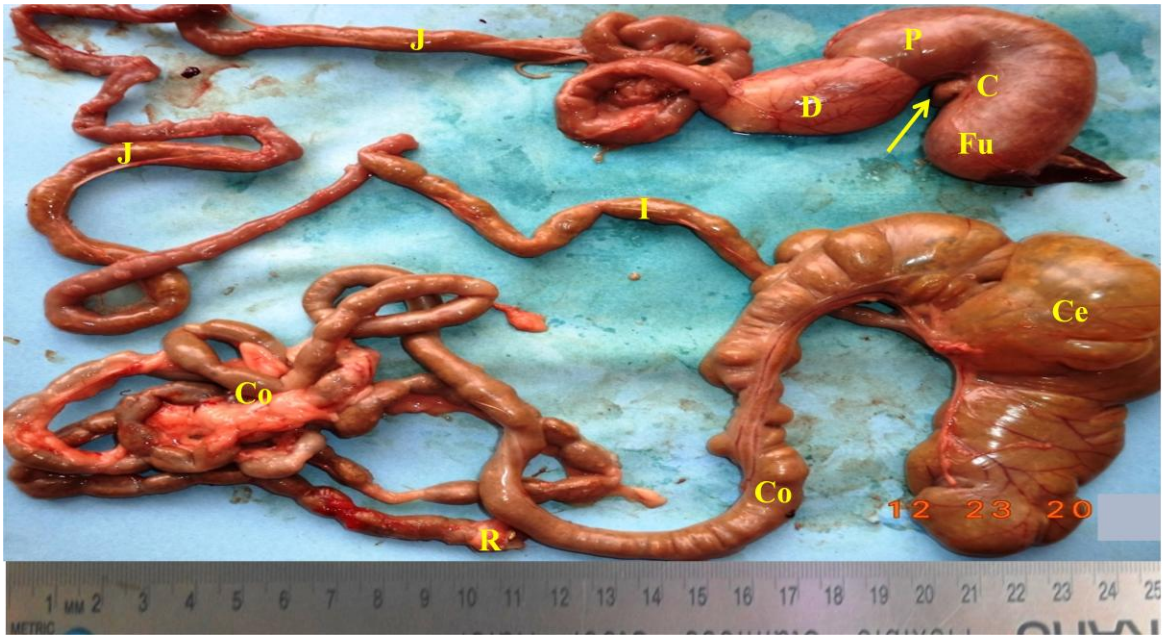


Plate II: Gastrointestinal tract of the Grasscutter showing the fundus (Fu), cardia (C), pylorus (P), duodenum (D), oesophagus (arrow), jejunum (J), ileum (I), colon (Co), rectum (R) and caecum (Ce).

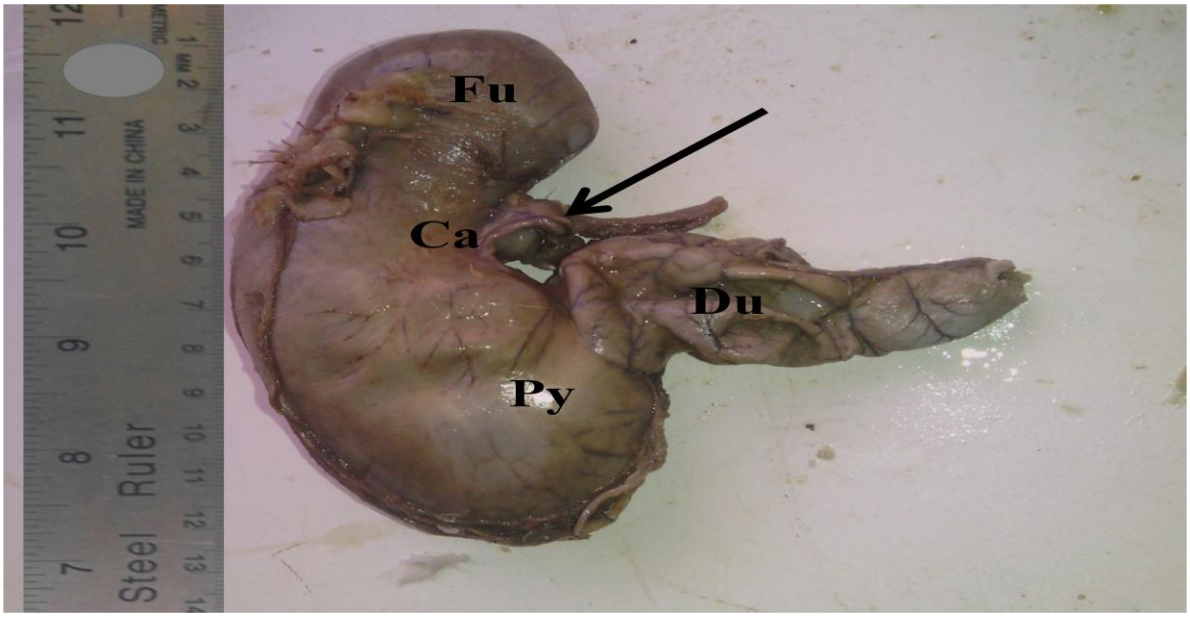


Plate III: Showing the cardiac (Ca), fundic (Fu) and pyloric (Py) regions of the stomach with the oesophagus (arrow) and duodenum (Du) attached.

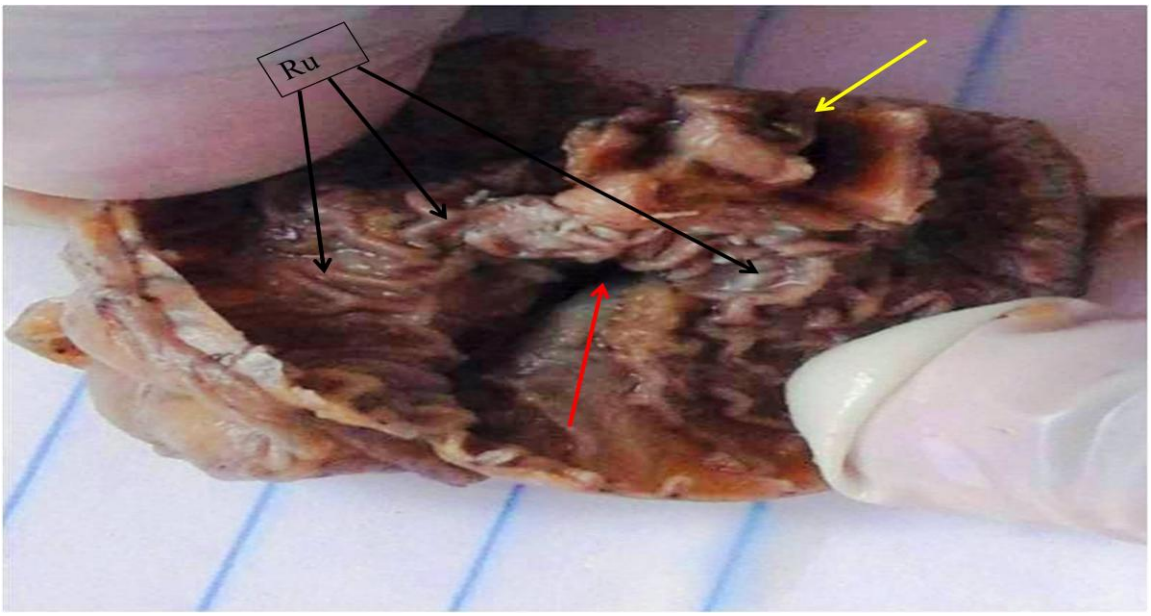


Plate IV: The internal surface of the stomach showing the rugal folds (Ru), oesophageal opening (yellow arrow) and pyloric anthrum (red arrow).

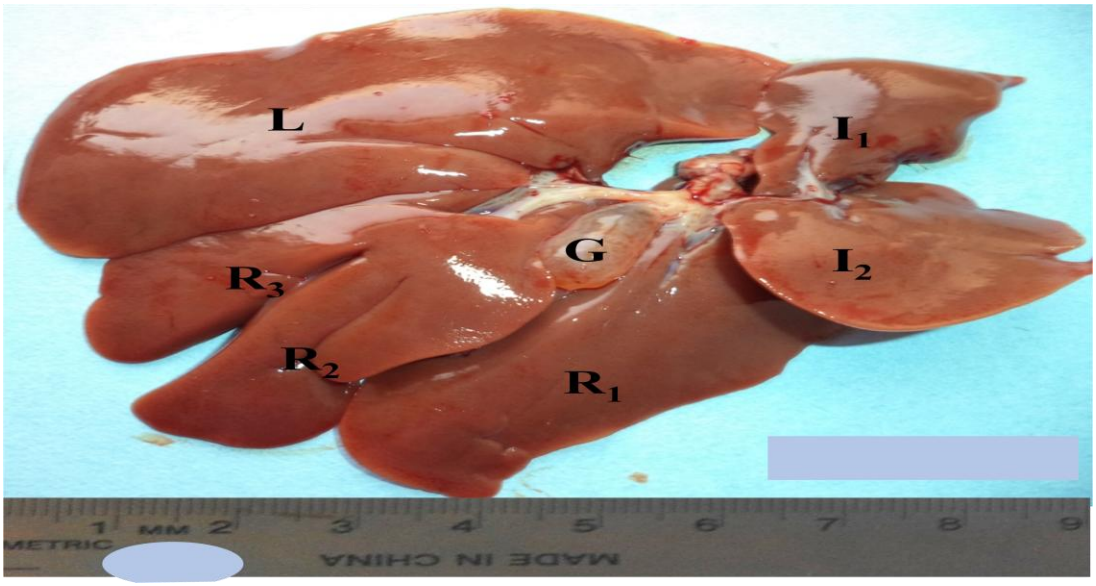


Plate V: Visceral surface of the liver showing left lobes (L), intermediate lobes (I_1, I_2), right lobe (R_1, R_2 and R_3) and gall bladder (G).

4.3 HISTOLOGICAL STUDIES

4.3.1 Stomach

The result of the histological studies showed that the stomach had three regions, the cardia, fundus and pylorus which revealed that all three regions had four distinctive layers; the mucosa, the submucosa, the tunica muscularis and the serosa. The epithelium of the three regions was lined by simple columnar cells. The tunica muscularis in all three regions was seen to be made up of an inner oblique, middle circular and an outer longitudinal layer of muscles.

The cardiac region had long gasstric pits with numerous glands noticeable around the entrance of the stomach as shown in Plate VI, these glands were branched and tubular especially those close to the transition zone (cardiac sphincter). The circular muscle layer of the muscularis externa thickened to form the cardiac sphincter.

The fundic region showed shorter gastric pits and had extensive rugae. The lamina propria of the mucosa was observed to contain numerous tubular glands with a variety of cells with the dominant cells being the chief cells as shown in Plate VII. The external muscle layer was seen to be thick and containing muscles arranged in different orientations.

The pyloric region was observed to have pits of similar length with those seen in fundic region. The pyloric glands were simple and tubular as shown in Plate VIII. The glands were seen to occupy about two-thirds of the mucosa with parietal cells interspersed among the several types of cells, the most notable being the chief cells which were numerous, forming the glands of this region as shown in Plate IX.

4.3.2 Small Intestine

The histological results from present study showed that the small intestine had four functionally distinguishable layers; mucosa, submucosa, muscularis externa and serosa. The epithelium lining the villi of all three regions was made up of simple columnar enterocytes with basally placed nuclei. There were lacteals, blood vessels and a few lymphoid cells within the lamina propria.

The result for the duodenum revealed tall leaf-like villi which were closely packed with the mucosa separated from the submucosa by a thin band of smooth muscle (muscularis mucosa). The duodenal glands of Brunner were seen to occupy the submucosa as well as part of the lamina propria. These glands were seen to be tubular and branched as shown in Plate X.

The result for the jejunum revealed long villi of varying lengths, finger-like in appearance but not closely packed. The lamina propria had hymphoid cells scattered all over, with the lacteals visible. The submucosa also housed some lymphoid tissues aggregate. The tubular intestinal glands (crypts of Lieberkühn) were located at the base of the mucosa as shown in Plate XI.

The result for the ileum showed that the villi were shorter. The villi were both finger-like and ridge-like in appearance and placed at different levels. The intestinal glands (crypts of Lieberkühn) were basally located in the lamina propria and were tubular in shape as shown in Plate XII. Large lymphoid tissue aggregation were visible (Peyer's patches) in some of the regions of the submucosa traversing to the base of the mucosa, reducing the thickness of the muscularis propria where it was located as shown in Plate XIII.

4.3.3 Large Intestine

The results of the present study showed that the large intestine was divided into three histological different regions; the caecum, colon and rectum.

The caecum which is the first part of the large intestine was seen to have simple columnar epithelial cells with basal nuclei. The mucosa was thrown into numerous folds and the lamina propria was seen to contain lymphoid cells around blood vessels. There were tubular intestinal crypts all over lamina propria of the mucosal layer. The submucosa was seen to contain an aggregation of lymphoid tissue with presence of blood vessels. The muscularis propria was thick with an inner circular and an outer longitudinal layer as shown in Plate XIV.

The histology of the colon revealed the presence of a simple columner epithelial cells with basal nuclei, the epithelial cells and the intestinal crypts were seen to contain numerous goblet cells interposed within the cells. The submucosa was separated from the mucosa by the lamina muscularis and the submucosa was seen to contain blood vessels in loose connective tissues. The muscularis externa layer was seen to contain an inner circular and outer longitudinal layer of smooth muscle; this was seen to be thick as shown in Plate XV.

The histology of the rectum revealed the presence of simple columnar epithelium with basal nuclei; long crypts of Lieberkühn were located in the limina propria. The crypts of the rectum presented numerous goblet cells. A thin lamina muscularis separated the muscosa from the submucosa. The muscularis external was thick and had a thicker inner circular and thinner outer longitudinal layer as shown in Plate XVI.

4.3.4 Liver

Histological studies revealed that the liver was made up of closely packed parenchymal hepatocytes. The cells were large, polyhedral with centrally placed round nuclei. The arrangement of the hepatocytes was in diffuse to radial pattern and the sinusoidal spaces were not very prominent except in some areas where they become more visible. The portal tracts were scattered randomly all over the liver parenchyma, it was seen to be made up of a bile duct, portal vein and an artery. There were central veins at various intervals draining the sinosoids. Blood cells (erythrocytes) were visible within the sinusoids. There were the non-parenchymal Kupffer cells noticeably interposed among the hepatocytes as shown in Plates XVII and XVIII.

4.3.5 Pancreas

Result from the histological studies showed that the pancreas was made up of lightly stained and darkly stained portions. The eosinophilic pancreatic islands (islets of Langerhans) were seen interposed within the pancreatic parenchyma and these cell clusters were of different sizes (lightly stained). The basophilic serous acinar cells were distributed all over the parenchyma and were darkly stained. The pancreatic parenchyma was seen to be divided into numerous lobules of different sizes by the connective tissues septa. The pancreas was seen to be highly vascularised and intralobular ducts were seen in-between the secretory acinar cells as well as the presence of interlobular ducts which they drain into. The presence of zymogen granules within the acinar cells can be seen as the darkly stained portion of the secretory acini as shown in Plates XIX and XX.

4.3.6 Gall Bladder

The result of the H and E stained gall bladder showed the presence of tall columnar epithelium with basally located nuclei. The muscularis externa was indistinct and visible as a discontinuous layer of smooth muscle beneath the epithelium. The muscle thickness was seen to vary and placed at different region of the gall bladder. There was no visible lamina propria. The loose connective tissue was seen to contain numerous blood vessels of different sizes. There was presence of an outermost serosa as shown in Plate XXI.

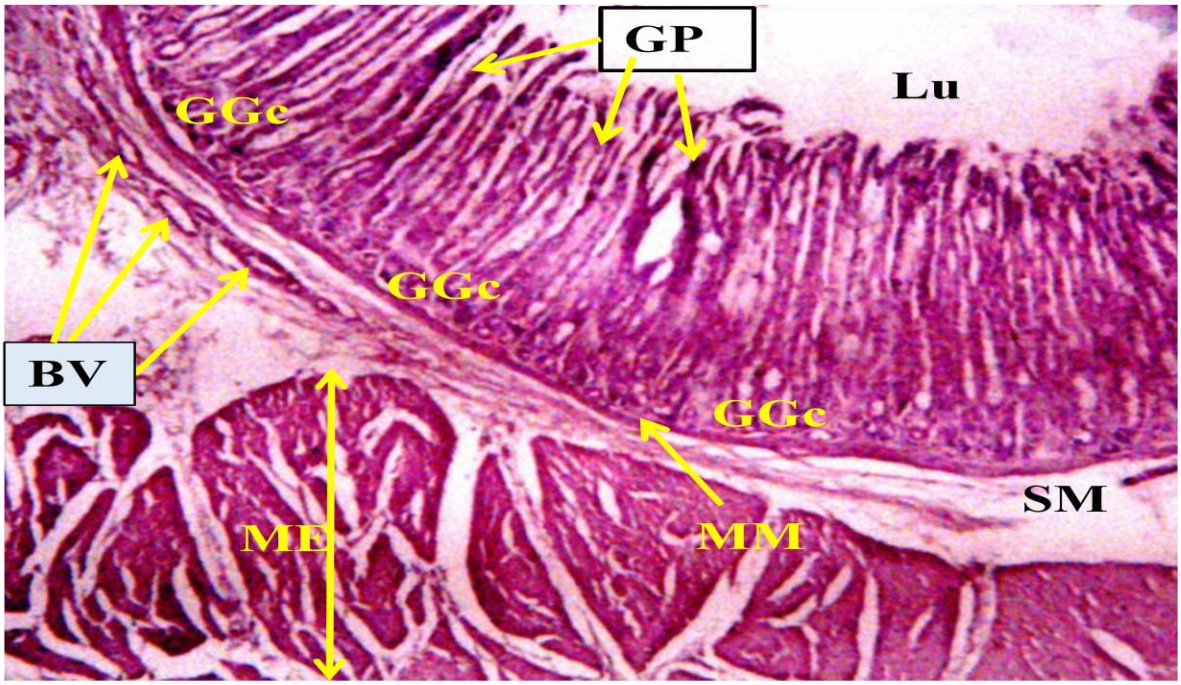


Plate VI: A transverse section of the cardiac region of the stomach (gastro-esophageal junction) showing the lumen (Lu), cardiac glands (GGc), muscularis mucosa (MM), blood vessels (BV) and submucosa (SM) and part of the muscularis externa. H and E × 40

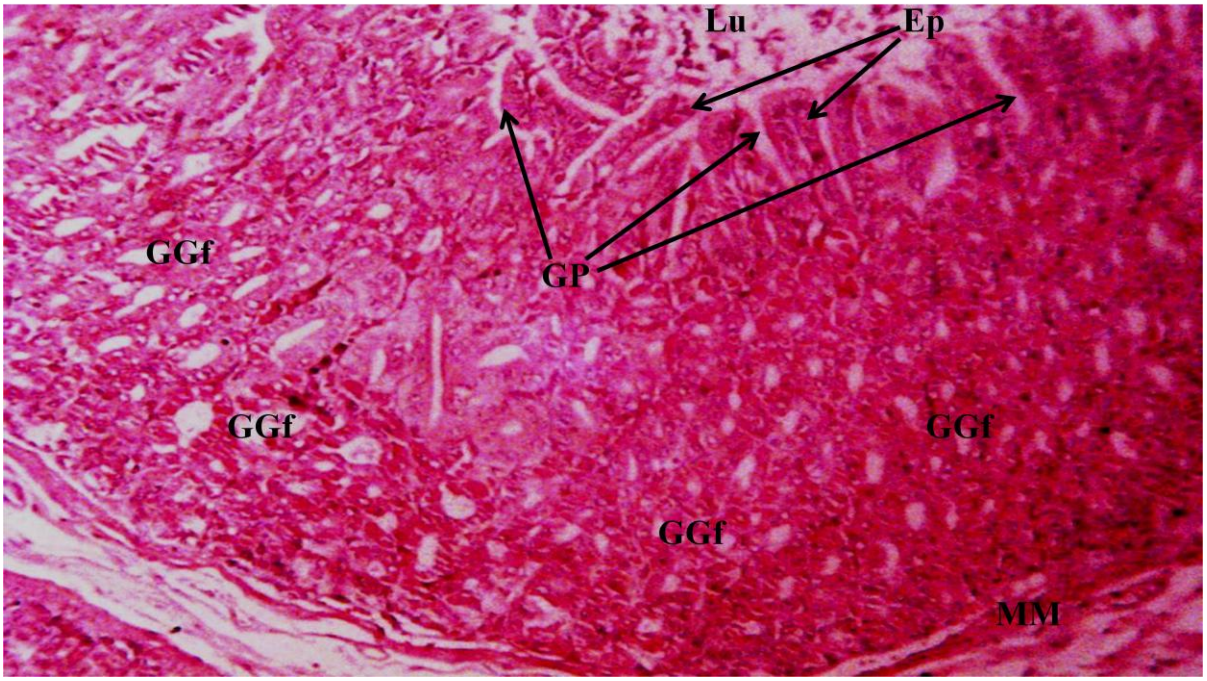


Plate VII: A transverse section of the fundic region of the stomach showing the lumen (Lu), epithelium (Ep), gastric pits (GP), fundic glands (GGf) and muscularis mucosa (MM). H and E × 100

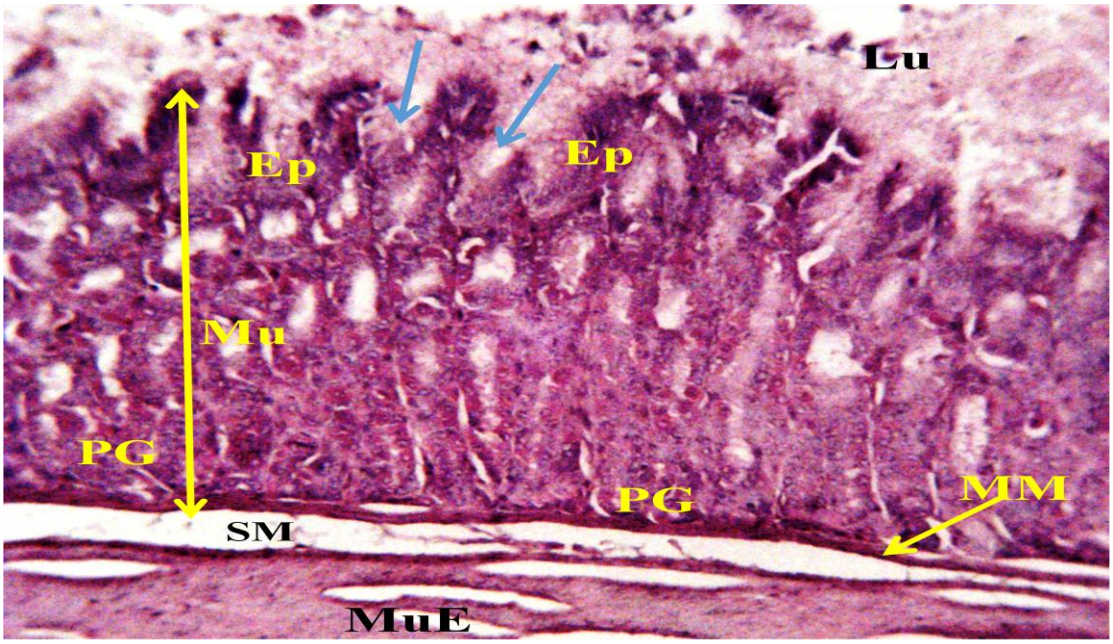


Plate VIII: A transverse section of the pyloric region of the stomach showing the lumen (Lu), gastric pits (arrows), pyloric glands (PG), muscularis mucosa (MM), submucosa (SM), muscularis externa (ME) and blood vessels (BV). H and E × 100

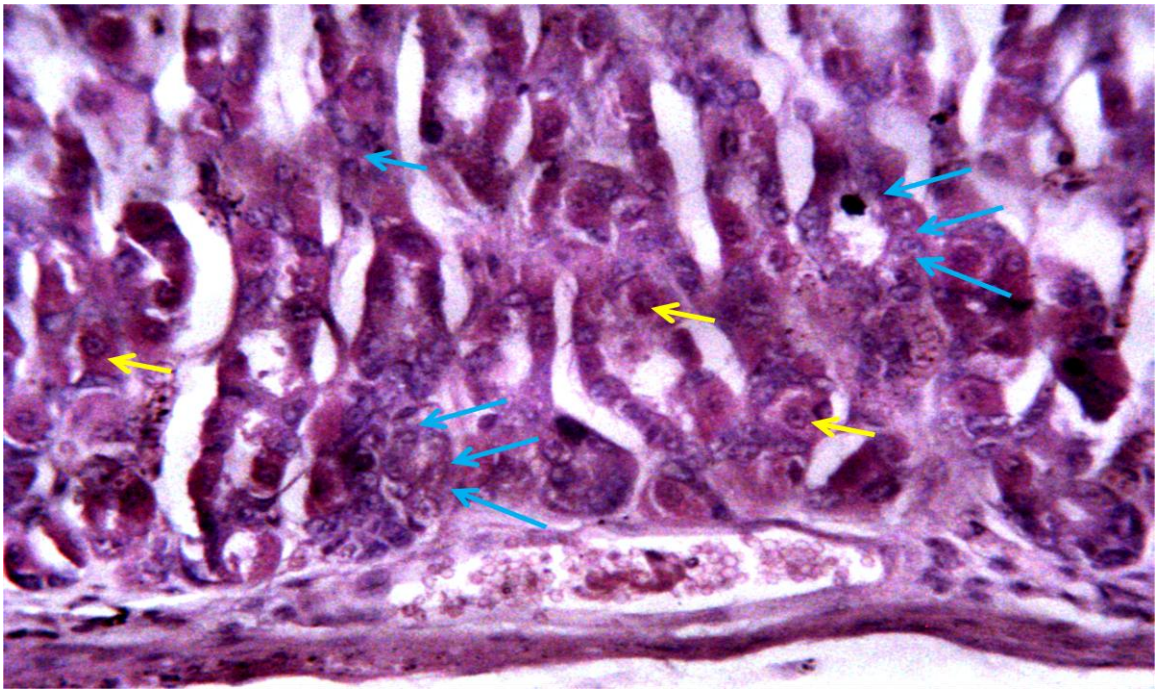


Plate IX: A transverse section of the pyloric region of the stomach showing the chief cells (blue arrows) and parietal cells (yellow arrows). H and E × 250

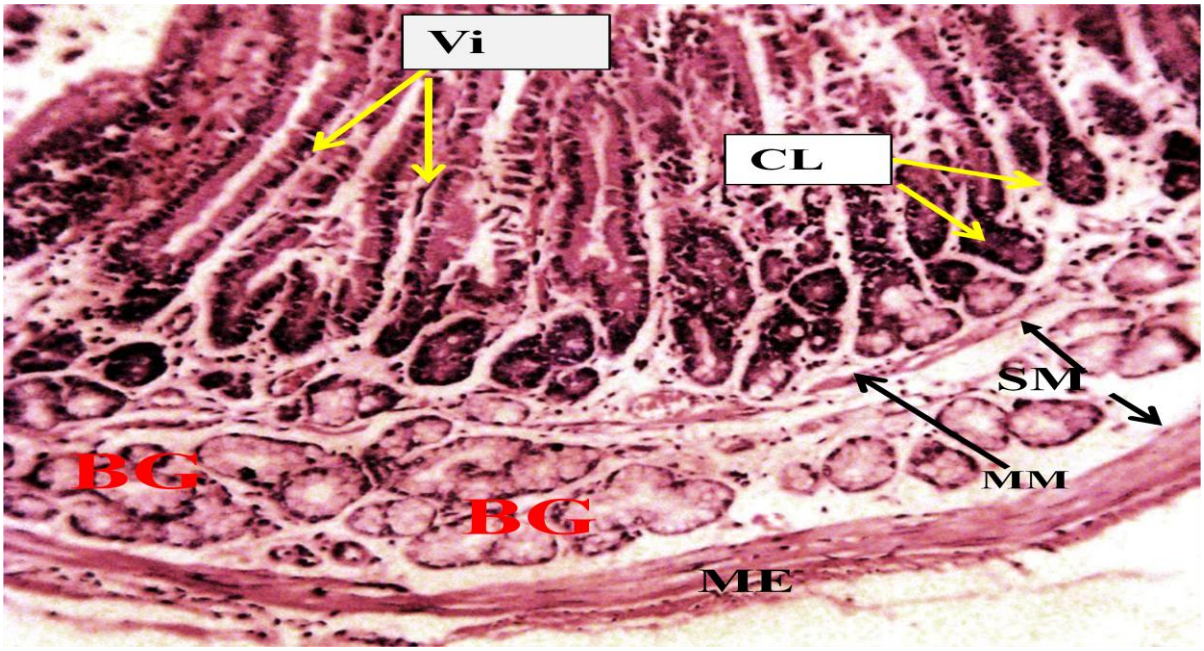


Plate X: A transverse section of the duodenum showing the Brunner's glands (BG), duodenal Villi (Vi), crypt of Lieberkühn (CL), muscularis mucosa (MM), submucosa (SM) and muscularis externa (ME). H and E × 100

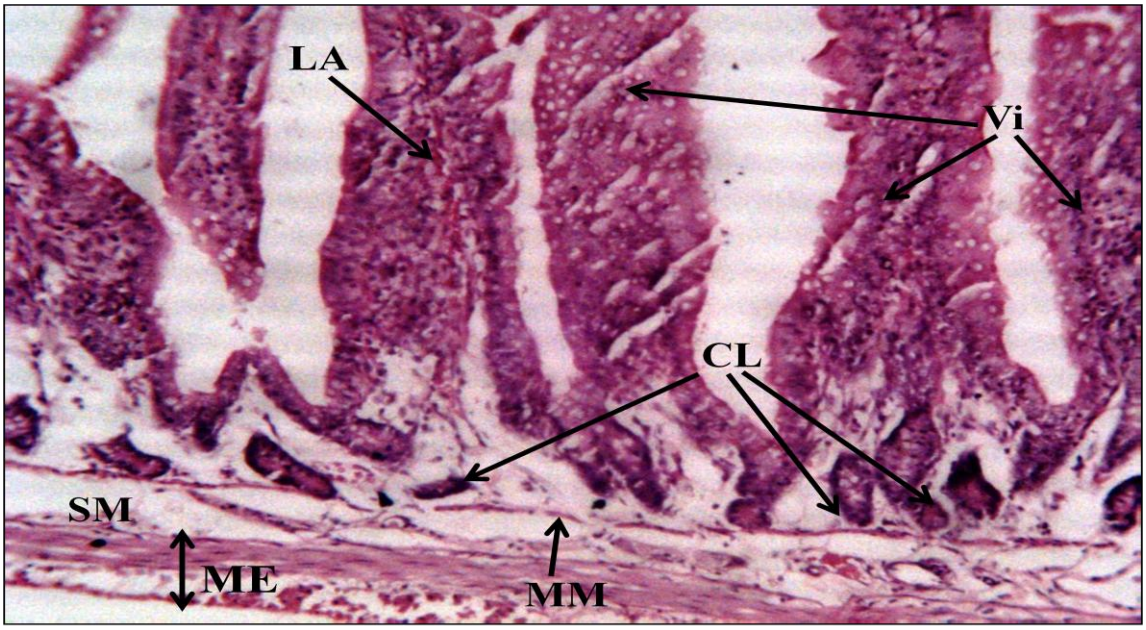


Plate XI: A transverse section of the jejunum showing the jejunal villi (Vi), lacteal (LA) crypts of Lieberkühn (CL), muscularis mucosa (MM), submucosa (SM) and muscularis externa (ME). H and E × 100

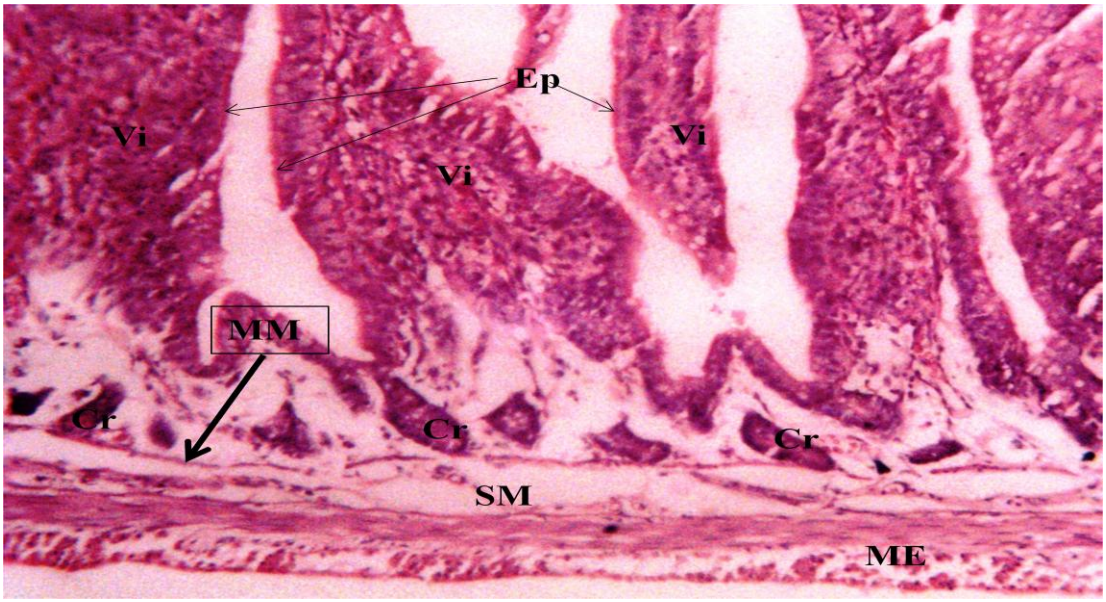


Plate XII: A transverse section through the ileum showing the simple columnar epithelium (Ep), ileal villi (Vi), crypts of Lieberkühn (Cr), muscularis mucosa (MM), submucosa (SM) and muscularis externa (ME). H and E × 100

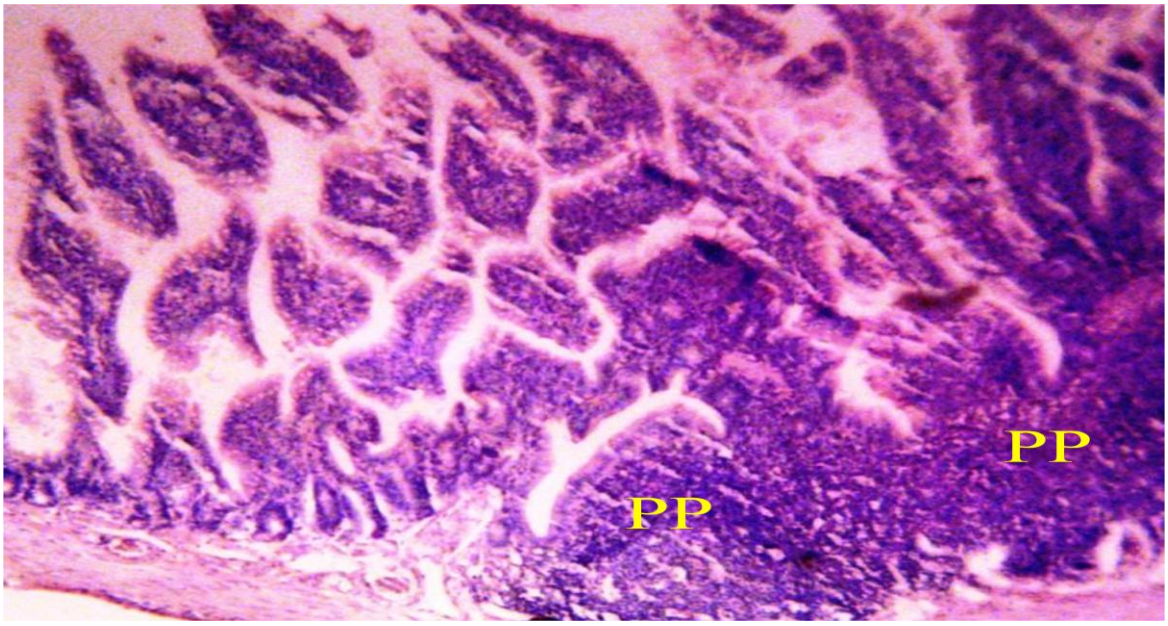


Plate XIII: A transverse section of the ileum showing the Peyer's patches (PP) in the submucosa. H and E × 40

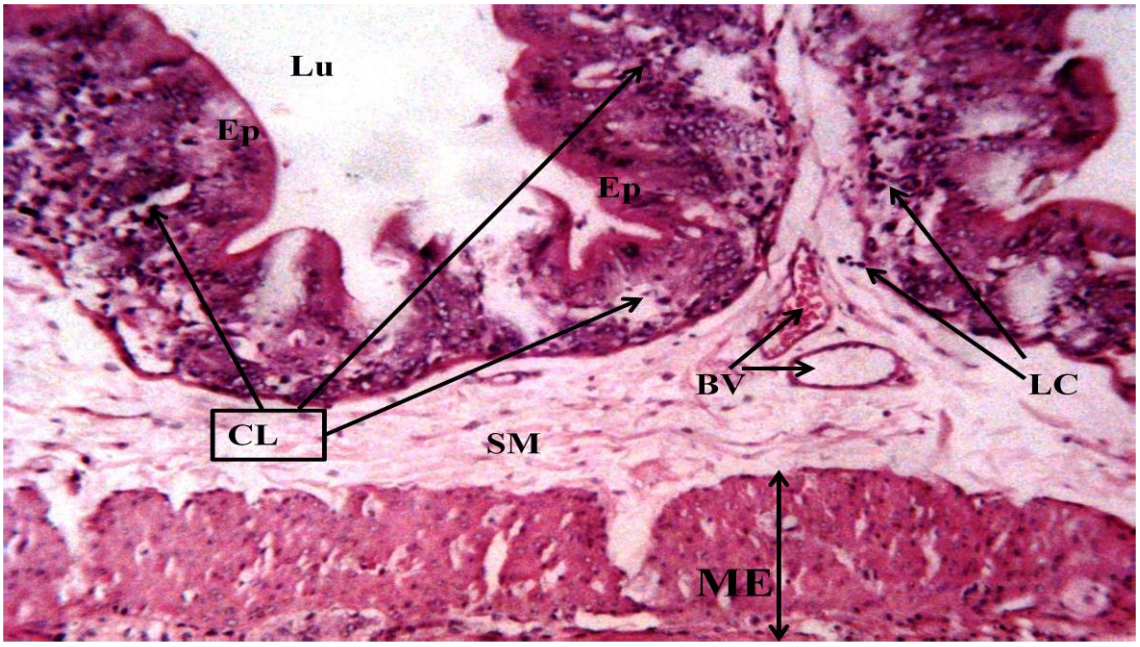


Plate XIV: A transverse section of the caecum showing lumen (Lu), simple columnar epithelium (Ep), blood vessel (BV), lymphoid cells (LC), crypts of Lieberkühn (CL) and submucosa (SM). HandE × 100

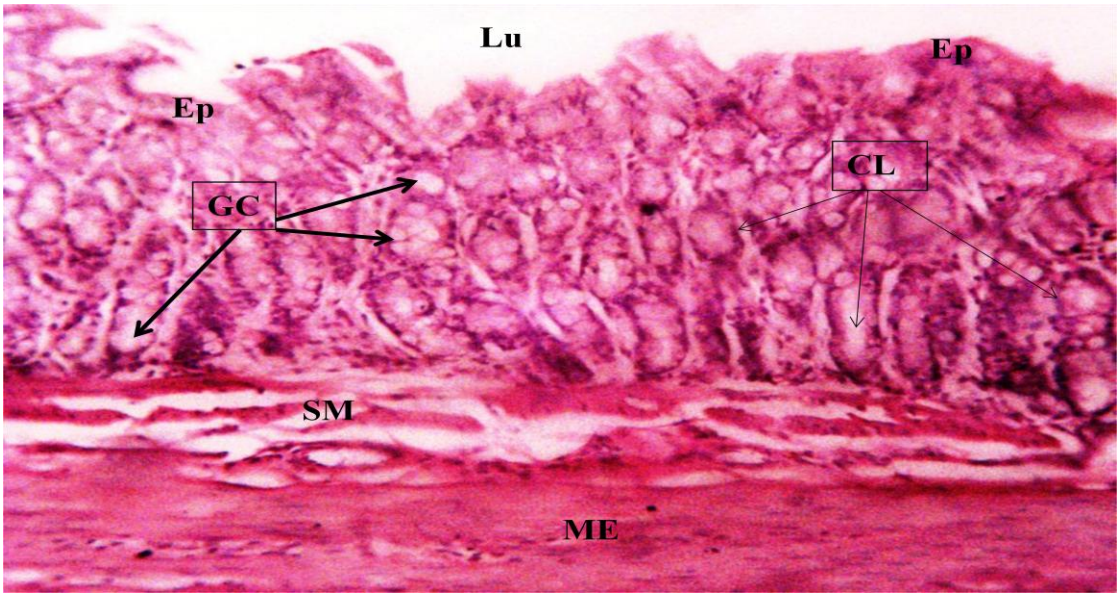


Plate XV: A transverse section of the colon showing lumen (Lu), epithelium (Ep), goblet cells (GC), crypts of Lieberkühn (CL), submucosa (SM) and muscularis externa (ME). H and E × 100

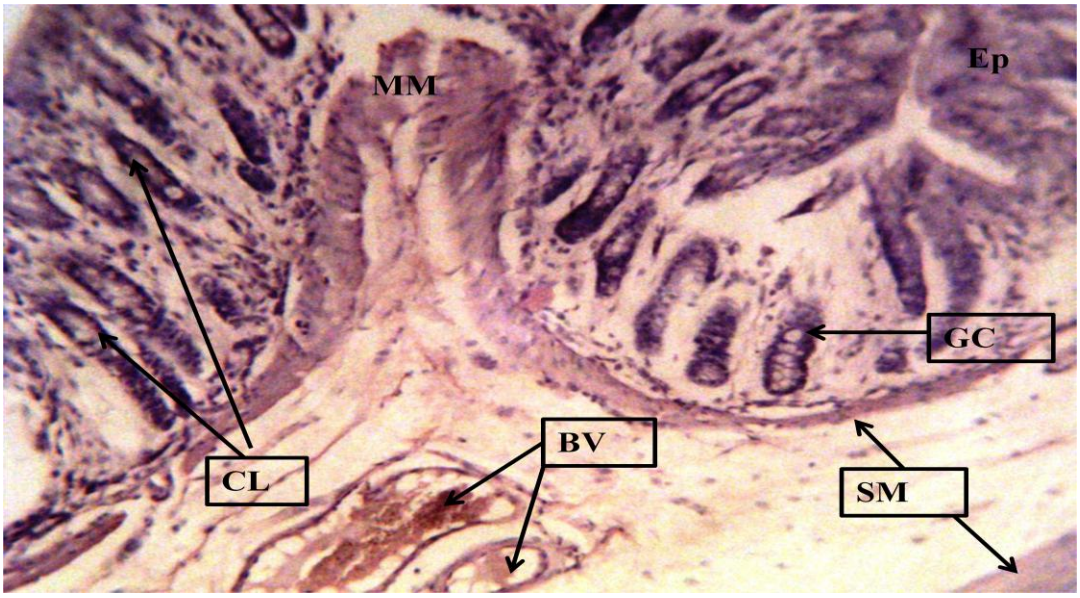


Plate XVI: A transverse section of the rectum showing the Epithelium (Ep), crypts of Lieberkühn (CL), muscularis mucosa, blood vessels of the submucosa (BV), submucosa (SM) and goblet cell (GC). H and E × 100

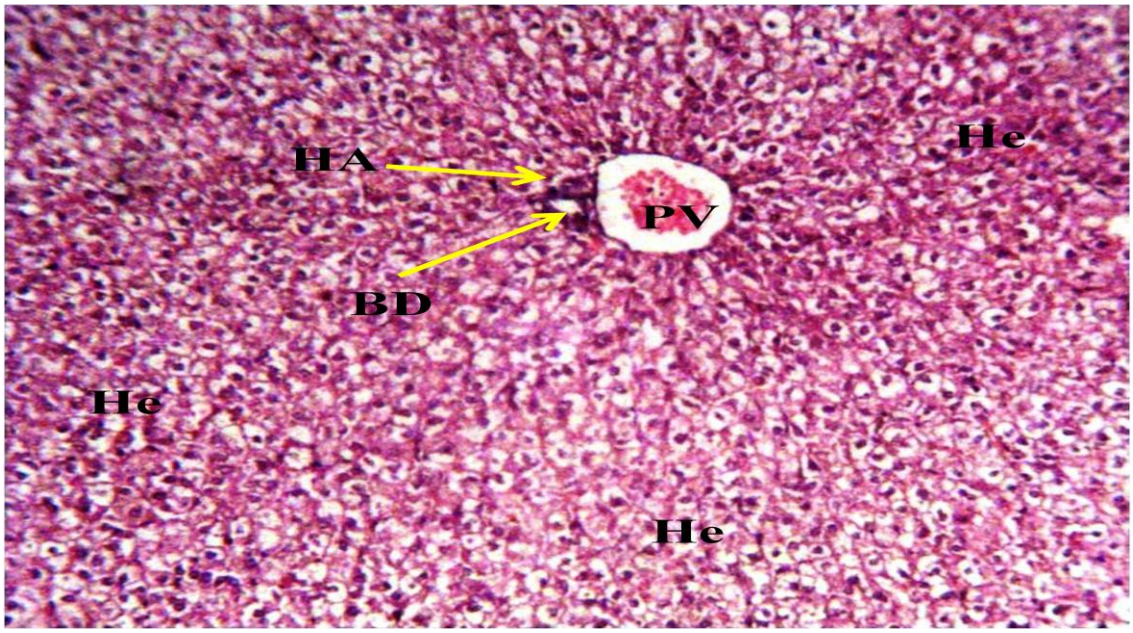


Plate XVII: A transverse section of the liver showing a hepatic portal vein (PV), hepatic artery (HA), bile duct (BD) and hepatocytes (He). H and E × 100

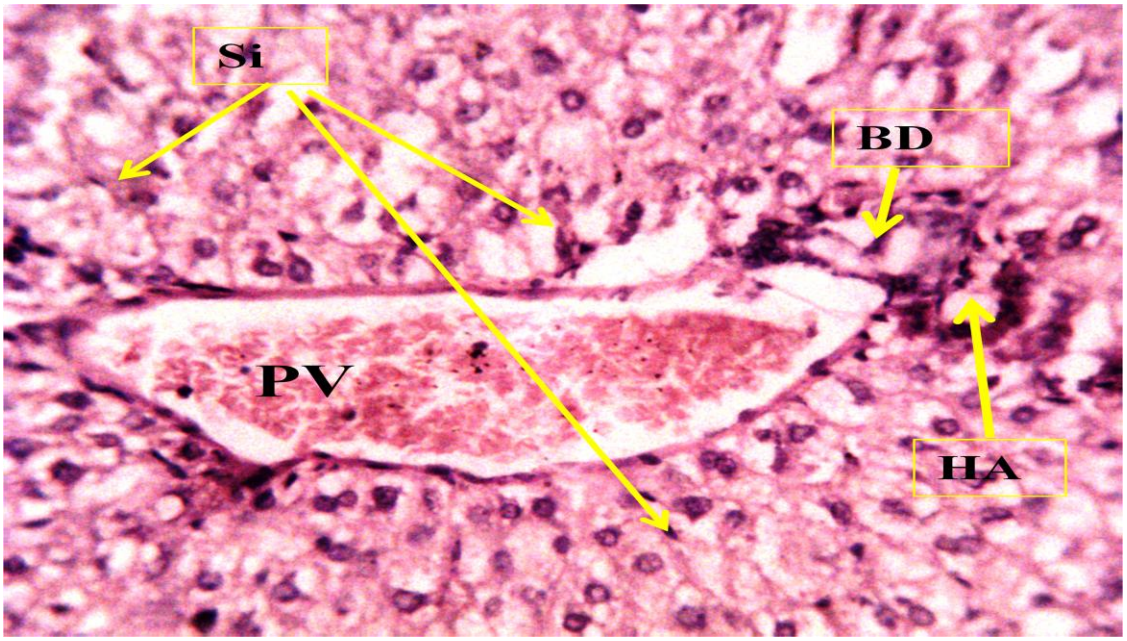


Plate XVIII: A transverse section of the liver showing Sinusoidal spaces (Si), Hepatic portal vein (PV), hepatic artery (HA), bile duct (BD). H and E × 250

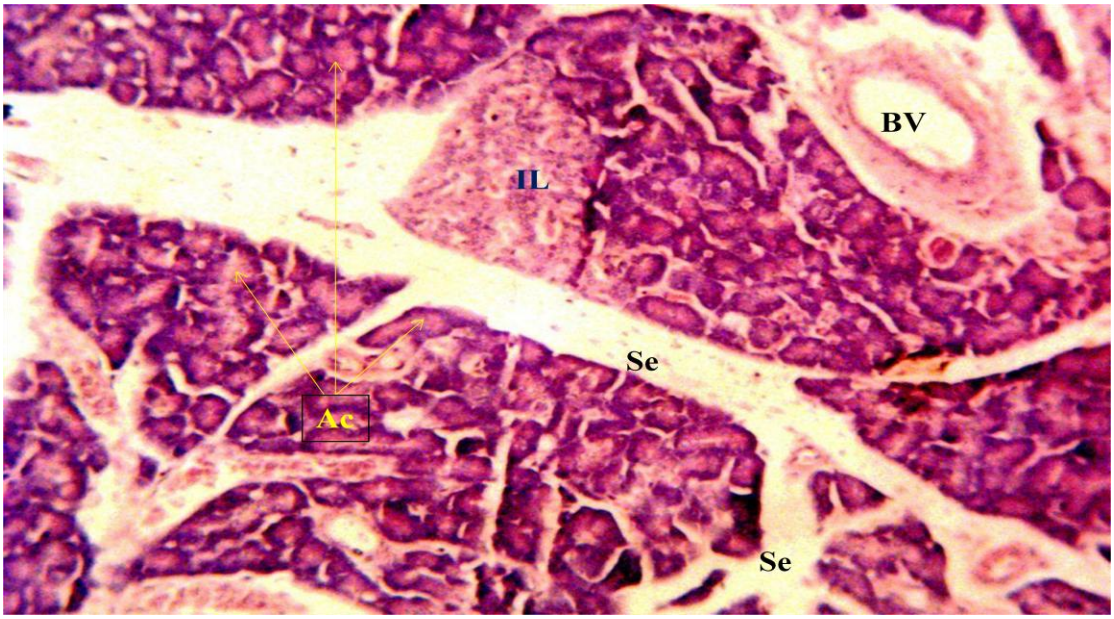


Plate XIX: A transverse section of the pancreas showing the islets of Langerhans (IL), blood vessel (BV) and secretory acini (Ac) and connective tissue septum (Se). H and E × 100

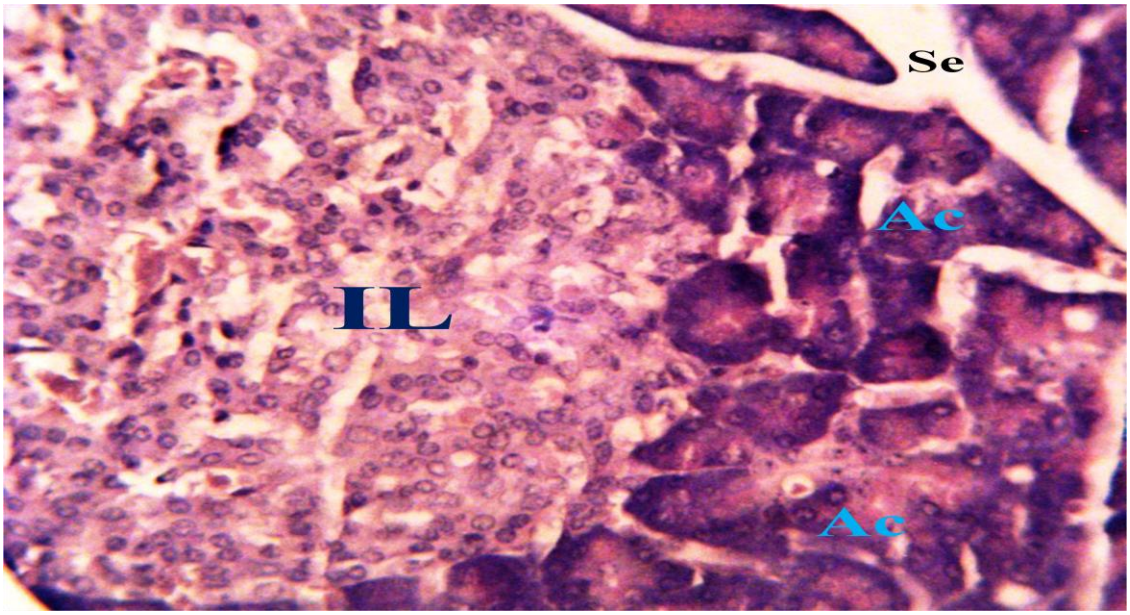


Plate XX: A transverse section of the pancreas showing the islets of Langerhans (IL), septum (Se) and secretory acini (Ac). H and E × 250

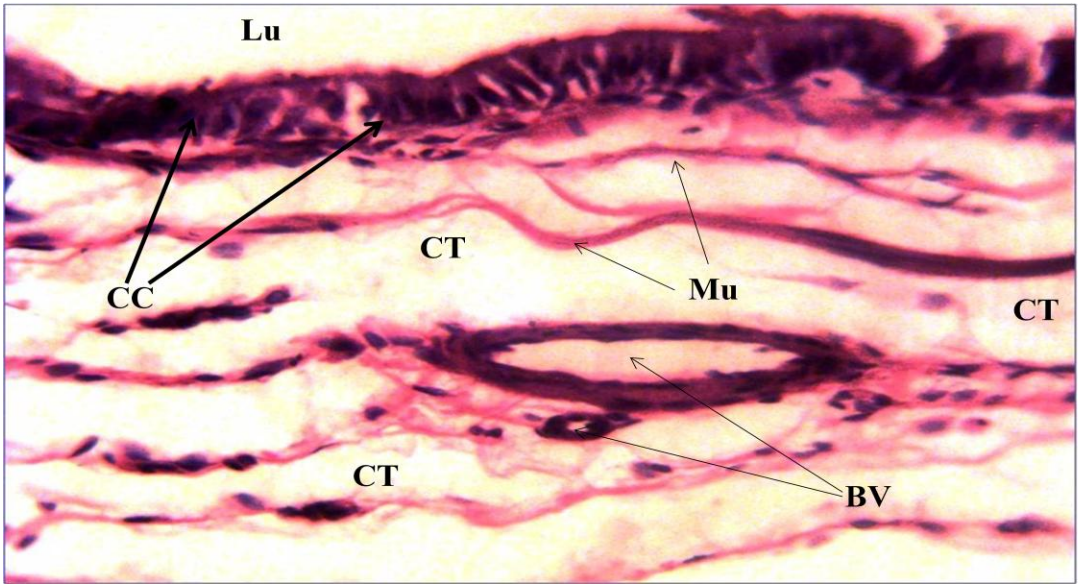


Plate XXI: A transverse section of the gall bladder showing the lumen (Lu), simple columnar cells of the epithelium (CC), muscle fibres (Mu), blood vessels (BV) and connective tissue (CT). HandE × 250

4.4 HISTOCHEMISTRY

The results for AB, PAS and AB/PAS staining technique of the stomach, small intestine, large intestine and gall bladder revealed the following:

4.4.1 Mucin Histochemistry of the Stomach

The results for the mucin histochemistry of the stomach revealed the presence of only neutral mucins in the cardiac, fundic and pyloric regions with the AB, PAS and AB-PAS techniques.

In the cardiac region of the stomach, the cardiac glands were AB negative and PAS positive, staining magenta which indicated the presence of neutral mucins while the surface mucous cells of the region were PAS positive only indicating presence of neutral mucins as shown in Plates XXII and XXIII. The AB-PAS technique confirmed the presence of neutral mucins in the cardiac region as shown in Plate XXIV.

In the fundic region of the stomach, staining revealed that the surface mucous cells lining the gastric pits to be AB negative and PAS positive that stained a deep magenta which indicated the presence of neutral mucin granules within these cells. The tubular fundic glands were negative for all the techniques. The result was the same for the AB-PAS technique which confirmed only PAS positive surface mucous cells as shown in Plates XXV, XXVI and XXVII.

In the pyloric region of the stomach, the surface mucous cells and pyloric glands were both AB negative while the surface mucous cells and pyloric glands were both PAS positive. AB-PAS technique confirmed the presence of only neutral mucins in the surface mucous cells and glands of the pyloric region as shown in Plates XXVIII, XXIX and XXX.

4.4.2 Mucin Histochemistry of the Small Intestine

Histochemical studies of the small intestine revealed the presence of both acid and neutral mucins with the AB, PAS and AB-PAS staining techniques, across the three segments (duodenum, jejunum and ileum). With AB-PAS technique, it was observed that some of the mucous secreting cells stained darker both in the surface epithelia and crypts, indicating combined acid and neutral mucins secretion in these cells.

In the duodenum, the Brunner's glands were positive for the AB and PAS techniques indicating presence of both acid and neutral mucins. Similarly, the surface mucous cells of the duodenal villi were positive for the AB and PAS techniques which indicated the presence of acid and neutral mucins. The mucin granules of the surface mucous cells were seen interspersed among the simple columnar enterocytes of the duodenal villi. The AB-PAS technique was used to confirm the presence of both acid and neutral mucins in the duodenum as shown in Plates XXXI, XXXII and XXXIII.

In the jejunum, the intestinal glands (crypts of Lieberkühn) were positive for the AB and PAS techniques, the mucus granules stained dark blue and magenta indicating the presence of both acid and neutral mucin granules respectively. Similarly, the surface mucous cells of the jejunum were AB and PAS positive, staining dark blue and magenta respectively. The mucin granules of the surface mucous cells were interspersed among the simple columnar enterocytes of the jejunal villi. The AB-PAS technique was used to confirm the presence of both acid and neutral mucins in the jejunum as shown in Plates XXXIV, XXXV and XXXVI.

In the ileum, the crypts of Lieberkühn were positive for the AB and PAS techniques, the mucus secreting cells stained dark blue and magenta indicating presence of both acid and

neutral mucins granules respectively. The surface mucous cells of the villi were positive for both AB and PAS, staining dark blue and magenta indicating presence of both acid and neutral mucins granules respectively. Like in the duodenum and jejunum, mucin granules of the surface mucous cells were interspersed among the simple columnar enterocytes of the ileal villi. The AB-PAS technique was used to confirm the presence of both acid and neutral mucins in the ileum as shown in Plates XXXVII, XXXVIII and XXXIX.

4.4.3 Mucin Histochemistry of the Large Intestine

The results for the histochemical studies of the large intestine revealed the presence of both acid and neutral mucins with the AB and PAS staining techniques, across the three segments (caecum, colon and rectum). With AB-PAS technique, it was observed that some of the mucous secreting cells stained darker both in the surface epithelia and crypts, indicating combined acid and neutral mucins secretion in these cells.

The epithelium of the caecum was positive for AB technique which revealed very few acid mucin granules stained as dark blue. The crypts of the caecum also showed little acid mucin secretion. The PAS technique was negative for both the surface mucous cells and the crypts of the caecum. The AB-PAS technique was used to confirm the presence of both acid and neutral mucins in the caecum but only acid mucins were present as shown in Plates XL, XLI and XLII.

The colonic epithelium was AB and PAS positive with the surface mucous cells staining dark blue and magenta respectively indicating the presence of acid and neutral mucins. The tubular colonic crypts were positive for both acid and neutral mucins. The acid mucin droplets were considerably more than the neutral mucins droplets in this region of the large

intestine. General mucin secretion was by far more than what was observed in the caecum. The AB-PAS technique was used to confirm the presence of both acid and neutral mucins in the colon as shown in Plates XLIII, XLIV and XLV.

For the rectum, the surface mucous cells were AB and PAS positive, staining dark blue and magenta respectively. The longer crypts of the rectum were also positive for acid and neutral mucins with the acidic mucins observed to be dominant over the neutral mucins. The AB-PAS technique was used to confirm the presence of both acid and neutral mucins in the rectum as shown in Plates XLVI, XLVII and XLVIII.

4.4.4 Histochemical Studies of the Liver

Histochemical studies to demonstrate the presence of glycogen in the liver using PAS and PAD staining techniques revealed a PAS positive result with hepatocytes staining magenta suggesting the presence of glycogen granules within their cytoplasm. In the PASD technique, the PAS positive result when treated with diastase showed numerous clear cytoplasms with the nuclei visible in the clear background, indicating a possible depletion of sugar stores in the liver as shown in Plates XLIX and L.

4.4.5 Mucin Histochemistry of the Gall Bladder

Histochemical studies on the gall bladder using AB, PAS and AB-PAS staining techniques were all negative, suggesting absence of both acidic and neutral mucins as shown in Plate LI.

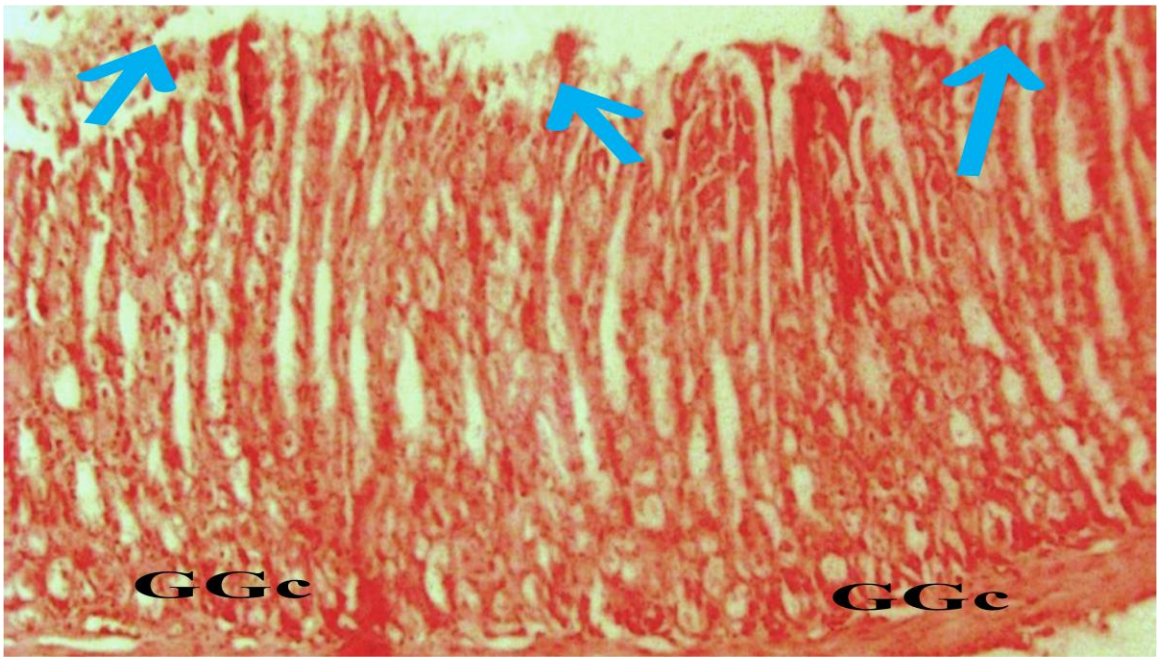


Plate XXII: A transverse section through cardiac region of the stomach showing AB negative epithelial cells (blue arrows) and cardiac glands (GGc). AB × 100

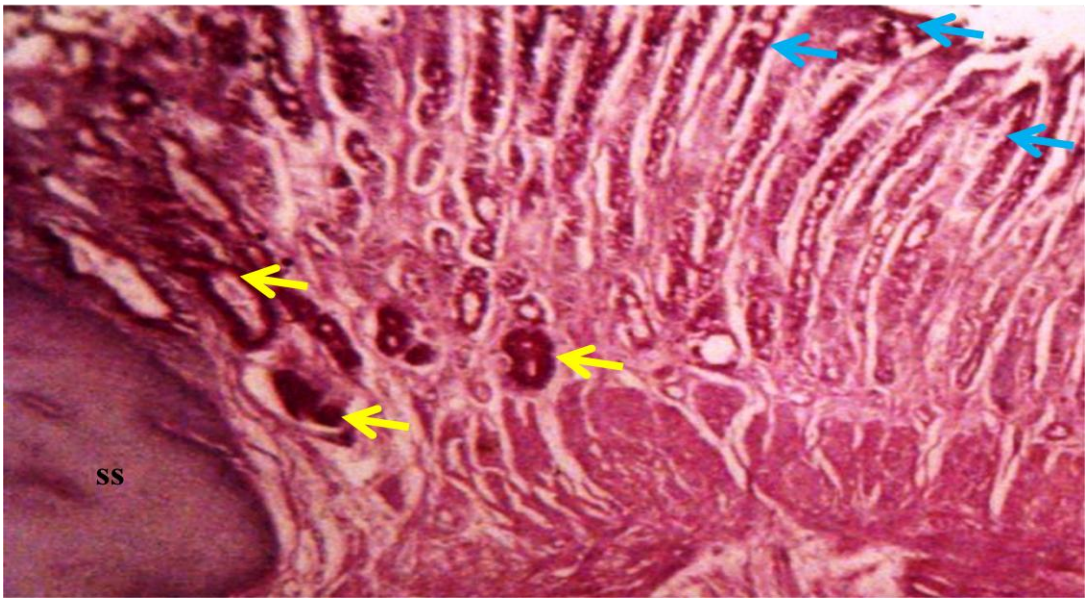


Plate XXIII: A transverse section through cardiac region of the stomach (gastro-esophageal junction) showing PAS positive surface epithelial cells (blue arrows), cardiac glands (yellow arrows) and stratified squamous epithelium of the Oesophagus (SS). PAS × 100

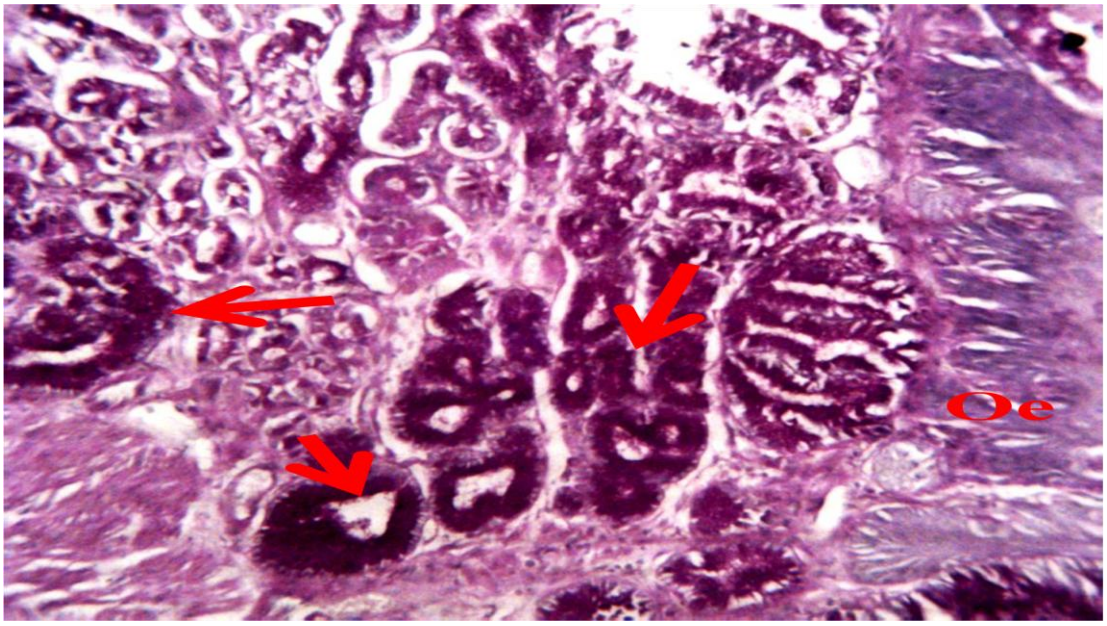


Plate XXIV: A transverse section through the cardiac region of the stomach showing PAS positive cardiac glands (red pointers) at the gastro-oesophageal junction and oesophagus (Oe). AB-PAS × 100

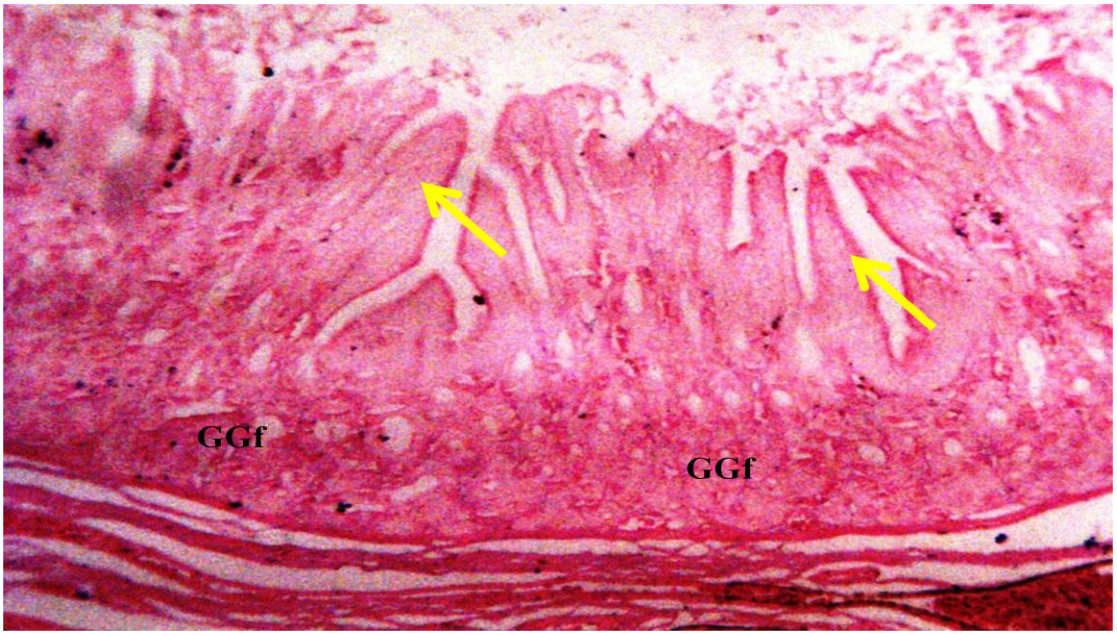


Plate XXV: A transverse section through the fundic region of the stomach showing AB negative surface mucous cells (arrows) and fundic glands (GGf). AB x100

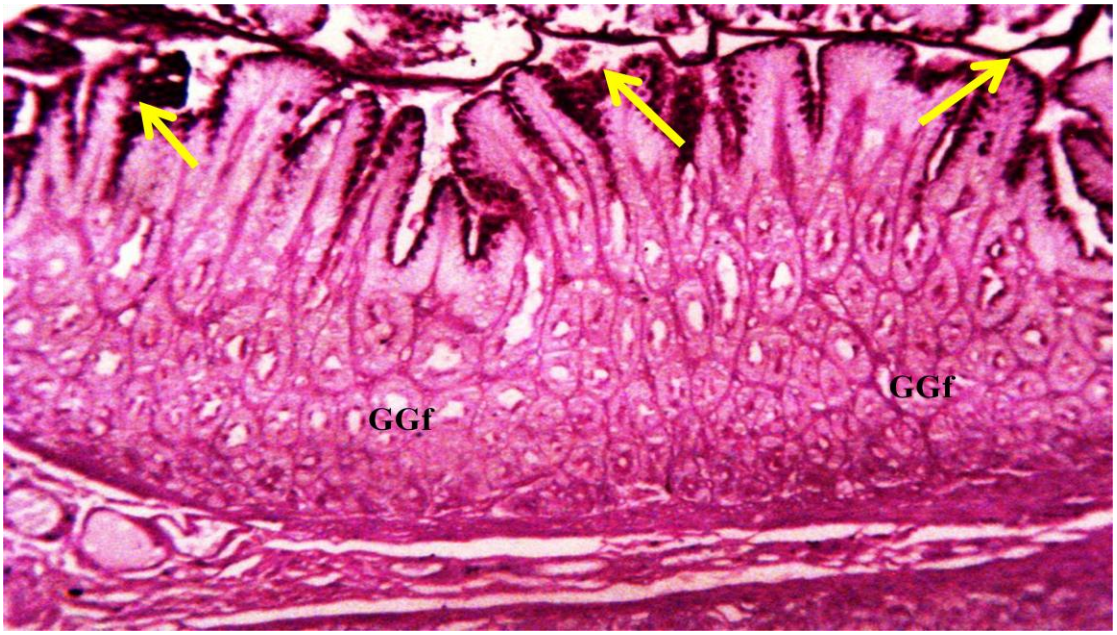


Plate XXVI: A transverse section through the fundic region of the stomach showing PAS positive surface mucous cells (arrows) and PAS negative fundic glands (GGf). PAS × 100

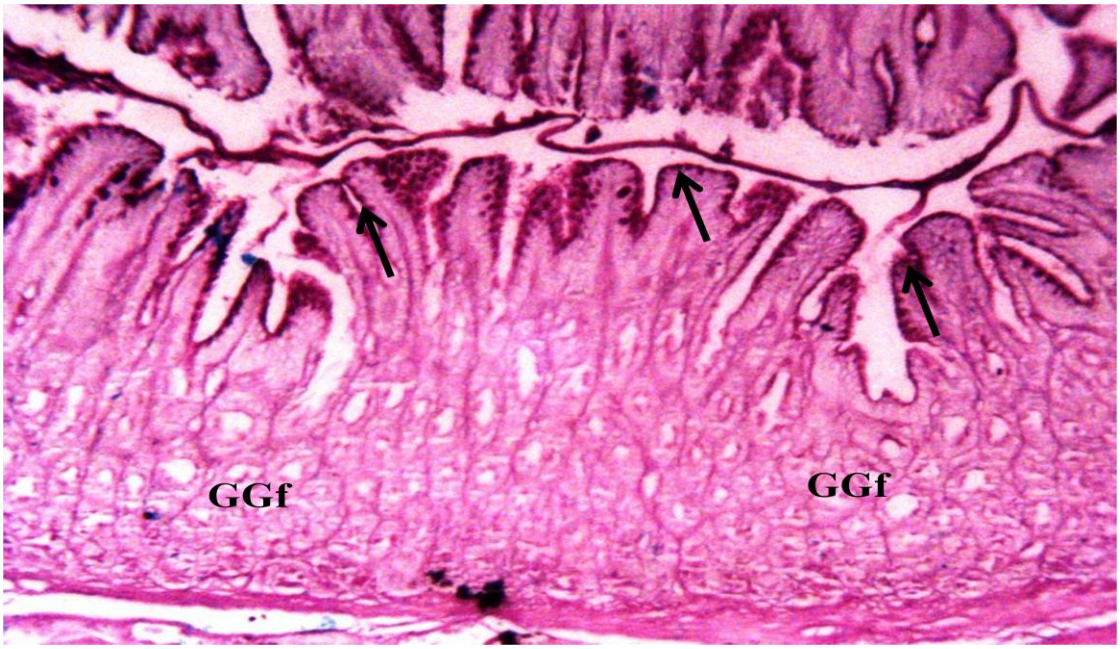


Plate XXVII: A transverse section through the fundic region of the stomach showing PAS positive surface mucous cells (arrows), AB and PAS negative fundic glands (GGf). AB-PAS × 100

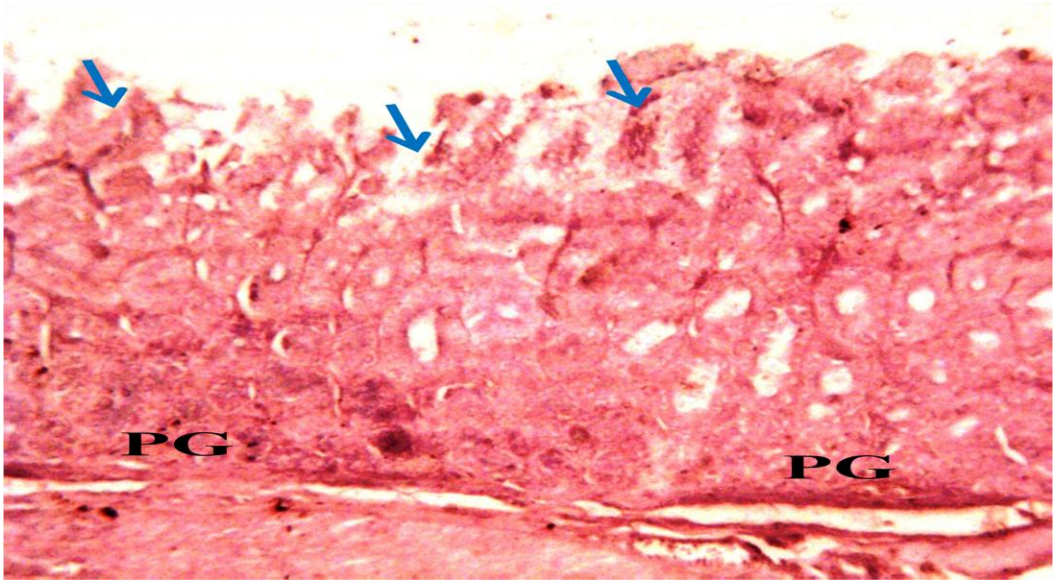


Plate XXVIII: A transverse section through the pyloric region of the stomach showing AB negative surface epithelial cells (arrows) and pyloric glands (PG). AB x100

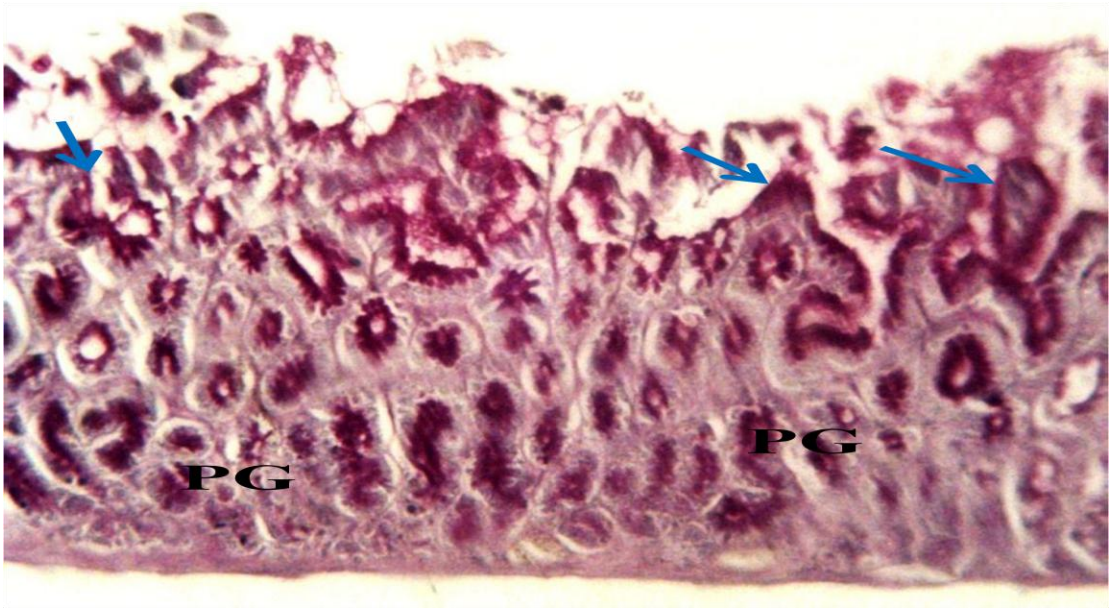


Plate XXIX: A transverse section through the pyloric region of the stomach showing PAS positive surface mucous cells (arrows) and pyloric glands (PG). PAS × 100

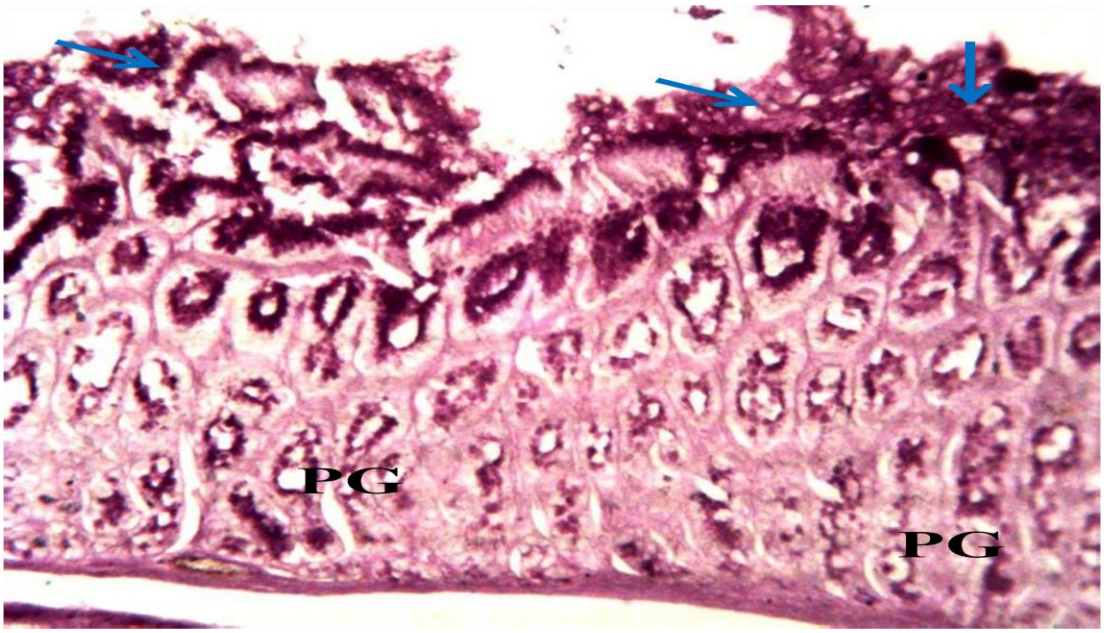


Plate XXX: A transverse section through the pyloric region of the stomach showing PAS positive surface mucous cells with mucous blanket covering the epithelial surface (arrows) and pyloric glands (PG). AB-PAS × 100

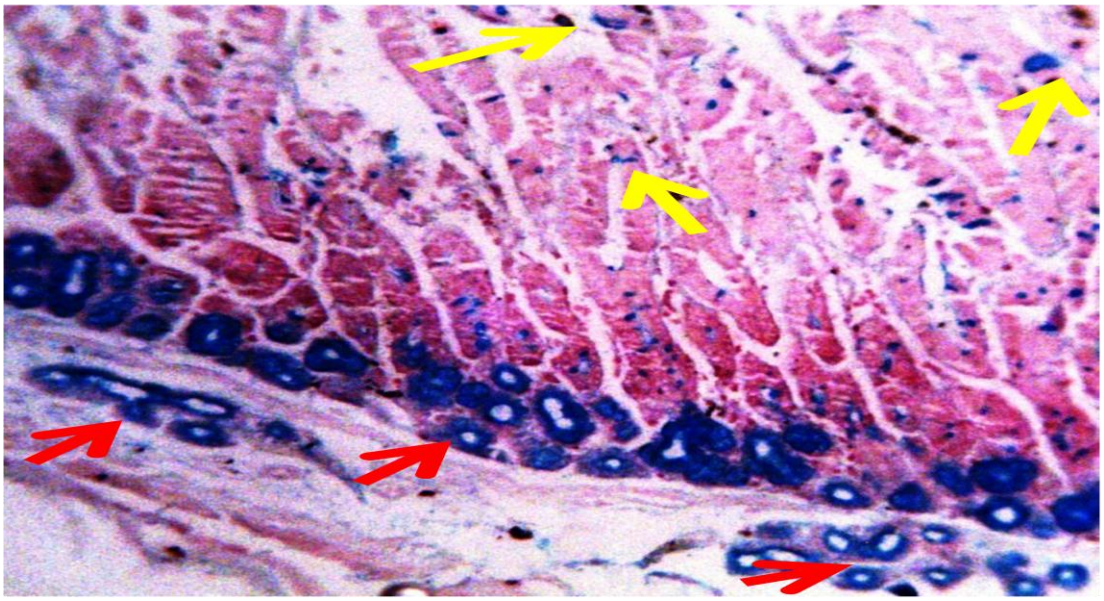


Plate XXXI: A transverse section of the duodenum showing AB positive surface mucous cells of the duodenal villi (yellow arrows) and Brunner's glands (red arrows). AB x100

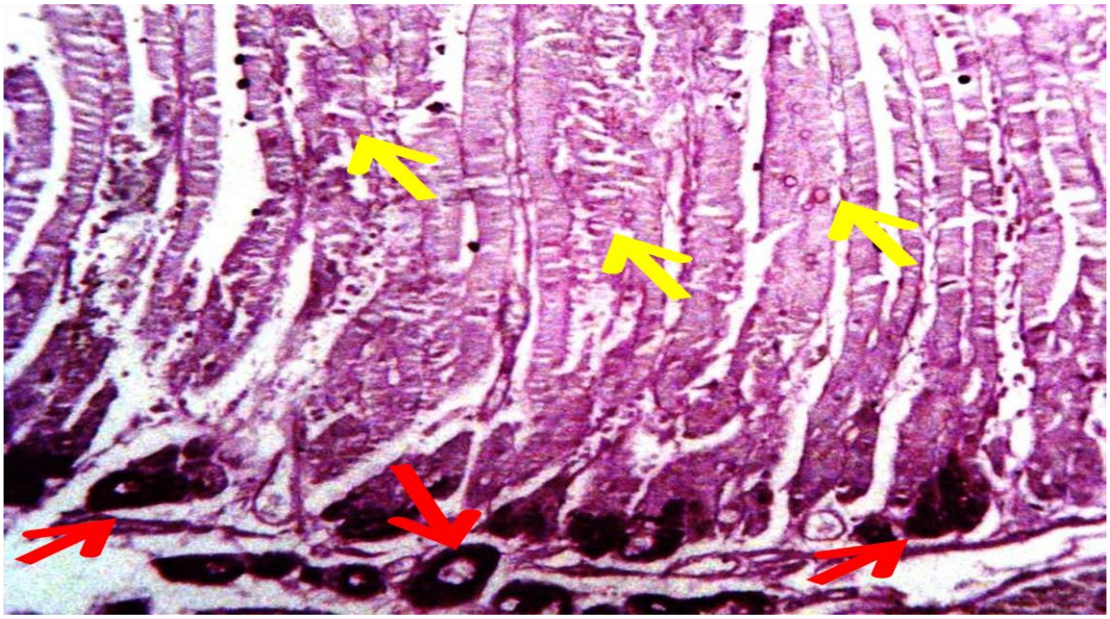


Plate XXXII: A transverse section of the duodenum showing PAS positive surface mucous cells of the duodenal villi (yellow arrows) and Brunner's glands (red arrows). PAS × 100

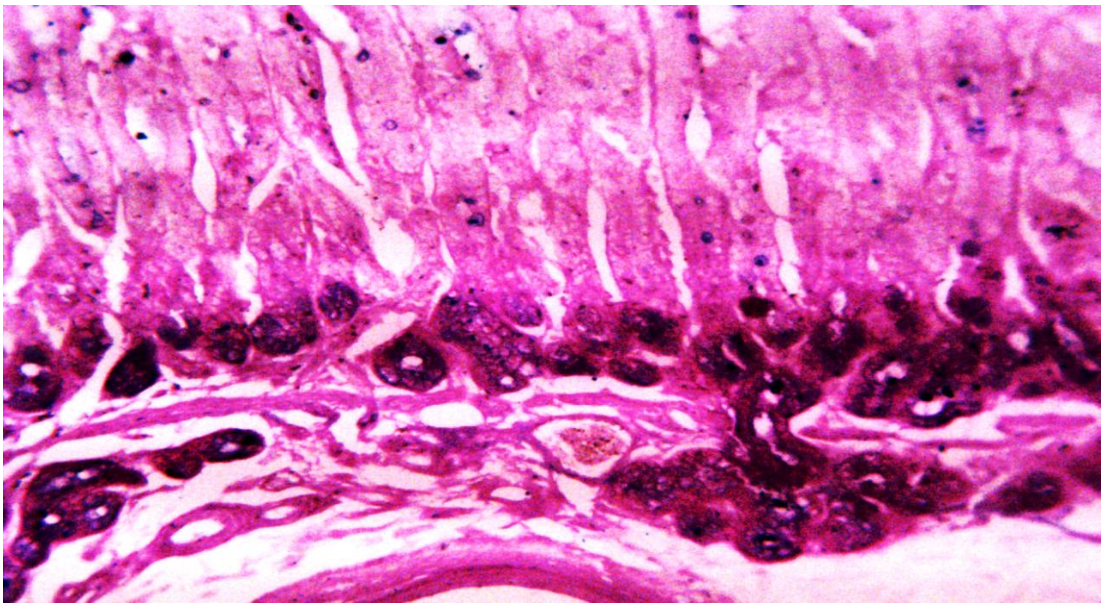


Plate XXXIII: A transverse section through the duodenum showing both AB positive (Blue droplets) and PAS positive (magenta droplets) surface mucous cells of the duodenal villi and Brunner's glands. AB-PAS × 100

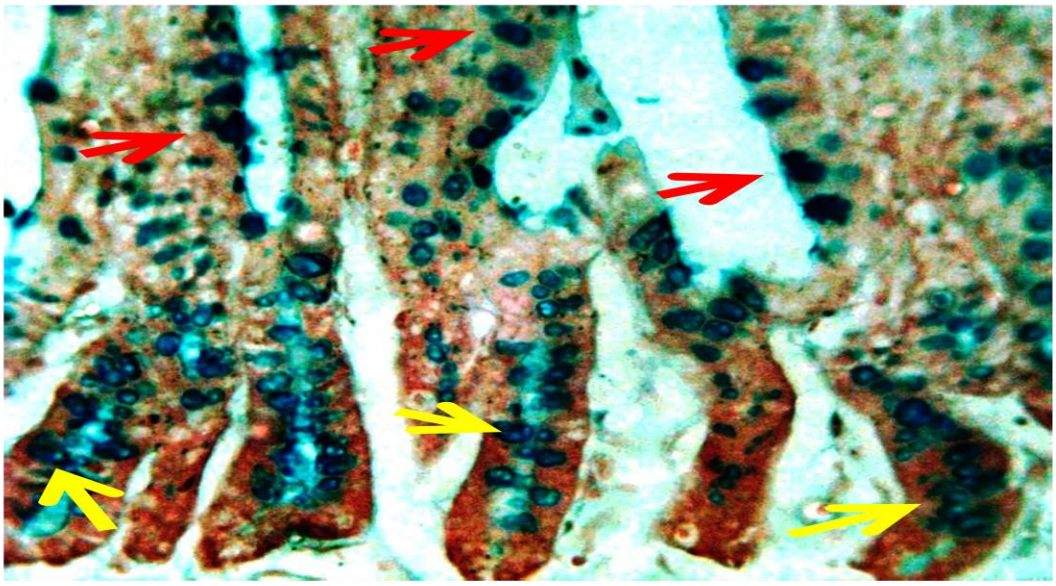


Plate XXXIV: A transverse section through the jejunum showing AB positive surface mucous cells of the jejunal villi (red arrows) and crypts of Lieberkühn (yellow arrows). AB × 250

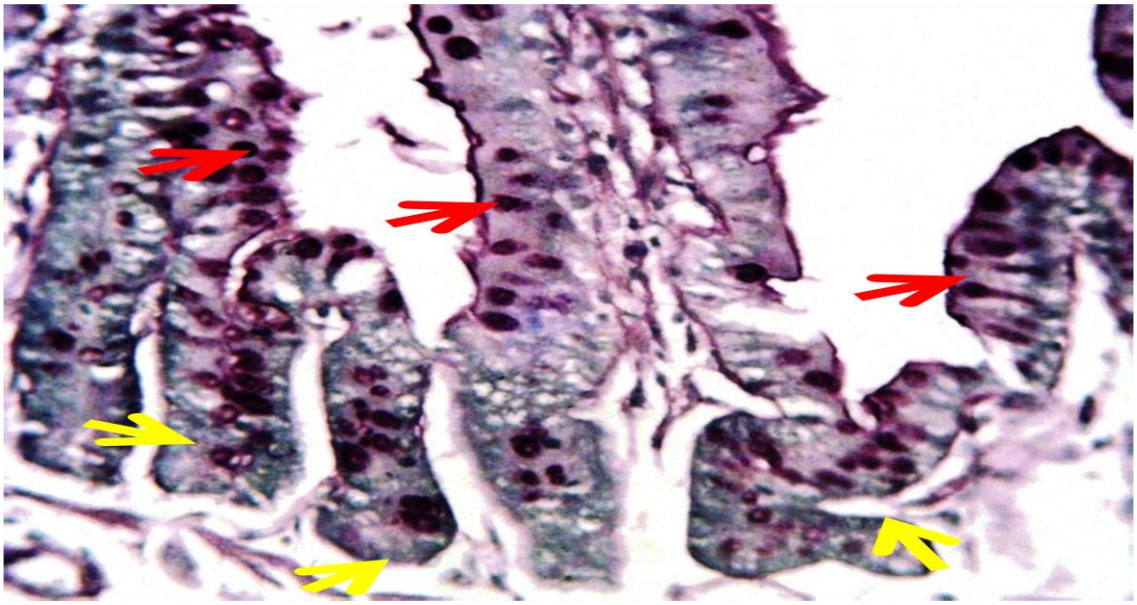


Plate XXXV: A transverse section through the jejunum showing PAS positive surface mucous cells of the jejunal villi (red arrows) and crypts of Lieberkühn (yellow arrows). PAS × 250

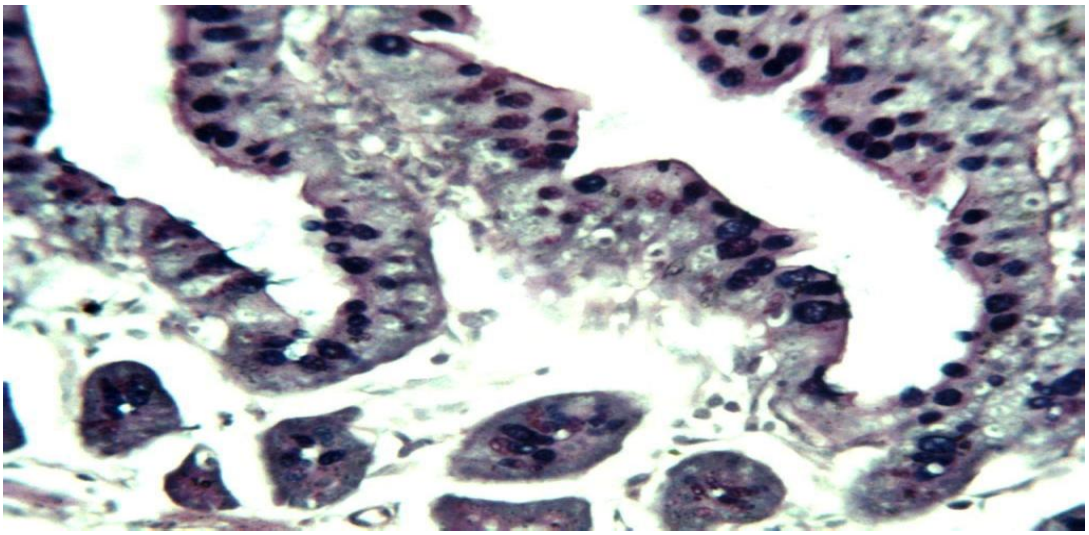


Plate XXXVI: A transverse section through the jejunum showing both AB positive (Blue droplets) and PAS positive (magenta droplets) surface mucous cells of the jejunal villi and intestinal crypts. AB-PAS × 250

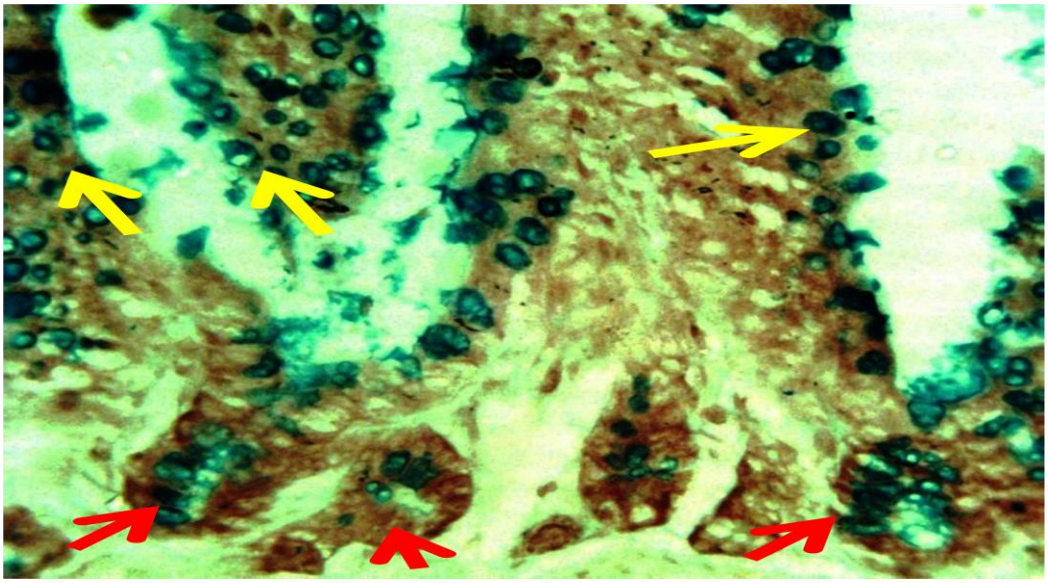


Plate XXXVII: A transverse section of the ileum showing AB positive surface mucous cells of the villi (yellow arrows) and crypts of Lieberkühn (red arrows). AB × 250

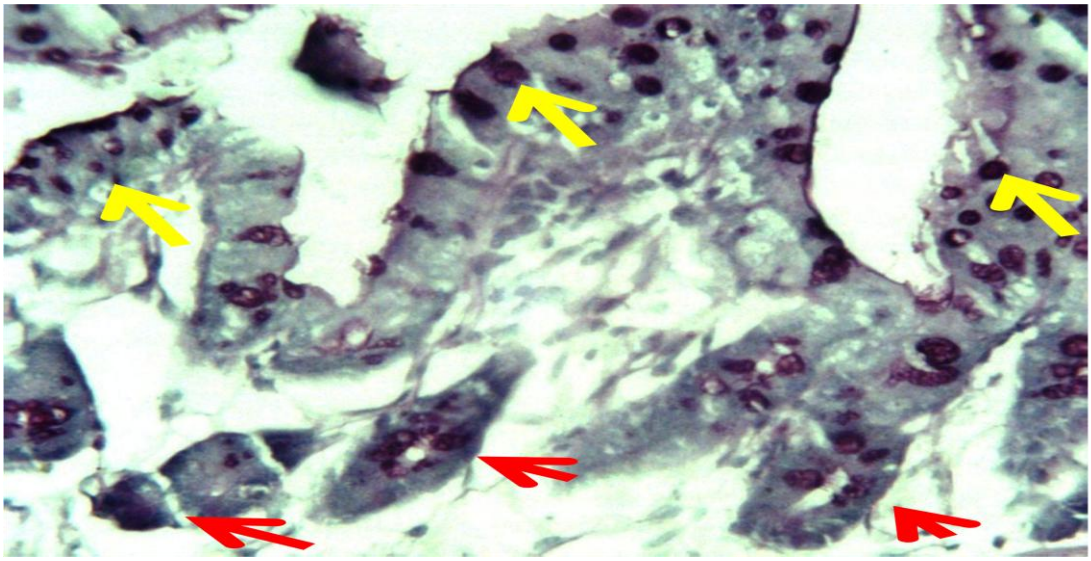


Plate XXXVIII: A transverse section of the ileum showing PAS positive surface mucous cells of the ileal villi (yellow arrows) and crypts of Lieberkühn (red arrows). PAS × 250

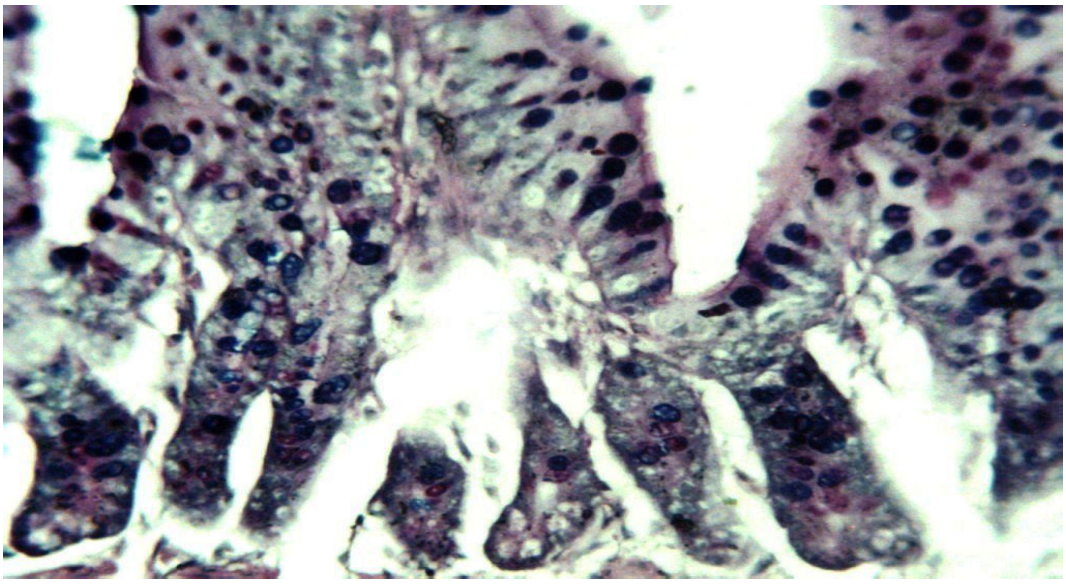


Plate XXXIX: A transverse section through the ileum showing AB positive (blue droplets) and PAS positive (magenta droplets) of the surface mucous cells of the ileal villi and crypts of Lieberkühn. AB-PAS × 250

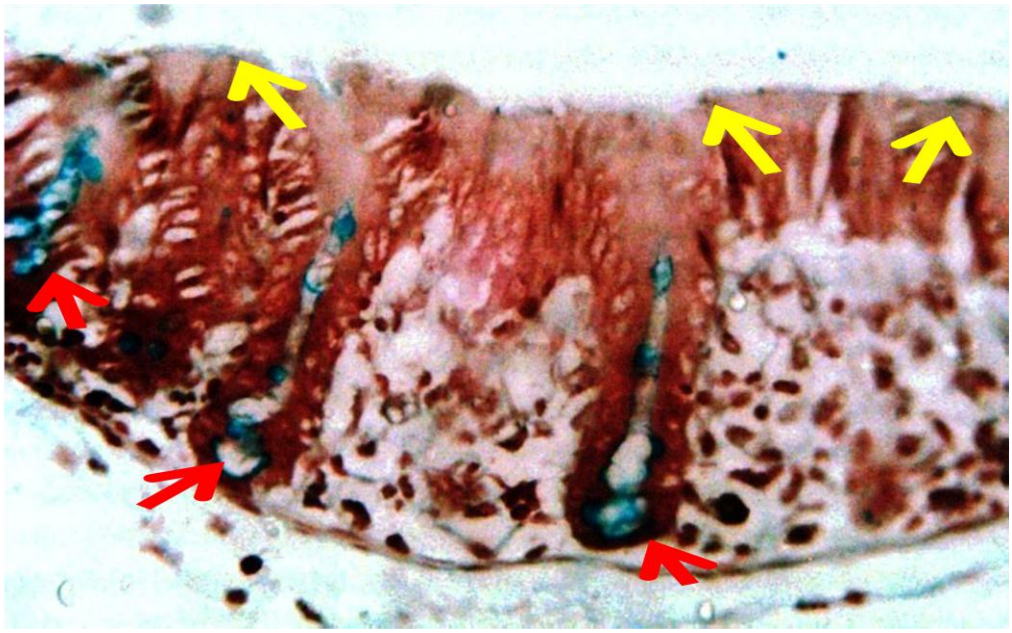


Plate XL: A transverse section of the caecum showing few AB positive surface mucous cells (yellow arrows) and crypts (red arrows). AB × 250

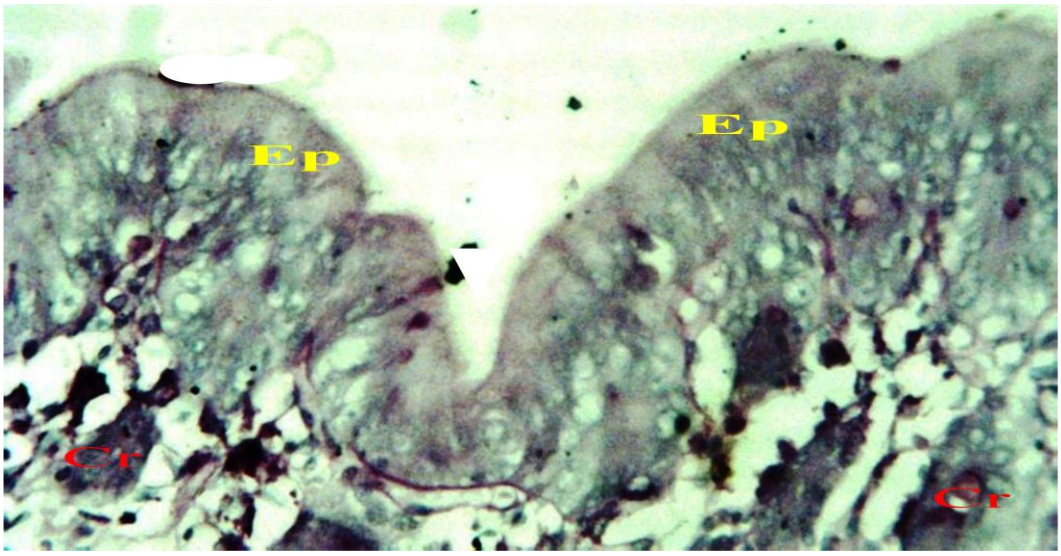


Plate XLI: A transverse section of the caecum showing PAS negative surface epithelial cells (Ep) and crypts (Cr). PAS × 250



Plate XLII: A transverse section of the caecum showing few AB positive crypts (blue droplets). AB-PAS × 250

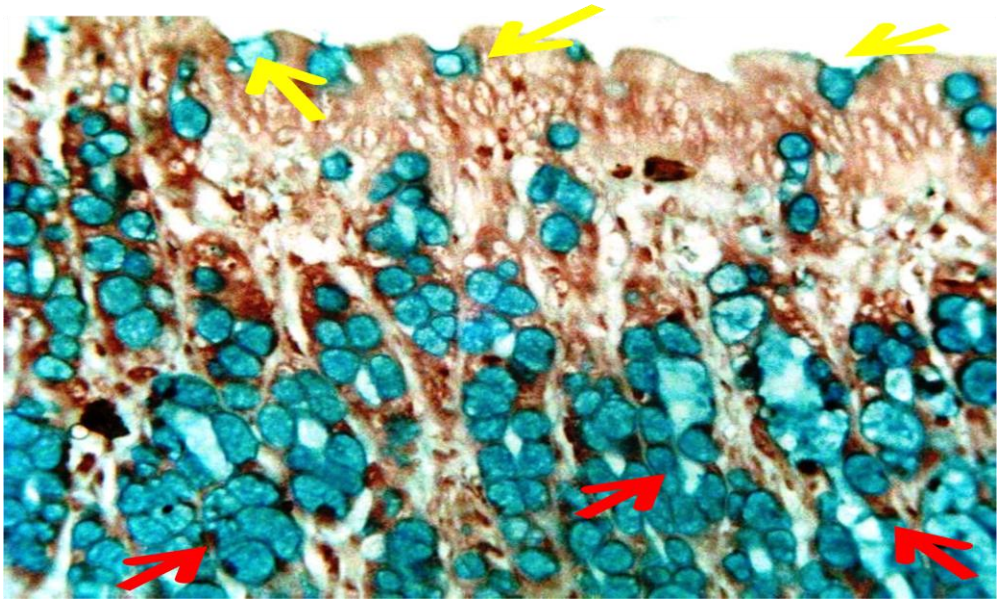


Plate XLIII: A transverse section of the colon showing AB positive surface mucous cells (yellow arrows) and colonic crypts (red arrows). AB × 250

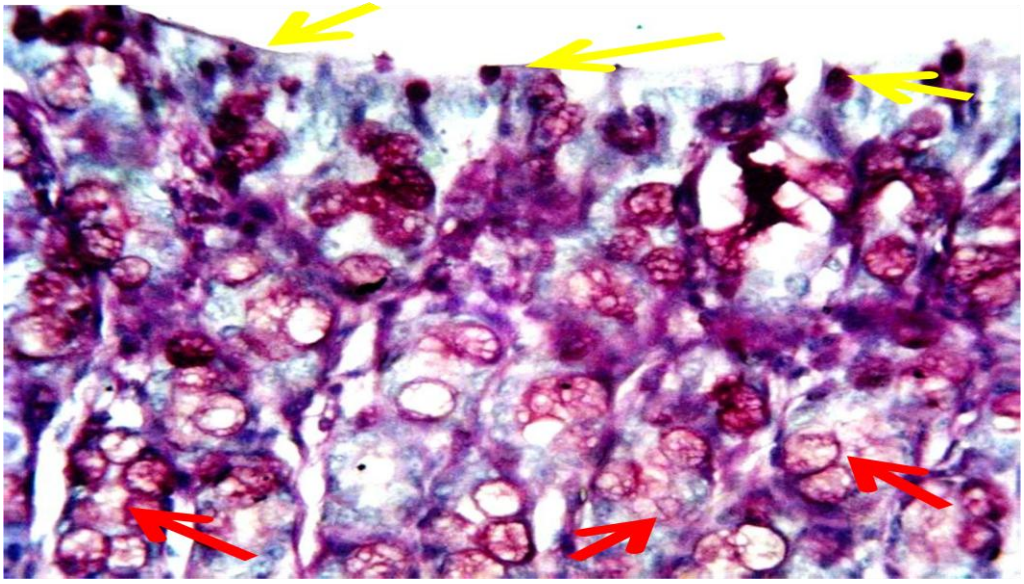


Plate XLIV: A transverse section through the colon showing PAS positive surface mucous cells (yellow arrows) and colonic crypts (red arrows). PAS × 250

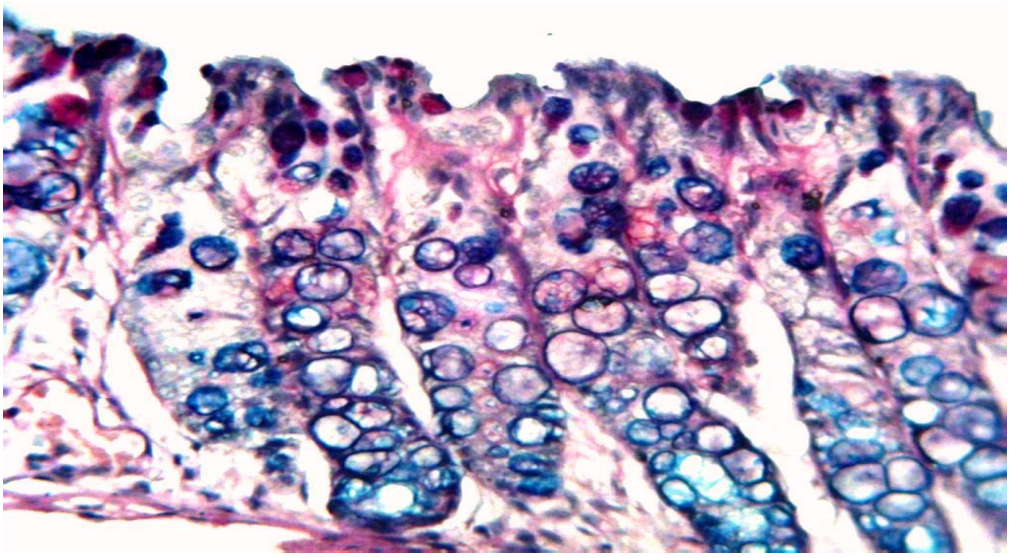


Plate XLV: A transverse section through the colon showing both AB positive (blue droplets) and PAS positive (magenta droplets) surface mucous cells and colonic crypts. AB-PAS × 250

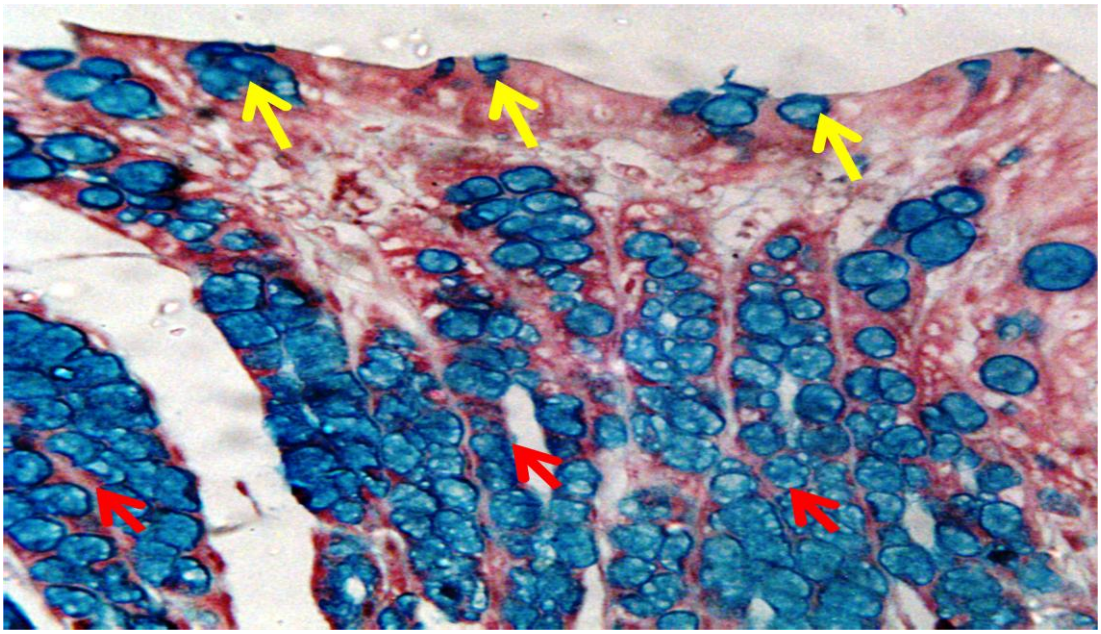


Plate XLVI: A transverse section of the rectum showing AB positive surface mucous cells (yellow arrows) and crypts (red arrows). AB × 250

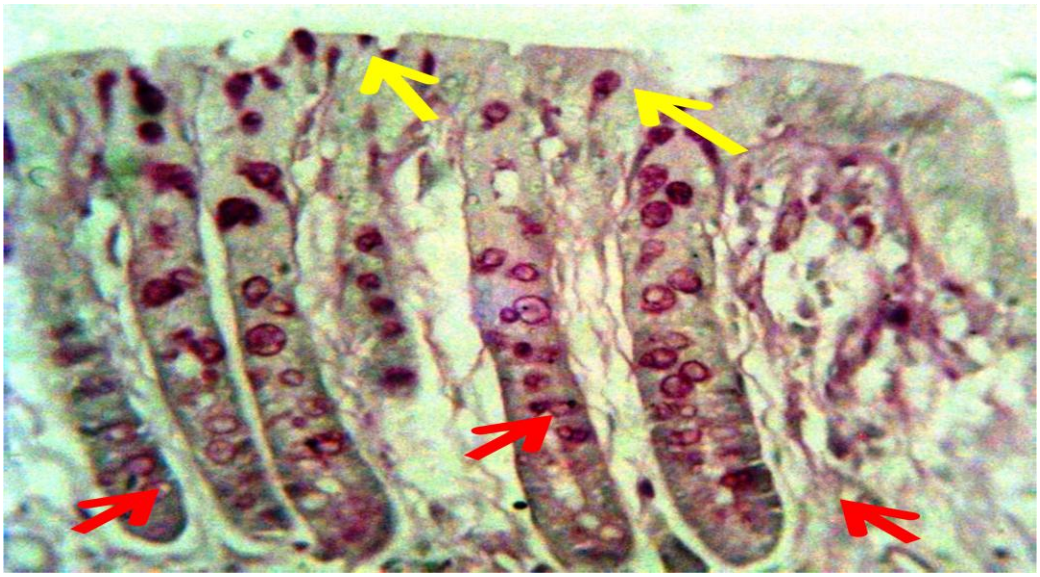


Plate XLVII: A transverse section of the rectum showing PAS positive surface mucous cells (yellow arrows) and crypts (red arrows). PAS × 250

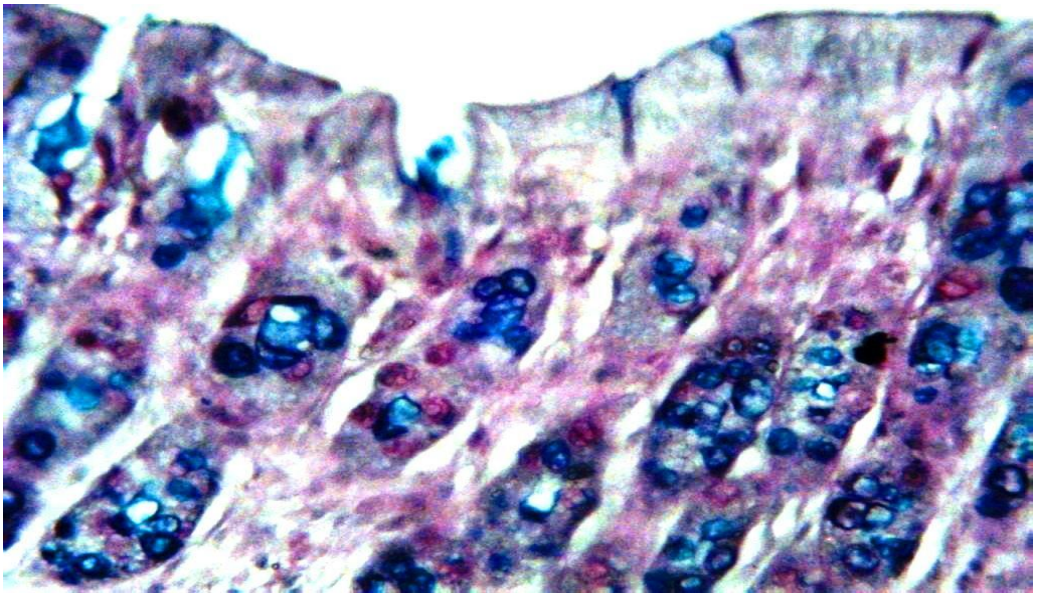


Plate XLVIII: A transverse section through the rectum showing both AB positive (blue droplets) and PAS positive (magenta droplets) surface mucous cells and rectal crypts. AB-PAS × 250

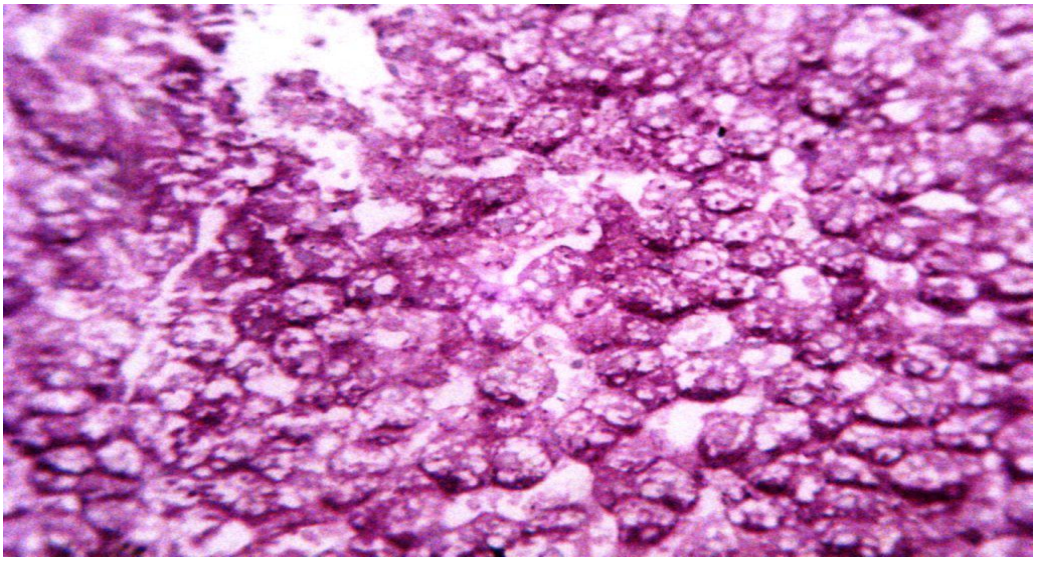


Plate XLIX: A transverse section of the liver showing numerous glycogen laden hepatocytes. PAS × 250

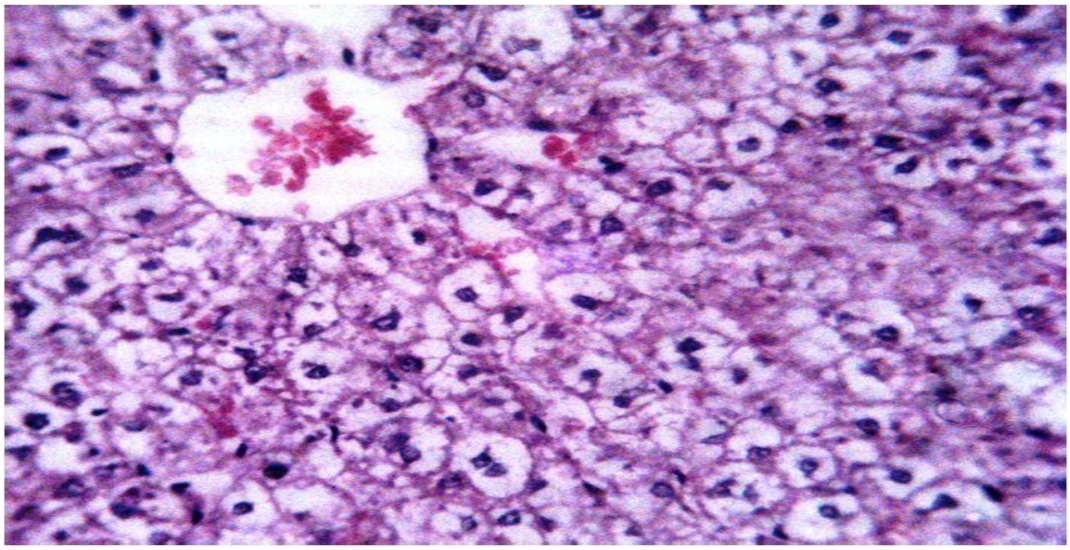


Plate L: A transverse section of the liver showing numerous clear cytoplasms of hepatocytes, suggesting a possible digestion of glycogen stores in those cells after diastase treatment. PASD x 250

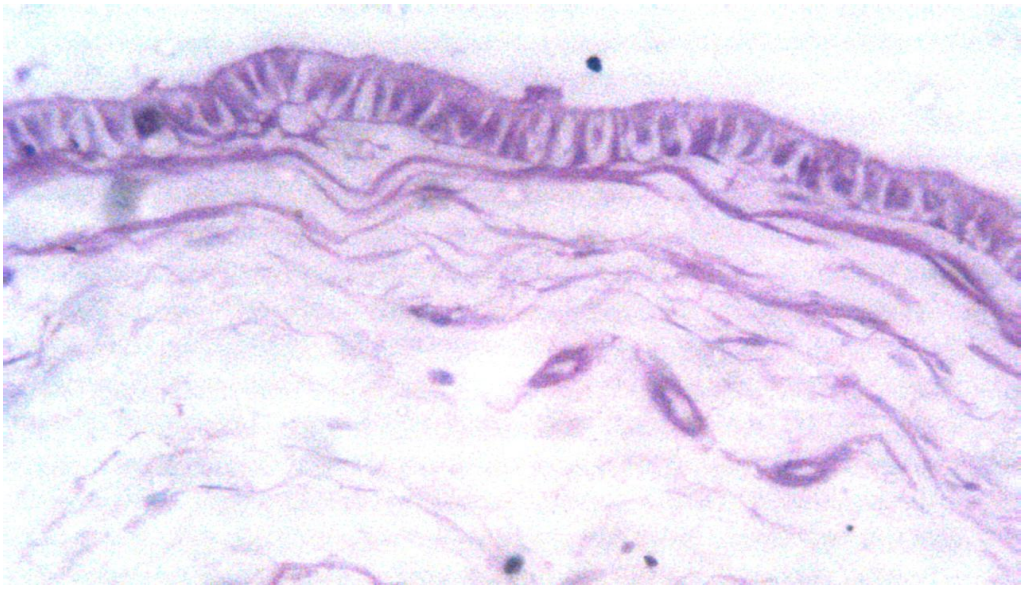


Plate LI: A transverse section of the gall bladder showing a negative result for both AB and PAS. AB-PAS × 250

CHAPTER FIVE

DISCUSSION

5.1. MORPHOLOGY AND MORPHOMETRY

In the present study, the mean weight of the male Grasscutter was found to be relatively but not significantly higher than that of the female Grasscutter, this agrees with the findings of Byanet *et al.* (2008). The mean length of the GIT in the present study for both male and female was observed to be lower than that reported by Byanet *et al.* (2008), but higher than that reported for the AGR by both Ali *et al.* (2008) and Nzalak *et al.* (2010). The stomach length in the present study was 10.35 ± 0.62 cm and 10.00 ± 0.10 cm in males and females respectively. Grossly, the stomach of the Grasscutter was seen to be divided into three regions; the cardia, the fundus and the pylorus which agrees with the findings of Byanet *et al.*, (2008), it also agrees with the findings of O'Malley (2005) who reported that the stomach of Rabbit had three distinct regions (cardia, fundus, pylorus). O'Malley (2005) also reported a J-shaped stomach in Rabbits which was in contrast with the inverted J-shaped stomach of the Grasscutter. The fundic region of the stomach in this study was thrown into prominent longitudinal folds (rugae). Eman and Haider (2012) had observed these rugae in the fundic region of the Rabbit stomach. These rugae help to increase the volume of the stomach. In the present study, it was observed that the small intestine of the Grasscutter was made up of the duodenum, jejunum and ileum which agree with the findings of Byanet *et al.* (2008) and Boonzaier (2012). Bob *et al.* (2012) reported a similar arrangement in Rabbits while Ali *et al.* (2008) and Nzalak (2010) had reported the same arrangement in AGR. The mean length of the small intestine in this study for both male and female Grasscutter was lower than that

reported by Byanet *et al.* (2008). In the large intestine, the caecum, colon and rectum were observed with the caecum being larger than any other component part of the GIT. The caecum of the Grasscutter like other monogastric herbivores serves as the site for microbial fermentation (Grant, 2010). It occupied most of the abdominal cavity, which was consistent with reports by Nzalak (2010) and Stan *et al.* (2014) in AGR and Rabbit respectively.

5.2. HISTOLOGY

Histologically, the stomach epithelium was lined by simple columnar cells. In the three regions of the stomach, glands were observed with specialized cells for the secretion of mucus and gastric juices for digestion. The parietal and chief cells located in the pyloric region play a huge part in chemical digestion. However, their location in this study was contrary to their location in the fundic region as reported by Byanet *et al.* (2011). The tunica muscularis was thick and had three layers; an oblique, a circular and longitudinal layer which played an important role in mechanical digestion. This agrees with the findings of Nzalak *et al.*, (2010), since the Grasscutter feeds more on plant materials, chemical digestion is of great necessity in order to breakdown the plant material.

The small intestine segments had tunics modified and differentiated to perform relevant functions. One notable difference was the presence of Brunners glands within the submucosa of the duodenum only, which agrees with the findings of Byanet *et al.* (2011). The villi of the small intestine in this study presented leaf-like, finger-like or ridge-like projections lined by simple columnar enterocytes and goblet cells, which is in line with the findings of Nzalak (2010), Byanet *et al.* (2011) and Boonzaier (2012). These projections increase the surface area for absorption of nutrients in the small intestine.

In the large intestine, the ceacum was seen to have fewer goblet cells in relation to the colon and rectum which agrees with the findings of Nzalak (2010) and Boonzaier (2012) contrary to the numerous goblet cells observed by Byanet *et al.* (2011). This decrease in number may be influenced by microflora present in the caecum (Sharma *et al.,* 1995). Nzalak (2010), Byanet *et al.* (2011) and Boonzaier (2012) all reported that the colon of rodents that they studied had simple columnar epithelium with microvilli, which performed the function of absorption. The result of the present study is no different as the colon was seen to have fecal balls along its entire length. The mucous secreting goblet cells of the region were numerous. There was noticeable thickening of the muscularis propria of the large intestine which may be related to the need for temporary storage and expulsion of digesta from the cecum and propulsion in the colon and rectum (Nzalak, 2010; Byanet *et al.,* 2011).

The liver was seen to be made of large polyhedral hepatocytes with central nucleus. The arrangement was more of a diffuse than radial pattern, although some areas showed a radial pattern of arrangement. Raskovic *et al.* (2011) and Ikpegbu *et al.* (2012) reported a similar arrangement in some teleosts. This may help in the rate of liver functions especially that of detoxification as more cells will be reached faster. The hepatocyte arrangement differed from the mammalian type, lacking discrete lobules. However, hepatic portal tracts were visible.

Nzalak (2010) had reported the pancreas of the AGR to be diffuse with part of it lying in the U-shaped bend of the duodenum, this correlates with what was observed in the present study. Histological study on the pancreas revealed both endocrine and exocrine tissues as lightly stained and darkly stained areas respectively. This agrees with the findings of Sheibani and Yali. (2006) and Ikpegbu *et al.* (2012) which revealed lightly stained eosinophilic Islets of Langerhans and darker basophilic serous acini, containing zymogen granules. The pancreas

was highly vascularised, which could play apart in quick and easy exchange of substances with the circulatory system.

The gall bladder in this study was located on the visceral surface of the liver. Its presence may suggest the need to regulate the emulsification of fats. The gall bladder was seen to be made up of tall columnar cells with microvilli which may suggest absorptive functions from the stored bile according to the findings of Ikpegbu *et al.* (2012).

5.3 HISTOCHEMISTRY

The mucin histochemical study of the stomach revealed that the cardiac and pyloric glands were both PAS positive with the fundic glands negative for both AB and PAS techniques. However, the surface mucous cells of the cardia, fundus and pylorus were seen to be PAS positive, indicating the presence of neutral mucins which serves to regulate the pH in the stomach and toxicity of substances (Stanforth, 2004; Nikumbh, 2012).

Histochemical study of the small intestine showed that all three segments were AB, PAS and AB-PAS positive. In the Brunner's glands of the duodenum, AB and PAS positive results were recorded indicating the presence of both acidic and neutral mucins respectively. This is in agreement with works done by Ndou (2007), Nzalak *et al.* (2010) and Boonzaier (2012) in mole rats, AGR and *Acomys spinosissimus* respectively. Both acid and neutral mucins increase the viscosity of the mucous gel and protect the epithelial surface (Bansil and Turner, 2006). The acid and neutral mucins granules observed in the Brunner's glands may facilitate the protection against bacteria as suggested by Cao and Wang (2009). Neutral and acid mucin secreting goblet calls were seen in both the villi and crypts with a few of the cells secreting both acid and neutral mucins (mixed mucins). The Acid mucin secretory cells in the jejunum

and ileum appeared to dominate the neutral mucins secreting cells which agree with the findings of Boonzaier (2012).

Histochemical studies of the large intestine revealed the presence of both acid and neutral mucins with the AB, PAS and AB-PAS staining techniques, across the three segments. These findings correlate with work done by Ndou (2007), Nzalak (2010) and Boonzaier (2012). The caecum was observed to have very few acid mucin droplets and complete absence of the neutral mucin which correlated with the few number of goblet cells in the caecum. This may be because of the large microflora in caecum (Sharma *et al.,* 1995). The colon had more of the acid mucin granules than the neutral mucin granules. This increase in acid mucin granules may suggest the need to increase the mucus gel viscosity since large bacterial colonies are present in the colon because acid mucin granules are known to increase the viscosity of the mucus (Macfarlane and Dillon, 2007) to better protect the epithelial surface. The rectum also maintained a similar mucin composition as the colon.

The PAS and PASD staining techniques employed for the liver revealed PAS positive result, with the PAS positive result changing after prior treatment with diastase (PASD), many clear spaces appeared which may suggest those clear spaces had contained glycogen (Faure *et al.,* 2002; Sheibani and Yali, 2006).

Histochemical study using AB, PAS and AB-PAS techniques were all negative indicating absence of mucins, this is in contrast with the findings of Ganesh *et al.,* (2007) and Ikpegbu *et al.* (2012) who reported the presence of acid and neutral mucins in the gall bladder epithelium of *Clarias gariepinus* B.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 SUMMARY

In the present study, the stomach of the Grasscutter was observed to be made up of cardia, fundus and pylorus. The fundic region was thrown into numerous longitudinal folds. Microscopic observations of the stomach revealed simple columnar cells lining the epithelium across all the regions, glands were observed with specialized cells for secretion of mucus and digestive juice with the parietal cells seen in the pyloric region. Neutral mucins were observed in the surface mucous cells of the three regions while all three regions were negative for acid mucins.

The small intestine was observed to be divided into duodenum, jejunum and ileum with all three segments having villi projecting into the lumen. Microscopic examinations revealed slight modifications in some tunics with the Brunner's glands observed in the submucosa of the duodenum. Histochemical studies of the small intestine revealed positive results for the surface mucous cells and glands with acid and neutral mucins present across all three segments.

The large intestine was made up of three segments (caecum, colon and rectum). The caecum was observed to contain fewer goblet cells when compared to the colon and rectum. A thick tunica muscularis was observed throughout the large intestine. Histochemical studies showed presence of acid and neutral mucins across the segments with acid mucins being the dominant across the large intestine.

The liver was observed to be made up of large polyhedral hepatocytes with basophilic central nuclei. The cells were arranged from a diffuse to radial pattern. The PAS and PASD staining techniques used suggested the liver played an active role in glycogen storage.

The pancreas in this study was observed to be diffuse and friable. Microscopically, it was observed to have both exocrine and endocrine tissues made up of serous acini cells and Islets of Langerhans respectively.

The gall bladder was seen to be made up of tall columnar epithelial cells. The smooth muscle layer was discontinuous and placed at different orientations. Histochemical studies showed absence of any mucins as the AB, PAS and AB-PAS techniques were all negative.

6.2 CONCLUSION

The Grasscutter showed a pattern in the distribution of mucins in the GIT with the stomach containing only neutral mucins while acidic mucins were dominant in the intestines, this could be as a result of their diet or feeding habit.

6.3 RECOMMENDATION

The present study could be used in comparative anatomical studies with other species of the Muridae family and even higher orders.

The ultrastructure of the mucin secreting goblet cells could be investigated with the aid of the electron microscopy.

Specific lectins could be used for more specific identification of mucin types in the GIT.

Further studies should be done to quantify the mucous secreting goblet cells in the various regions of the gastrointestinal tract.

Other staining techniques should be employed to ascertain exactly the type of acid mucin (weak or strong) present in the different regions of the gastrointestinal tract such Alcian blue at pH 1.0 and 2.5, Alcian blue-Aldehyde fuchsin and Alcian blue-high iron diamine techniques.

There is a need to look at the mucin histochemistry of other rodents and compare to that of the Grasscutter.

6.4 CONTRIBUTIONS TO KNOWLEDGE

Mucin histochemistry of the stomach revealed absence of acid mucins while neutral mucins were present across the three regions of the stomach.

Glycogen was demonstrated in the liver cells (hepatocytes) using Periodic Acid Schiff with Diastase control.

Parietal cells were observed in the pyloric region of the stomach as opposed to the existing reports of their presence in the fundic region of the stomach in Grasscutter.

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