Anatomical Studies on the Alimentary Tract of the Egyptian Typhlopid Snake*Rhamphotyphlops Braminus*

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Abstract: The alimentary tract of the typhlopid snake, *Ramphotyphlops braminus* was anatomically, histologically and scanning electron microscopically investigated. The gut is differentiated anatomically into the basic ophidian pattern. The absence of constrictions between the oesophagus and stomach, as well as duodenum and ileum was noticed. The large intestine lacks caecum. The mucosal surface of the oesophagus is thrown into numerous prominent longitudinal folds leaving long furrows in between. These folds showed irregular patterns. The gastric mucosa forms large number of primary longitudinal folds. Small furrows were detected in between. The luminal surface of the columnar cells appeared pentagonal. Simple tubular gastric glands open into the gastric pits. The luminal surface of the small intestine appeared as irregular wavy mucosal folds in the fashion of Zig-Zag pattern. The mucosal folds of the small intestine contained short and blunt projections representing the villi. Pentagonal absorptive columnar cells and goblet cells were detected in between these columnar epithelial cells. The internal surface of the large intestine is provided with many irregular wavy folds which anastomose with each other to form complex reticulated pattern leaving shallow cup-shaped depressions. The mucosal surface of the large intestine consisted of simple columnar cells which have pentagonal shape. Many goblet cells were detected between these columnar cells.

The wall of the oesophagus, stomach, small and large intestine is built up of serosa, muscularis, submucosa and mucosa. The oesophageal mucosa is consisted of simple columnar epithelium. The oesophageal glands were compeletely absent. The gastric mucosa is consisted of simple columnar epithelial cells. The mucosa of small intestine is composed of two types of cells including absorptive and goblet cells. The mucosal epithelium of the large intestine is represented by simple columnar epithelial cells and scattered goblet cells. Mucosa of both small and large intestine lacks intestinal glands as well as crypts of lieberkhün. The PAS-Positive materials, mucopolysaccharides (acid and neutral), and total proteins were histochemically investigated in the different regions of the alimentary tract. PAS-Positive materials are evident in the mucosa of oesophagus, gastric glands of stomach and goblet cells of small and large intestine. High content of both acidic and neutral mucins is displayed in the mucosal epithelium of oesophagus and stomach, gastric glands and goblet cells of small and large intestine. High protein content was detected in the muscularis and mucosa of oesophagus , stomach , small and large intestine.

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1.Introduction

Morphology of gastrointestinal tract (GIT) has been studied extensively (**Dehlawi and Zaher, 1985 a&b**; **Farag and Al-Robai, 1986**, **Zaher *et al.*, 1987**). Studies on the ophidian GIT have been restricted only to *Natrix tessellata* (**Abo-Taira *et al.*, 1988 b**). **Khamas and Reeves (2011)** studied the morphology of the oesophagus and stomach of the gopher snake *Pituophis catenifer*. **Zaher *et al.* (1990a)** studied the morphological characters of *Mabuya quinquetaeniata quinquetaeniata*.

A review on the histological studies on the alimentary tract of different members of order squamata revealed that most of these studies were dealt with the suborder lacertilia (**Dehlawi and Zaher, 1985a&b**). On the other hand, little attention has been paid to the members of suborder ophidia. Most of the available works were dealt with certain parts of the tract (**Ferri *et al.*, 1974; Heyder, 1974 and Anwar and Mahmoud, 1975**). However, Other investigations were dealt with the study of the histology of the whole alimentary tract of certain species of reptiles such Some american turtles (**Ballmer, 1949**); *Mabuya quinquetaeniata* (**Amer and Ismail , 1976**); *Uromastyx philbyi*(**Farag, 1982**) and *Acanthodactylus boskianus* (**Dehlawy and Zaher, 1985b**).The microscopic structure of the tract organs of some snakes was investigated by **Heyder (1974)** on *Typhlops vermicularis* and **Abdeen *et al.* (1994)** on *Malpolon monspessulanus*, *Coluber florulentus* and *Tarpophis obtusus*. **El-Bakry *et al.*, 2012** studied *Natrix tessellata* (Family colubridae).

The histological structure of the gut of *Varanus niloticus* was studied by**Ahmed *et al.* (2009)**.

Dehlawi and Zaher (1985a) studied the histology of the alimentary tract organs in the gecko *Pristurus rupestris*. The histology of the tract organs of the gecko *Gekko japonicus* was studied by **Oidumi and Ishihara (1964)**. The histological studies of the alimentary tract of some lizards were carried-out by several authors including the work of **El-Toubi and Bishai (1958)** on *Uromastyx aegyptiaca*; **Bishai (1959)** on *Varanus griseus*; **Anwar and Mahmoud (1975)** on two egyptian lizards *Mabuya quinquetaeniata* and *Chalcides ocellatus*; **Amer and Ismail (1976)** on *Agama stellio*; **Farag (1982)** and **Dehlawi *et al.* (1988a)** on *Uromastyx philbyi*, **Dehlawi and Zaher (1985b)** on *Acanthodactylus boskianus*; **Zaher *et al.* (1989a& 1990c)** on *Mabuya brevicollis* and*Chalcides sepoides* respectively. The macroscopic structure of the reptilian alimentary tract has been subjected to extensive studies. Reports on this subject were presented by **Abo-Taira *et al.* (1988a & 1988c)** on *Acanthodactylus boskianus* and *Tarentola annularis* respectively.**Zaher *et al.* (1989b& 1991a)** studied the anatomy of the alimentary tract in *Stenodactylus slevini* and *Echis carinatus* respectively. Other studies were made on *Scincus officinalis* (Family Scinidae, **El-Toubi, 1936**), *Uromastyx aegyptia* (Family Agamidae, **El-Toubi and Bishai,1958**), *Varanus griseus* (Family Varanidae, **Bishai, 1959**),*Chamaeleon vulgaris*(Family Chamaeleontidae, **Bishai, 1960**)while **Zaher *et al.*, 1990 b** and **El-Bakry *et al.*, 2012** studied the alimentary tract of *Mabuya brevicollis*and *Scincusscincus* (Family Scincidae) respectively.

Some investigations have described the distribution of mucosubstances throughout the gastrointestinal tract (**Dehlawy *et al.*, 1987 a&b).** The distribution of carbohydrates and proteins in the alimentary tract was studied by **Amer *et al.* (1987b)** in the snake *Echis carinatus*. Reports on this subject was presented by **Abdeen *et al.* (1990 b)** on the snake *Cerastes vipera*.**Abdeen *et al.*, (1992)** studied the distribution of carbohydrates and proteins in the oesophageal and gastric mucosal epithelium of the three snakes; *Malpolon monspessulanus*, *Coluber florulentus* and *Tarbophis obtusis* (family colubridae). Extensive histochemical studies have been carried-out on the alimentary tract organs of reptiles. The distribution of carbohydrates in the gut mucosa of the snake *Naja nigricolis* was carried-out by **Badr El-Din (1991)**. The histochemical characterization of the alimentary tract of ophidia has been studied on *Echis carinatus* (**Amer *et al.*, 1987 a**), *Cerastes cerastes* (**Abo-Taira *et al.*, 1989**) and *Eryx Colubrinus* **(Zaher *et al.*, 1990 d).**

Biomy (2010) studied the histochemical characterization of the alimentary tract of the insectivorous reptile, *Scincus scincus*. **Ahmed *et al.* (2009)** studied the histochemical characterization of the gut of *Varanus niloticus*. The localization and distribution of carbohydrates , proteins, lipids and nucleic acids in the mucosal coat of the reptilian gut has been extensively described (**Zaher *et al*, 1995**).

The histochemical distribution of mucopolysaccharides in the alimentary tract mucosa of the gecko *Tarentola annularis* was studied by **Amer *et al.* (1987a)**. The mucopolysaccharides, lipids and proteins in the tract mucosa of the lizard *Acanthodactylus scutellatus* was subjected to such study by **Amer *et al.* (1988)**. Some histochemical studies have been carried-out on the alimentary tract organs of reptiles. **Dehlawi and Zaher (1985a &1987b)** studied the histochemical distribution and localization of carbohydrates in the alimentary tract of the gecko *Pristurus rupestris* and the lizard *Agama adramitana*, respectively. A comparative histochemical study on the distribution of carbohydrates in the gut mucosa of the lizard *Uromastyx philbyi*.

Anwar and Mahmoud (1975) resolved the distribution of carbohydrates and proteins in mucosa of the ileum and rectum of *Mabuya quinquetaeniata* and *Chalcides ocellatus*. **Taib and Jarrar (1983& 1985)**and **Taib (1984)** studied the histochemistry of the alimentary tract of *Chalcides levitoni*, *Mauremys caspica*, *Chalcides ocellatus* and *Uromastyx microlepis*, respectively. **Abdeen *et al.*(1990a)** studied the histochemical distribution of carbohydrates and proteins in *Eumeces schneideri* (Lacertilia, scincidae).

2.Material and Methods

Animals:-

The animals used in the present work is *Rhamphotyphlops braminus* (family typhlopidae); were collected from Ashmon, Menofia, Egypt and were identified according to (**Benton , 1997 and Saleh , 1997**).

The Alimentary tract:-

Gross Anatomy:-

Three Animals were sacificed to illustrate the different regions of the alimentary tract (Oesophagus, Stomach and intestine).

Scanning Electron Microscope Studies:-

For SEM, ten animals were anesthetized by chloroform and dissected quickly. The different regions of the alimentary tract (oesophagus, stomach, small and large intestine) were cut longitudinally to expose their luminal surface, directly fixed in 2.5% gluteraldehyde in Sorensen's Phosphate Buffer (pH 7.3) for 4 hrs and optionally followed by postfixation with osmium tetroxide (**Jeffree and Read, 1991**).The fixed tissue is then dehydrated with graded series of ethanol. The specimens were then dried in vaccuum at critical point drying. The dry specimen is usually mounted on a specimen stub using conductive double-sided adhesive tape, sputter-coated with gold using SPI sputter coater and viewed in JSM- 6510 LA(JEOL).

Histological and Histochemical Studies:-

For histological studies of alimentary tract; ten animals were anesthetized by chloroform, orally injected with 10% neutral buffered formalin and dissected quickly. The different regions of the alimentary tract (oesophagus, stomach, small and large intestine) were directly fixed in 10% neutral buffered formalin solution, then washed and dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin. Sections were cut at 5µm thickness and stained with Erich's haematoxylin and eosin (**Mallory, 1944**) for general histological studies,Masson trichrome stain (**Drury *et al*., 1976**)for detection of connective tissue fibers, Periodic Acid Schiff (PAS) for detection of the presence of carbohydrates (**McManus, 1946**), Acid and neutral mucopolysaccharides were demonstrated by the Alcian blue-PAS method (**Mowry, 1956**) and the mercuric bromophenol blue method (**Mazia *et al*., 1953**)For displaying the total proteins were adopted. These sections were photographed using letiz microscope.

3.Results

Morphological Observations:-

Ramphotyphlops braminus used in the present study has cylindrical body (Fig , 1). The head and the tail tip are nearly similar. It is often mistaken for earth worms, except it's not segmented. The body is shiny black or brown with slightly lighter on the ventral side. The rigid cylindrical body bears uniform cycloid scales dorsally and ventrally. It has vestigial eyes in the form of black spots beneath the scales on the head (Fig, 1). The mouth is crescent-shaped and ventrally located. The dentition is reduced to few short recurved teeth on the maxilla of the upper jaw. The tail is short with terminal spine.

Anatomical Observations:-

In the present study the alimentary tract of the blind snake Rhamphotyphlops *braminus* is a narrow elongated more or less straight tube (Fig, 2), the buccal cavity leads to short pharynx which open into oesophagus. The oesophagus is a narrow straight tube lying dorsal to trachea. The oesophagus opens posteriorly into ashort tubular stomach(Fig, 2). There is no anatomical constriction between the oesophagus and stomach. The latter assumes an elongated outline corresponding to body shape. The stomach opens into the the intestine which is differentiated into small intestine and large intestine (Fig, 5). There is no anatomical constriction between the small and large intestine. The large intestine is short and straight ends by the cloaca which is a common chamber, receiving products from the digestive, urinary and reproductive systems. The cloaca opens into transverse cloacal aperature. The liver is the largest internal organ (Figs, 2 & 3) . It is elongated and bilobed , the two lobes are fused together at their inner borders. The gall bladder is a spherical dark thin sac (Figs, 4) . A characteristic of snakes is that there is a relatively long distance between the caudal tip of the liver and the gall bladder.

Scanning Electron Microscopic observations:-

The oesophagus

The mucosal surface of the oesophagus is thrown into numerous prominent longitudinal folds leaving long furrows in between. These folds showing irregular pattern of arrangement. Minute circular openings of the empty mucous cells are also detected (through the pores the mucous cells discharge their contents). Increased mucous cell activity is demonstrated by the deposition of mucous masses (Figs, 6 & 7).

The stomach

The topographical study of the mucosa of the stomach reveals that the gastric mucosa forms large number of primary longitudinal folds. Small furrows can be detected between the longitudinal folds. The luminal surface of the columnar cells appeared pentagonal. Simple tubular gastric glands open into gastric pits were observed(Figs, 8 & 9).

The small intestine

The luminal surface of the small intestine is Characterized by the presence of irregular wavy mucosal folds which are arranged in Zig-Zag pattern. The mucosal folds of the small intestine is in the form of short and blunt projections presenting the villi. Pentagonal absorptive columnar cells can be seen also goblet cells were detected in between these columnar epithelial cells.The apices of the discharging goblet cells appeared as shallow depressions(Figs, 10 & 11).

The large intestine

The internal surface of the large intestine is provided with many irregular wavy folds which anastomose with each other to form complex reticulated pattern leaving shallow cup-shaped depressions. The mucosal surface of the large intestine consisted of simple columnar cells which have pentagonal shape. Many goblet cells were detected between these columnar cells(Figs, 12 & 13).

Histological Observations:-

The wall of the alimentary tract of *Ramphotyphlops braminus* is differentiated to the same basic four layers of other vertebrates. These layers are from the peripheral surfaces inwards; serosa, muscularis, submucosa and mucosa.These layers show different degrees of development in various parts of the alimentary tract. The serosais found as a thin layer covering the whole alimentary canal and consists of a single layer of simple squamous epithelium.

The oesophagus

In the oesophagus of the studied species, the serosa is followed by muscularis which is built up of smooth unstraited muscle fibers. These muscles are differentiated into an outer circular (less continous) and inner longitudinal (more continuous) smooth muscle layers. In the oesophagus, the muscularis is very thin as movement of the whole body muscles are responsible for passage of food from oesophagus to stomach. The submucosa consists of connective tissue which is supplied with blood vessels. The submucosa is involved in the structure of oesophageal folds. The muscularis mucosa is in the form of thin circular muscle layer. The oesophageal mucosa is thrown into numerous tapering longitudinal folds of variable lengths. The mucosal epithelium of the oesophagus is completely free from glands. The mucous membrane of the oesophagus is simple, lined by simple columnar epithelium. The oesophageal mucosa of *R.braminus*consisted of single type of cells, mucous secreting cells, which are large club-shaped, each consists of an inner basal part and an outer hyaline portion. The basal part contains a flat nucleus which is intensively stained with Haematoxylin(Figs, 14 & 15). Collagenous fibers are widely distributed in lamina propria of mucosa (Figs, 22 &23).

The stomach

In the present study, the stomach is covered by serosa which is lined with a simple flat epithelium and surrounds the muscularis. The muscularis is composed of an outer thin layer of smooth circular muscle fibers and an inner thick and well developed layer of smooth longitudinal muscle fibers. The narrow submucosa is followed by the muscularis mucosa. The gastric muscularis mucosa is well-developed and built up of circular muscle fibers. The muvosa of the stomach is thrown into wavy longitudinal folds. The gastric mucosa consisted of the surface mucosal epithelium, lamina propria and gastric glands. The mucosal epithelium of the stomach is lined by simple columnar epithelium with cylindrical cells. The nuclei of the mucosal epithelium are oval in shape, basal in location and stained dark blue with haematoxylin. The lamina propria is composed of connective tissue which is situated between the muscularis mucosa and the mucosal epithelium extending to the gastric folds. The gastric glands are embedded in the lamina propria and open into the gastric folds via the gastric pits (Figs, 16 & 17). Collagenous fibers are scarcely distributed in lamina propria of mucosa (Figs, 24 &25).

The small intestine

There is no morphological differences between the dudenum and ileum. The serosa is followed by the muscularis layer. The muscularis of the small intestine has an outer thin layer of smooth circular muscle fibers (more continuous) and an inner thicker one of smooth longitudinal muscle fibers (less continuous). The submucosa is supplied with blood vessels and capillaries as well as lymph spaces. Moreover, it is involved in the intestinal villi. The muscularis mucosa is in the form of continuous thin circular muscle layer. The mucous membrane of the small intestine is thrown into large numbers of high narrow longitudinal folds called intestinal villi, some of these folds take a zigzag shape. The mucosal epithelium of the small intestine is composed of two kinds of cells; absorptivecells and goblet cells. The mucosa of the small intestine shows complete absence of the intestinal glands and glandular crypts (Figs, 18 & 19). Collagenous fibres are widely distributed in submucosa while moderately distributed between both longitudinal and circular layers of muscularis(Figs, 26 &27)

The large intestine

The serosa is followed by the muscularis. The muscularis of the large intestine is formed of an outer thin longitudinal muscle layer and inner slightly thicker circular muscle layer. The submucosa is formed of connective tissue rich in blood vessels. The muscularis mucosa is formed of continuous thin circular muscle layer. The mucosal epithelium of the the large intestine is built up of simple columnar cells with several scattered goblet cells. The nuclei of the former cells are large in size, oval in shape and basal in position. The goblet cells are limited in number and have the same pattern as those of small intestine. Glandular crypts are not observed in the mucosa of large intestine(Figs, 20 & 21). Collagenous fibers are scarcely distributed in submucosa and between both longitudinal and circular layers of muscdularis of the large intestine (Figs, 28 & 29).

Histochemical Observations:-

The application of periodic Acid Sciff 's (PAS) technique on the oesophagus of *Ramphotyphlops braminus*recorded that the mucosa was heavily loaded with PAS positively stained materials, while the muscularis and submucosa appeared moderately positive (Fig, 30). On the other hand, high polysaccharides content was detectable in the mucosal epithelium and gastric glands of the stomach. However, muscularis and submucosa were moderately stained (Fig, 31). The small intestine displayed an intense amount of PAS-positive materials in the mucosal epithelium and intestinal goblet cells (Fig, 32).

Moreover, the mucosal epithelium and goblet cells of the large intestine exhibited an intense PAS-positive reaction (Fig, 33).

The application of PAS-Alcian blue staining technique for both neutral mucins (PAS –positive) and acidic mucins (Alcian- blue positive) displayed high content of neutral and acidic mucins in the mucosal epithelium of oesophagus, while muscularis and submucosa are moderately stained (magenta color) (Fig, 34).The stomach exhibited high content of mixed red (neutral) and blue (acidic) mucopolyasccharides (magenta) in the mucosal epithelium and gastric glands while muscularis, submucosa and lamina propria of gastric mucosa were moderately stained with a mixture of Alcian-blue (acidic mucins) and PAS (neutral mucins) (Fig, 35). Moreover, high content of neutral and acidic mucins were observed in the goblet cells of the small intestinal mucosa. The muscularis and submucosa of the small intestine are moderately stained magenta (Fig, 36). In case of large intestine, high PAS-Alcian blue positive materials in magenta color (neutral and acidic mucins) were observed in the goblet cells. The muscularis and submucosa of the large intestine are moderately stained magenta (Fig, 37).

The application of Bromophenol blue staining technique indicates the presence of high protein content in the muscularis and mucosa of oesophagus, stomach, small intestine and large intestine. The lamina propria of gastric mucosa, gastric glands and mucosal epithelium of the stomach were strongly loaded with proteins stained materials (Figs, 38 - 41).

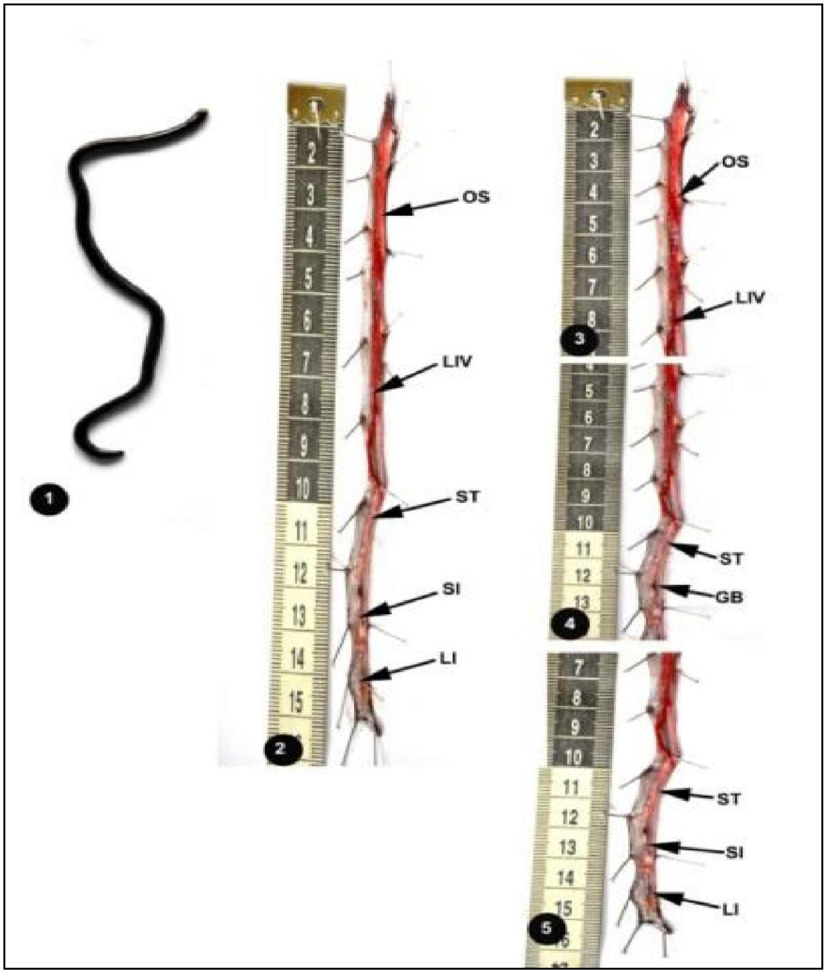
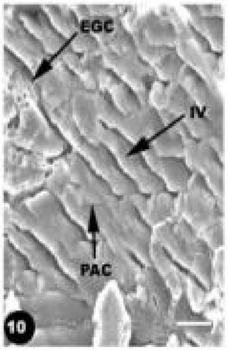
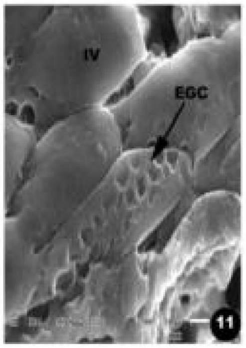
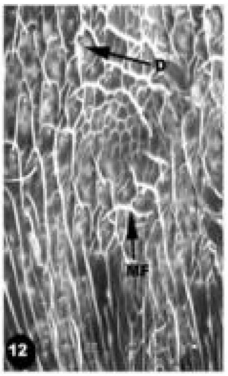


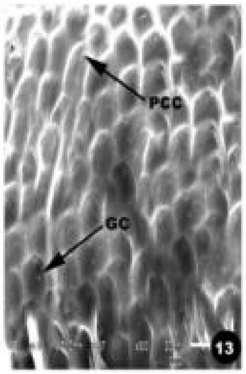
Fig. 1: External features of the blind snake, *Ramphotyphlops braminus.*
Fig. 2: Dissection of the blind snake, *Ramphotyphlops braminus* showing oesophagus (OS), stomach (ST), small intestine (SI), large intestine (LI) and liver (LIV).
Fig. 3: Dissection of the blind snake, *Ramphotyphlops braminus* showing oesophagus (OS) and liver (LIV).

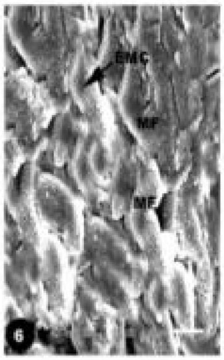
Fig. 4: Dissection of the blind snake, *Ramphotyphlops braminus* showing stomach (ST) and gall bladder(GB).
Fig. 5: Dissection of the blind snake, *Ramphotyphlops braminus* showing stomach (ST), small intestine (SI) and large intestine (LI).

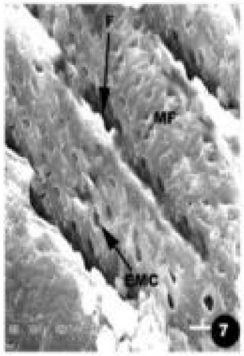


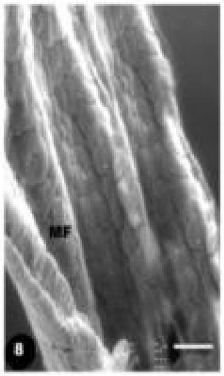












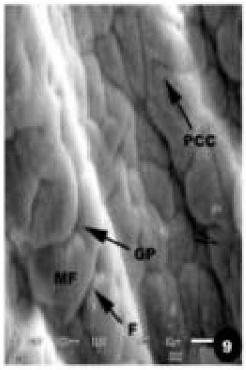
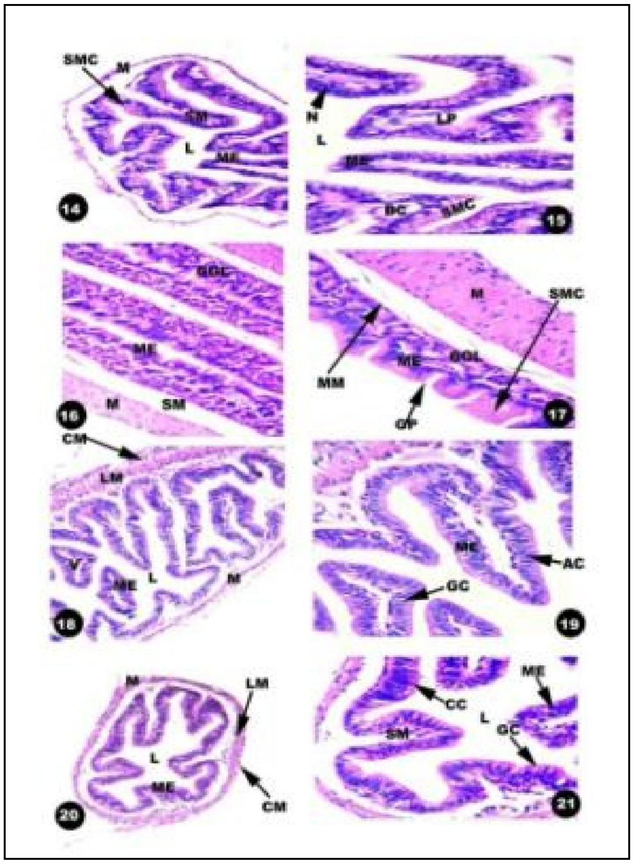


Fig. 6: SEM of the oesophageal mucosa of the blind snake, *Ramphotyphlops braminus* showing mucosal folds (MF) and empty mucous secreting cells (EMC). (Scale bar, 50µm).
Fig. 7: Higher magnification of the previous section showing mucosal folds (MF), empty mucous secreting cells (EMC) and long furrows (F). (Scale bar, 10µm).
Fig. 8: SEM of the gastric mucosa of the blind snake, *Ramphotyphlops braminus* showing mucosal folds (MF). (Scale bar, 100 µm).
Fig. 9: Higher magnification of the previous section showing mucosal folds (MF), small furrows (F), gastric pit (GP) and pentagonal columnar cells (PCC). (Scale bar, 50µm).
Fig. 10: SEM of the mucosa of the small intestine of the blind snake, *Ramphotyphlops braminus* showing intestinal villi (IV), pentagonal absorptive cells (PAC) and empty goblet cells (EGC). (Scale bar, 50 µm).
Fig. 11: Higher magnification of the previous section showing intestinal villi (IV) and empty goblet cells (EGC). (Scale bar, 10 µm).
Fig. 12: SEM of the mucosa of the large intestine of the blind snake, *Ramphotyphlops braminus* showing mucosal folds (MF) and depressions (D). (Scale bar, 50 µm).
Fig. 13: Higher magnification of the previous section showing pentagonal columnar cells (PCC) and goblet cells (GC). (Scale bar, 20 µm).



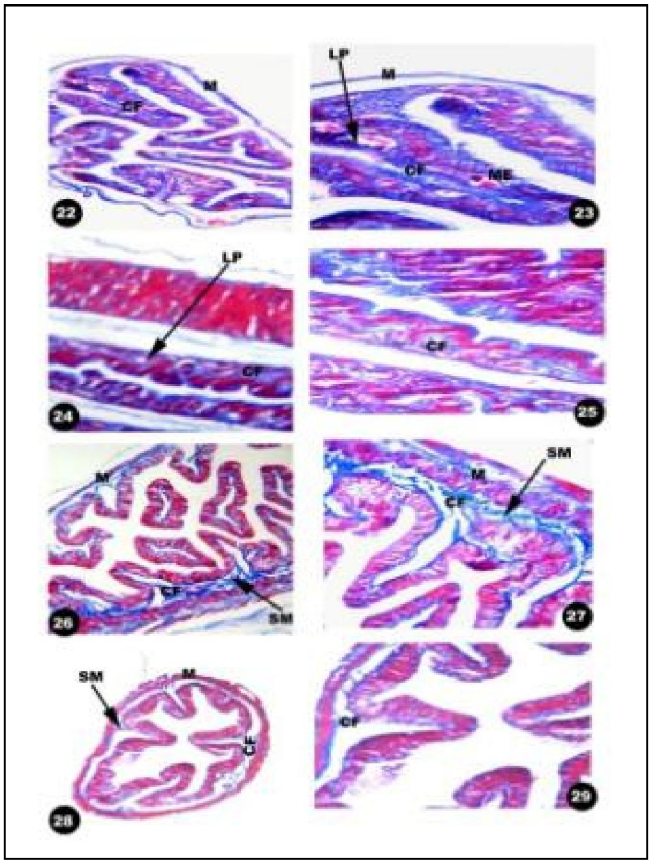


Fig. 14: T.S. of the oesophagus of the blind snake, *Ramphotyphlops braminus* showing muscularis (M), submucosa (SM), surface mucous cells (SMC), mucosal epithelium (ME) and lumen (l). H.E., X 200.

Fig. 15: Higher magnification of the previous section showing lamina propria (LP) containing blood capillaries (BC), surface mucous cells(SMC) with their blue stained nuclei (N), mucosal epithelium (ME) and lumen (L). H.E., X 400.

Fig. 16: T.S. of the stomach of the blind snake, *Ramphotyphlops braminus* showing muscularis (M), submucosa (SM), mucosal epithelium (ME) and gastric glands (GGL). H.E., X 200.

Fig. 17: Higher magnification of the previous section showing muscularis (M), mucosal epithelium (ME), surface mucous cells (SMC) , gastric pits (GP), gastric glands (GGL) and muscularis mucosa (MM). H.E., X 400.

Fig. 18: T.S. of the small intestine of the blind snake, *Ramphotyphlops braminus* showing muscularis (M) with an outer circular muscle fibres (CM) and inner longitudinal one(LM), mucosal epithelium (ME) , intestinal villi (V) and lumen (L). H.E., X 200.

Fig. 19: Higher magnification of the previous section showing mucosal epithelium (ME) of the intestinal villi containing both absorptive cells (AC) and goblet cells(GC). H.E., X 400.

Fig. 20: T.S. of the large intestine of the blind snake, *Ramphotyphlops braminus* showing muscularis (M) with an outer circular muscle fibres (CM) and inner longitudinal one(LM), mucosal epithelium (ME) and lumen (L). H.E., X 200.

Fig. 21: Higher magnification of the previous section showing submucosa (SM), mucosal epithelium (ME), goblet cell (GC), columnar cell (CC) and lumen (L). H.E., X 400.

Fig. 22: T.S. of the oesophagus of the blind snake, *Ramphotyphlops braminus* showing collagen fibres (CF) and muscularis (M). Masson's trichrome., X 200.

Fig. 23: Higher magnification of the previous section showing collagen fibres (CF), muscularis (M), lamina propria of mucosa (LP) and mucosal epithelium (ME). Masson's trichrome., X 400.

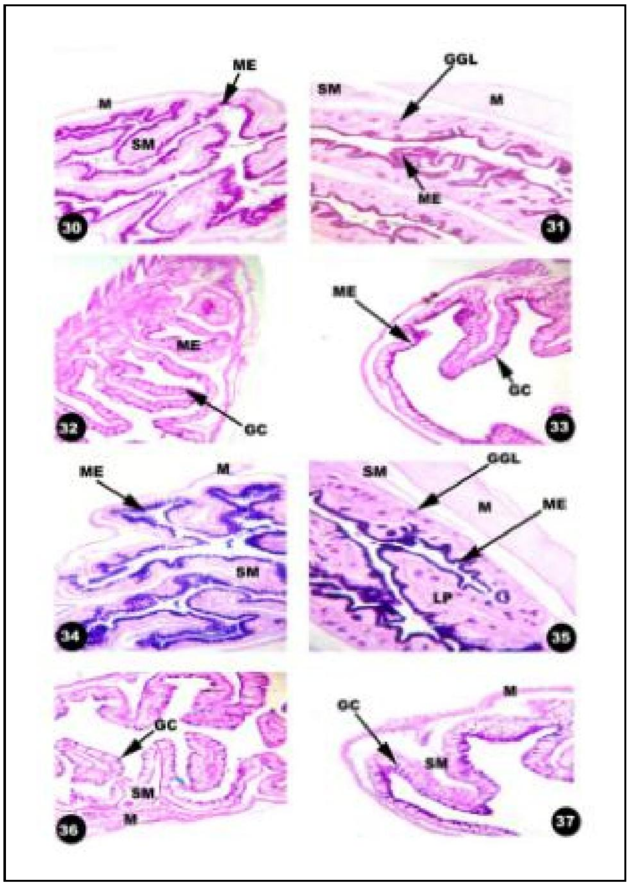
Fig. 24: T.S. of the stomach of the blind snake, *Ramphotyphlops braminus* showing collagenous fibres (CF) and the lamina propria of mucosa (LP). Masson's trichrome., X 200.

Fig.25: Higher magnification of the previous section showing collagen fibres (CF). Masson's trichrome., X 400.

Fig. 26: T.S. of the small intestine of the blind snake, *Ramphotyphlops braminus* showing collagen fibres (CF), muscularis (M) and submucosa (SM). Masson's trichrome., X 200.

Fig. 27: Higher magnification of the previous section showing collagen fibres (CF) , muscularis (M) and submucosa (SM). Masson's trichrome., X 400.

Fig. 28: T.S. of the large intestine of the blind snake, *Ramphotyphlops braminus* showingcollagenous fibres (CF), muscularis (M) and submucosa (SM). Masson's trichrome., X 200.
Fig. 29: Higher magnification of the previous section showing collagen fibres (CF). Masson's trichrome., X 400.



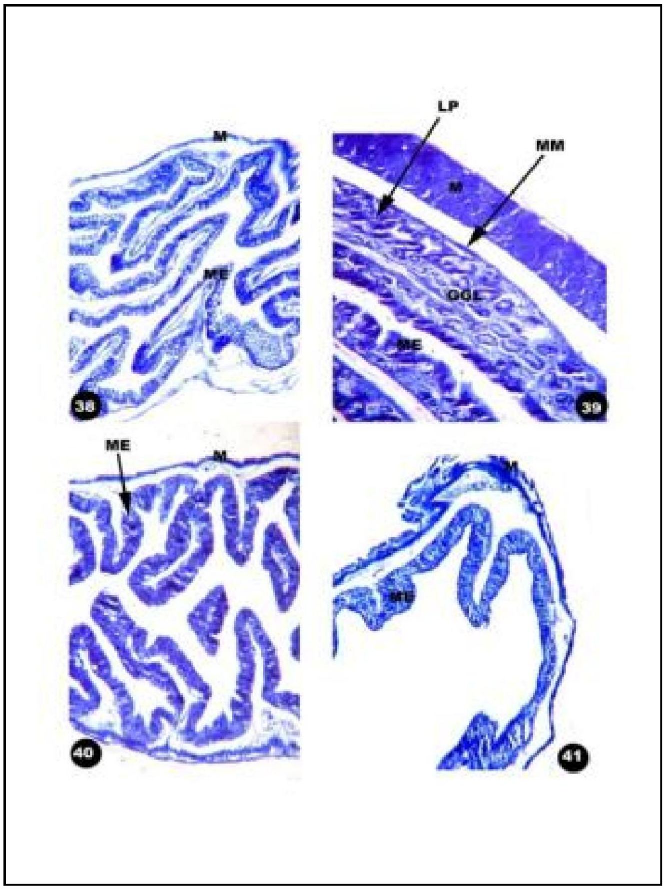


Fig. 30: T.S. of the oesophagus of the blind snake, *Ramphotyphlops braminus* displaying intensely high polysaccharide content in the mucosal epithelium(ME). The muscularis (M), submucosa (SM) appear weakly stained. PAS., X 200.
Fig. 31: T.S. of the stomach of the blind snake, *Ramphotyphlops braminus* showing high polysaccharide content in the mucosal epithelium(ME) and gastric glands (GGL) while muscularis (M) and submucosa (SM) are moderately positive.PAS., X 200.
Fig. 32: T.S. of the small intestine of the blind snake, *Ramphotyphlops braminus* showing an strongly high amount of PAS-positive materials in the mucosal epithelium (ME) and intestinal goblet cells (GC). PAS., X 200.
Fig. 33: T.S. of the large intestine of the blind snake, *Ramphotyphlops braminus* displaying the mucosal epithelium (ME) and goblet cells (GC) exhibiting intensely high positive reaction. PAS., X 200.
Fig. 34: T.S. of the oesophagus of the blind snake, *Ramphotyphlops braminus* displaying strongly high content of neutral and acidic mucins in the mucosal epithelium (ME). The muscularis (M) and submucosa (SM) are weakly stained (magenta colour). PAS-Alcian blue., X 200.
Fig. 35: T.S. of the stomach of the blind snake, *Ramphotyphlops braminus* showing intensely high content of mixed red (neutral) and blue (acidic) polysaccharides (magenta) in mucosal epithelium (ME) and gastric glands (GGL). muscularis (M), submucosa (SM), lamina propria of mucosa (LP) are weakly stained. PAS-Alcian blue., X 200.
Fig. 36: T.S. of the small intestine of the blind snake, *Ramphotyphlops braminus* denoting high content of neutral and acidic mucins in the goblet cells (GC). The muscularis (M) and submucosa (SM) are moderately stained magenta . PAS-Alcian blue., X 200.
Fig. 37: T.S. of the large intestine of the blind snake, *Ramphotyphlops braminus* displaying high PAS-Alcian blue positive materials in magenta colour (neutral and acidic mucins) in the goblet cells (GC). The muscularis (M) and submucosa (SM) are weakly stained magenta. PAS-Alcian blue., X 200.
Fig. 38: T.S. of the oesophagus of the blind snake, *Ramphotyphlops braminus* displaying moderate amount of protein content in muscularis layer (M) and mucosal epithelium (ME). Bromophenol blue., X 200.
Fig.39: T.S. of the stomach of the blind snake *Ramphotyphlops*, *braminus* showing strongly high protein content in muscularis layer (M),muscularis mucosa (MM), lamina propria of mucosa (LP), gastric glands (GGL) and mucosal epithelium (ME). Bromophenol blue., X 200.

Fig. 40: T.S. of the small intestine of the blind snake, *Ramphotyphlops braminus* disclosing strongly high protein content in muscularis layer (M) and mucosal epithelium (ME). Bromophenol blue., X 200.

Fig. 41: T.S. of the large intestine of the blind snake, *Ramphotyphlops braminus* displaying high protein content in muscularis layer (M) and mucosal epithelium (ME). Bromophenol blue., X 200.

4. Discussion

In the present study, the anatomical observations of the alimentary tract of *Ramphotyphlops braminus* detect the absence of an external demarcation between the oesophagus and the stomach. The absence of such constrictions seems to be a common feature for the suborder ophidia (**Zaher *et al*., 1991 a& b**). Moeover, the absence of such constrictions seems to be found in some lacertilian insectivorous members (**Zaher *et al*., 1990c).** On the contrary, the presence of constrictions between the oesophagus and the stomach was referred in *Uromastyx aegyptia* (**El-Toubi and Bishai, 1958**) and in turtles (**Luppa, 1977**). Obviously, the absence of constrictions between the oesophagus and the stomach is thus, closely related to the type of food, where it allows an easier passage for the chitinized insects or whole preys to the stomach since the examined snake feed mostly on small insects and soil invertebrates.

In the present snake, no pyloric constriction are found between the stomach and the duodenum. Such anatomical observation was also reported in other studied snakes (**Abo-Taira *et al*., 1988b; Dehlawi and Zaher, 1989 and Afifi *et al*., 1990**). This could be a common distinction of suborder ophidia. However, such constrictions are the most common feature in the suborder lacertilia (**Zaher *et al*., 1987 and Abo-Taira *et al*., 1988a**).

As generally known, the large intestine of reptiles is constructed of caecum, colon and rectum or sometimes only colon and rectum (**Abo-Taira *et al*.,1988a and Zaher *et al*.,1990 b**). In the present investigation the large intestine devoids of any external differentiation into caecum, colon or rectum. The anatomical indiscrimination of colon and rectum is detected in other reptiles (**Al-Nassar, 1976)**. However, its existence is recorded in most lizards and snakes (**Zaher *et al*.,1987 and Abo-Taira *et al*., 1990**). The variable appearence of the large intestine in the different species of reptiles seems to be associated with the mode of feeding or/and the difference in the geographical distribution of the animals.

The present observation reveals the absence of caecum in the large intestine. This character was also detected in other described carnivorous species (**Abdeen *et al*., 1994**). Hence the existence of caecum in the large intestine characterizes the insectivorous lacertilian species. The only exception is that of *Diplometopon zarudnyi* (**Al-Nassar, 1976**). Meanwhile, a well developed caecum is present in the herbivorous agamid lizard *Uromastyx aegyptia* (**El-Toubi and Bishai, 1958**). Evidently, the presence or absence of a caecum in the large intestine of reptiles is related to the type of food. The presence of large caecum in herbivorous reptiles is used for storing the food which takes a long time for complete digestion. In addition, it is adapted histologically for absorption of water present in the food mass. In carnivorous reptiles there is no need for such caecum as digestion takes place quickly by the strong digestive enzymes.

The shortest organs of the gastrointestinal tract of *R. Braminus* are the stomach and the large intestine. The shortening of both stomach and large intestine is one of the main ophidian features . This is quite possible, since all snakes are carnivorous animals, while in both insectivorous and herbivorous reptiles these two organs display a great elongation (**El-Toubi and Bishai, 1958and El- Bakry *et al*., 2012).**

The relative long oesophagus in the examined snake reflexes a good adaptation for its mode of feeding, since it swallows the whole preys and this needs to pass easily with the help of a sufficient amount of mucous secretions. This is available during the passage of the prey through the long oesophagus before being arrived to the stomach to be digested.

In the present snake, as examined by SEM, the mucosal surface of the oesophagus is thrown into numerous prominent longitudinal folds leaving long furrows in between. These folds showing irregular pattern while Fingerprint-like microridges of oesophageal mucosa have been described in fishes (**Grau *et al*.,1992**). The gastric mucosa of thepresent species forms large number of primary longitudinal folds. Small furrows were detected in between these longitudinal folds.**Grau *et al*. (1992)** found that the stomach of the *Amberjack Seriola dumerili* displayed a large number of primary longitudinal folds with secondary folds. In the present species, the luminal surface of the small intestine is characterized by the presence of irregular wavy mucosal folds of Zig-Zag pattern. The mucosal folds of the small intestine forms short and blunt projections presenting the villi. The columnar epithelium of the intestinal mucosa may have an absorptive function as reported by **Sis *et al*. (1979)**, **Clarke and Witcomb (1980)** and **Albrecht *et al*. (2001)**. These researchers also reported that numerous mucous-secreting goblet cells were present among the columnar cells in *O. angorae*.

Mucosal folds of luminal surface of the small intestine giving a honeycomb appearence incase of crocodiles and snakes (**Jacobshagen, 1937**).

According to **Patt and Patt (1969)**, typical intestinal villi appeared firstly in birds, though a few fish have finger-like projections somewhat like the intestinal villi of mammals. In the small intestine of *Xenodon merremii* there are no crypts of lieberkühn or submucosal glands of Brünner. This appeared in reptiles generally (**Slijper, 1946)**. The intestinal absorptive and goblet cells are generally similar to those of fish, amphibia, reptiles, birds and mammals (**Barrington, 1957).**

In the present species,the internal surface of the large intestine is provided with many irregular wavy folds which anastomose with each other to form complex reticulated pattern leaving shallow cup-shaped depressions. **Mir and Channa (2010)**, reported that mucosal surface of the large intestine of *Schizothorax curvifrons* is thrown into irregularly arranged mucosal folds, minor mucosal folds and the concavity being totally absent. The secretion of mucin to the exterior through pores has been reported by various workers in different teleosts **(Moitra and Ray, 1979).**

In the present snake, the mucosal epithelium of the oesophagus is represented by simple columnar epithelium. However, in Uromastyx *aegyptia* (**El-Toubi and Bishai, 1958**), *Chamaeleon vulgaris* (**Bishai, 1960**) and *Uromastyx philibyi* (**Farag, 1982**), the mucosal membrane of only the anterior region of the oesophagus consisted of simple epithelial cells, while the posterior region is formed of a stratified one. The oesophageal mucosa consisted of simple ciliated columnar epithelial cells and goblet cells in other reptilian species studied by **Mohallal and Rahmy (1992)**.

In the present snake, the oesophageal mucosa consists of a single type of cells which give positive reaction to PAS-Alcian blue stain and this points the mucous nature of these cells. Accumulation of acid mucopolysaccharides in these cells played a major role in the production of mucin which facilitates the process of swallowing preys. These cells resemble the goblet cells of mammals (**El-Beih *et al*.,1987**).

The oesophageal mucosa of the studied species is characterized by the absence of glands. This finding confirms that found in *Natrix natrix* and *Vipera berus* (**Pryzystalski, 1980**), *Agama adramitana* (**Farag and El-Robai, 1986**), *N. Tessellata* (**Abo-Taira *et al*., 1988b**), *C. florlentus* (**Dehlawi and zaher, 1989**), *Echis carinatus* (**Afifi*et al*., 1990**) and *Uromastyx aegyptius*(**Abo- Eleneen, 2010).** However, the oesophageal glands were previously reported in other reptilian species. The nature of such oesophagheal glands was subjected to different points of views. **El-Toubi and Bishai (1958)** demonstrated three different types of oesophagheal glands in *Uromastyx aegyptia* which are termed, mucous, oxyntic and mucous-oxyntic glands. Mucous and oxyntic glands in the posterior region of the oesophagus of *Chamaeleon vulgaris* were described by **Bishai (1960)**. In addition, **Dilmuhamidov (1975)** confirmed the presence of tubuloalveolar mucous glands in the oesophagus of *Testudo graecea* and *Testudo horsfieldii* respectively. However, **Farag and El-Robai (1986)** revealed the presence of two types of glands in the oesophagus of *Scincus hempichi*, mucous secreting and the other cells with acidophilic granules. This may confirms that the presence or absence of oesophageal glands are independent to the mode of feeding. Additionally, there is no link between the animal 's systematic position and the presence or absence of such glands.

The present work revealed that the oesophageal mucosa of the studied species is thrown into several longitudinal folds. This character is common for all the described reptiles including *Uromastyx aegyptia* (**El-Toubi and Bishai, 1958**) and *Pristurus rupestris* (**Dehlawi and Zaher, 1985a**).

The stomach of the examined species is characterized by the presence of a relatively thick gastric muscularis layer which is obviously a good adaptation for breaking up food to small pieces through strong muscular contractions.

The present microscopic investigation shows the complete absence of glands and crypts from the mucosa of the small intestine. This finding confirms the observations of the other authors in the study of the alimentary tract of snakes (**Dehlawi and Zaher 1989 and Zaher *et al*., 1991 a&b**). This feature can represent a common character of the ophidian gut. Similar data has been observed in *N. natrix* and *V. Berus* (**Prezystalski, 1980**). It is to be mentioned that the lieberkhün crypts was found in some reptilian species such as *U. aegyptia* (**El-Toubi and Bishai, 1958**) and the amphisbaenian *Diplometopon zarudnyi* (**Al-Nassar, 1976**).

As examined microscopically the large intestine of the present snake devoids of any glandular crypts. Such a condition is concordant to what were recorded by **Farag (1982)** and **Dehlawi and Zaher (1985a)** in *Pristurus rupestris*. On the contrary, the glandular mucosa were recorded in *Anguis fragilis* (**Greschik, 1917**).

The presence of large amount of PAS positive materials in the cells of oesophageal and gastric mucosal epithelium represents histochemical feature for oesophageal and gastric mucosa of the examined species.The present study indicated the presence of exaggerated amount of PAS positive materials in the goblet cells of the small and large intestine. This histochemical feature represents a common character for the intestine of squmates (**Dehlawi *et al*., 1988a and Zaher *et al*., 1990d**). The mucosa of oesophagus, stomach, small and large intestine are positively reacted with PAS indicating the presence of neutral polysaccharides responsible for the production of energy required for the process of peristaltic movement.

The oesophageal mucosa of this snake is positively reacted with PAS-Alcian blue stain so sufficient amount of mucous secretion is produced allowing an easier passage of the prey to stomach. The gastric glands and mucosal epithelium of the stomach of the studied species exhibited high content of mixed mucopolysaccharides denoting the mucous nature of their secretion. This secretion facilitates the passage of the digested food from stomach to small intestine. Such character seems to be one of the most specialized properties of snakes (**Dehlawi and Zaher, 1989 and Zaher *et al*., 1991a&b**) and lacertilian stomach (**Farag and Al-Robai, 1986**). Such property is considered as a common character for the stomach of squamate reptile.

Zaher *et al*., (1995) reported the presence of gastric acid and neutral mucosubstances in *Chamaeleon vulgaris* and *Chamaeleon basiliscus***.** The same results are also reported in other investigated reptilian species (**Kenawy and El-Dawoody, 1991**). **Badr El-Din, (1991)** described only gastric acid mucosubstances in the stomach of *Uromastyx philibyi* and *Naja nigricolis*, respectively. on the other hand, **Abo-Taira *et al*., (1989)** and **Zaher *et al*., (1990d)** observed gastric mucosubstances in *Cerastes cerastes and Eryx colubrinus*, respectively. Furthermore, **Abdeen *et al.* (1990b)** mentioned that the ophidian gastric mucosa contained mainly acid mucosubstances with some neutral ones. Finally, neutral mucosubstances beside some acid ones were detected in the gastric mucosa of the Viper *Echis carinatus*, **Amer *et al.* (1987a)**. On the other hand, the mucosal goblet cells of the small and large intestine of the investigated species are positively reacted with PAS-Alcian blue stain. Moreover, the presence of moderate amount of neutral mucosubstances in the intestinal columnar epithelial cells represented, more or less, a common histochemical appearence to the intestine of of squamata species (**Abdeen *et al*., 1990b**).The positive response of the mucosal epithelial cells of the large intestine of the investigated species proves that the abundant secretion of mucin of these cells. This may be considered as a well adaptive character of the large intestine to perform its function of water absorption and as defecation (**Thiruvathukal and Kuriakosa, 1965**).

The presence of a considerable amount of proteinic substances along the alimentary tract mucosa of the present snake is, somewhat , similar to that found in the viper *E. carinatus* (**Amer *et al*., 1987 b**) and the viper *C. cerastes* (**Abo-Taira *et al*., 1989**). The slight variations in the protein content seems to be dependent on the locality of the animal rather than on the mode of feeding or the taxonomic position.

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