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Histological and Histochemical Study of Large and Small Intestine of *Hydrophis cyanocinctus* in Minab Beaches

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Abstract

The aim of the current study was to investigate the histological and histochemical characteristics of large and small intestines of sea snake, *Hydrophis cyanocinctus*. Six adult sea snakes were collected from Minab beaches, located near the sea of Oman, in November, euthanized with chloroform, dissected and alimentary canal removed, tissues preparaed for sectioning and staining with Hematoxylin and Eosin (H&E) and Periodic Acid Schiff's (PAS) methods, and observed with a light microscope equipped with a dinolite lens. Histological evaluation showed that the walls of each organ was formed of mucosa, submucosa, muscularis and serosa. The wall of the small intestine has numerous circular folds with zigzag patterns. Epithelium of small intestine is composed of simple long columnar and goblet PAS positive cells. The epithelial layer of large intestine was formed of long simple columnar cells with acidophilic cytoplasm. Also, PAS positive goblet cells were found scattered in epithelial tissue. In histometry, the epithelial length and width in primary of small intestine was larger than other parts. Goblet cells with 15.36 ± 1.12 were more frequent in the large intestine. Thickness of both internal and external muscular layers were the most at the end of small intestine ($p \leq 0.05$).

Keywords: *Sea snake, Hydrophis cyanocinctus, Intestine, Histology*

1. Introduction

Sea snakes are the largest and most diverse group of marine reptiles amongst 7500 different species of reptiles which are very important in the food chains and conservation of ecosystems (Amber et al., 2015). *H. cyanocinctus* has the vastest distribution in the Persian Gulf and western areas of the Oman Sea (Rezaie-Atagholipour et al., 2012). It has been suggested that reptiles could be used as a better model for studying physiological regulation of the digestive process than mice, rabbits and pigs (Ahmed et al., 2009). The alimentary canal of the reptiles

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contains all the structures presented in other higher vertebrates (Vasaruchapong et al., 2014.). Histological and physiological study of the alimentary canal in reptiles showed the type of the food and feeding habits (Secor and Diamond, 1998). Research showed information on histological study of intestine is scarce (Hewitson and Darby, 2010). The aim of this paper was to analyze the histological and histochemical structures of the alimentary canal of *H. cyanocinctus*.

2. Materials and Methods

For this study, six *H. cyanocinctus* from Minab beaches located near the sea of Oman were gathered by trawl. The snakes were euthanized by chloroform (Unver and Saraydın, 2012). Samples were examined for abnormal signs such as infection, bleeding and skin parasite, disectesd, and alimentary canal was removed to be prepared with routine procedures of tissue preparation. Each part from different regions of intestine was taken, cleaned with saline solution and fixed in Bouin solution then washed and dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin. Six µm thick sections were prepared, stained with haematoxylin and eosin for general studies and with PAS (to detect carbohydrate and mucosa compounds) and observed and photographed under light microscope (An et al., 2014). All data are presented as (Means ± Se). Data are checked for normality by Kolmogorov–Smirnoff test and analyzed for statistical differences by one - way ANOVA. Differences between and within groups were tested using one-way variance analysis followed by the Tukey's comparison test. Differences were considered significant when P value was lower than 0.05. The statistical analysis was performed using the SPSS (version 18, Chicago, USA) (Edwards et al., 2013).

3. Results

Small intestine had circular folds with intestinal villi. In this area, circular folds were long, narrow, irregular and with zigzag -shaped pattern. The lumen of the small intestine was narrow that caused the presence of extremely long and coiled villi. The mucosa of the small intestine was covered with simple columnar epithelium. Two basic types of cells were presented in the intestinal lining epithelium, columnar and goblet cells. The columnar cells had large elongated nuclei situated at the base of cells and goblet cells secreted mucinous substances and reacted strongly with PAS. Lamina propria contained connective tissue cells and blood vessels. Muscularis mucosa separated the lamina propria of the submucosa and narrow strands of it entered circular folds. Submucosa was rich of blood vessels and connective tissue cells. Muscularis layer formed of circular and longitudinal layers. Circular layer was thicker and located on the inside but longitudinal layer was thinner and located on the outside. The outer thin layer of squamous cells was the serosa that is shown in (Figs 1- 3).

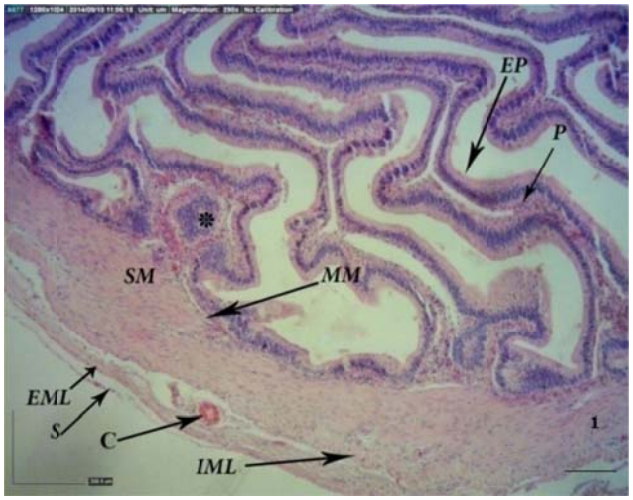

Fig. 1: The walls of the small intestine of sea snake H. cyanocinctus: Epithelial (EP), Lamina propria (L), Muscularis mucosa (MM), Submucosa (SM), Lymphoid communities (*), Internal muscular layer (IML), External muscular layer (EML), Capillary (C) and Serous (S), (H & E, x290)

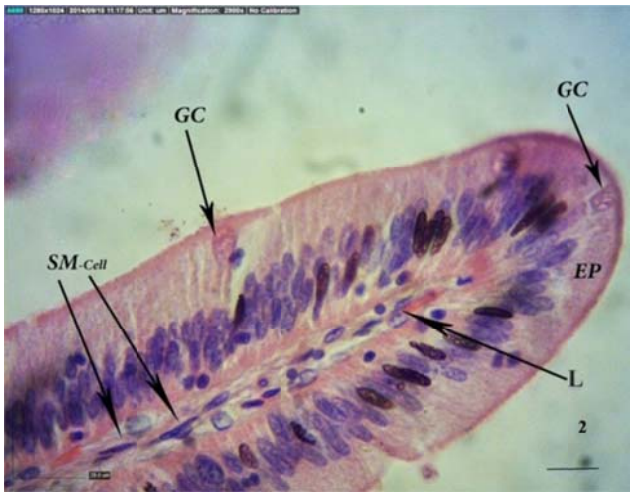

Fig. 2: The circular folds of small intestine of sea snake *H. cyanocinctus*: Simple columnar epithelium (EP), Goblet cells (GC), Lamina propria (L) and Smooth muscle cells (SM-CELL), (H & E, x2900).

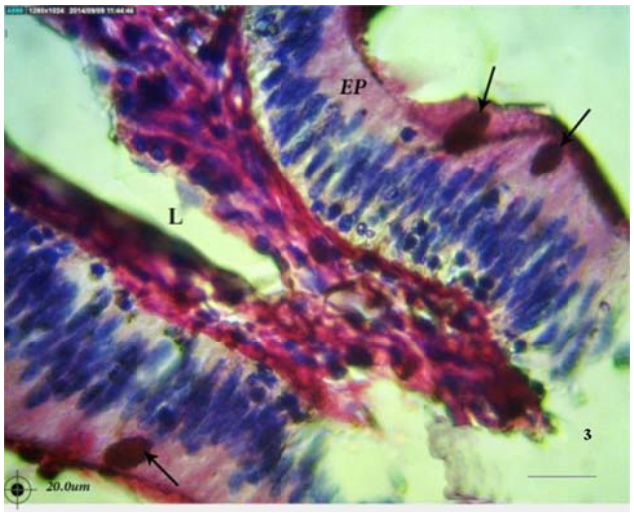

Fig. 3: The goblet cells of epithelium of sea snake *H. cyanocinctus*: Goblet cells (arrowhead), Epithelial (EP) and Lamina propria (L), (PAS, x2900).

3.1. Large Intestine

Long simple columnar cells with acidophilic cytoplasm cover the epithelial of large intestine. Goblet cells were found scattered in the epithelial tissue. Columnar and goblet cells stained very well with PAS stain. Connective tissue was located underneath the epithelia Lamina propria containing connective tissue and blood vessels and infiltrated into center of each intestinal villi. At the base of each intestinal fold, muscularis mucosa was located and folded into internal and external layers. Immediately, underneath muscularis mucosa, connective tissue of submucosa was peresent. Muscularis was observed in two layers, both made of smooth muscle layers. Circular thick layer was inside and longitudinal muscle layer with less thickness outside. In the outermost layer serosa was present (Figure 4 - 6).

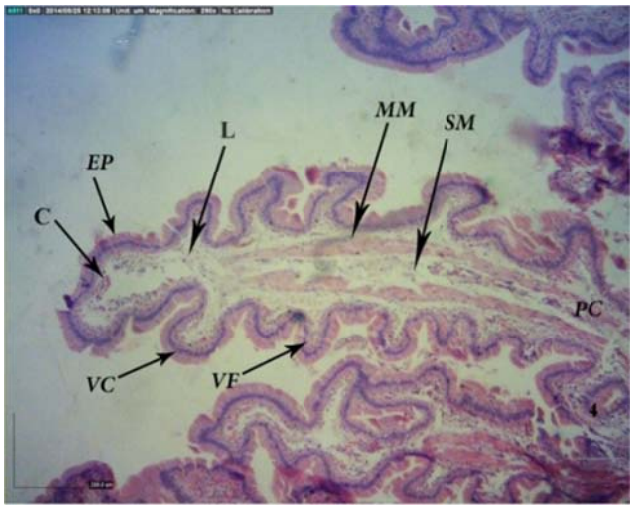

Fig. 4: The plicae circulares (PC) of large intestine of sea snake *H. cyanocinctus*: Simple columnar epithelial (EP), Lamina propria (L), Capillaries (C), Villi fingered (VF), Villi club (VC), Muscularis mucosa (MM) and Submucosa (SM), (H & E, 290).

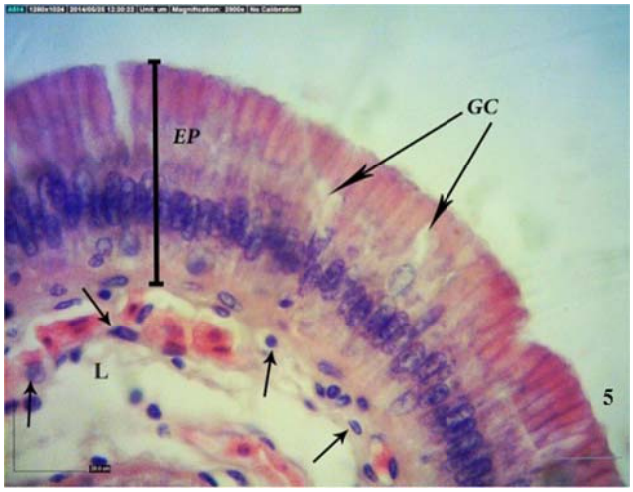

Fig. 5: The epithelium of large intestine of sea snake *H. cyanocinctus*: Simple columnar epithelium (EP), Goblet cells (GC), Lamina propria (L) and Connective tissue cells (arrowhead), (H & E, 2900).

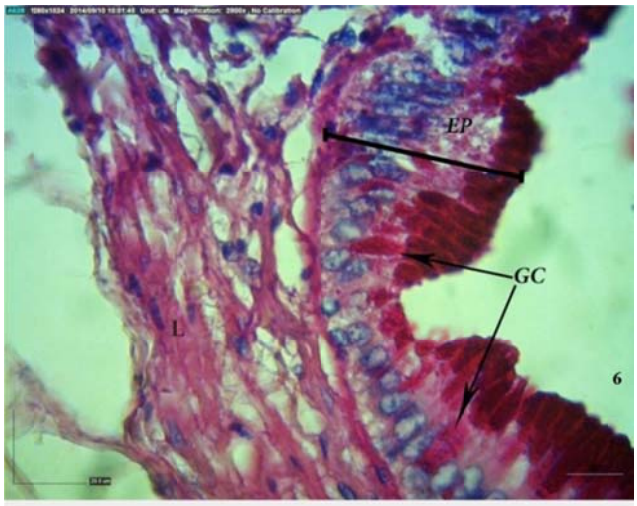

Fig. 6: The epithelium of large intestine of sea snake *H. cyanocinctus*: Simple columnar epithelium (EP), Goblet cells (GC), Lamina propria (P), (PAS, 2900).

Histometrical results showed that epithelium length and width in the primary of the small intestine were greater than other parts of it. Goblet cells with 15.36 ± 1.12µm were the longest. Thickness of both internal and external muscular layers were the most at the end of small intestine ($p \leq 0.05$) (Table 1).

4. Discussion

Results of histological and histochemical studies showed that the structure of intestines of sea snake *H. cyanocinctus* was similar to that of other reptiles with minor differences (Abdeen et al., 2013; Close and Cundall, 2014; De Oliveira et al., 2008). The walls of the intestines were composed of four original layers: mucosa, submucosa, muscularis and serosa from inside to the outside which is in agreement with other studies (Abo- Eleneen, 2010, Albrecht et al., 2001, Damotharan et al., 2010). In the present study, the mucosa of small intestine showed circular folds which was covered by tiny intestinal villus and epithelial tissue cells. Researchers reported that the mucosal folds of the small intestine form short and blunt projections presenting the villi (El- Bakry et al., 2012). The Zig–zag pattern of intestinal mucosa can be an adaptation for increasing the Scale of absorption in the present snake. The mucosal lining of the small intestine consisted of simple columnar long cells. This type of epithelial tissue has been reported for all common reptiles (Cakici and Akat, 2013). In addition to columnar cells in the epithelial tissue of the small intestine of sea snake, the goblet cells were also presented in large numbers. The columnar epithelium of the intestinal mucosa may have an absorptive function (Elliott, 2007).

Table 1: Mean of histometrical factors (μm^2) of different parts of intestine of *H. cyanacinctus*. Each factor was compared with others.

	Primary of small intestine	**End of small intestine**	**Large intestine**
Epithelial length	51.96±7.09*	47.50±9.25**	46.75±2.98***
Epithelial width	98.78±2.57*	76.01±1.24**	71.39±3.54***
Goblet cells	4.94±1.03*	7.92±1.23**	15.36±1.12***
Muscular thickness (Internal layer)	103.50±2.25*	143.06±13.48**	39.51±3.72***
Muscular thickness (External layer)	94.20±3.75*	106.62±4.35**	28.78±1.57***

* $p \leq 0.05$

** $p \leq 0.01$

*** $p \leq 0.001$

In lower part of the epithelial tissue of *H. cyanocincyus* small intestine, connective tissue and blood vessels were present, and muscularis mucosa was narrow and composed of a layer of smooth muscle cells. Similar to other reptiles, muscularis layer of small intestine was smooth and consisted of two layers (Putterill and Soley, 2003). There were no glands in the small intestine of *H. cyanocincyus* as indicated by Holmberg et al., 2002b. The mucosal epithelium of large intestine was built up of simple columnar cells with several sporadic goblet cells and both reacted with PAS, especially columnar cells that were highly stained. The positive response of the mucosal epithelial cells of the large intestine of the investigated species proves the abundant secretion of mucin of these cells. This may be considered as a well adaptive character of the large intestine to function in its environment (Firmiano et al., 2011). Abdeen et al. (2013) while studied the large intestine of the *Ramphotyphlops braminus* snake reported that in mucosa, a thin layer of muscle was present, which is the same layer as muscularis mucosa in this study. Then, was the submucosa layer, which is equivalent to the connective tissue, rich in blood vessels. Muscularis was made of a thick layer of longitudinal cells on the inside and a thin layer of circular cells on the outside. Serosa was located in the outermost part of the wall. Similar to most reptiles, the intestinal gland in the large intestine was not found in *H. cyanocinctus* (Gasperetti, 1988; Hamdi et al., 2014).

Reference

Abdeen, MA., Mostafa, AN., Abo-Eleneen, ER., Elsadany, AD., 2013. Anatomical studies on the alimentary tract of the Egyptian Typhlopid snake, *Rhamphotyphlops braminus*. Journal of American Science 9: 12-21.

Abo- Eleneen, RE., 2010. Comparative histological and histochemical studies on the mucosa of the digestive tract of the herbivore *Uromasatyxae gyptius* and the carnivore Varanusniloticus. Journal of the Egyptian German Society of Zoology 60: 1- 35.

Ahmed, YA., El-Hafez, A., Zayed, AE., 2009. Histological and histochemical studies on the Esophagus, stomach and small intestines of *Varanus niloticus*. Journal of Aeterinary Anatomy 2: 35-48.

Albrecht, MP., Ferreira, MF., Caramasch, EP., 2001. Anatomical features and histology of the digestive tract of two related neotropical omnivorous fishes (*Caraciformes; Anostomidae*). Journal of Fish Biology 58: 419-430.

Amber, K., Gillett, M., Lyndal, H., Jon, H., Paul, C., 2015. Haematological and biochemical reference intervals for three species of hydrophiine sea snakes (*Hydrophis curtus*, *H. elegans* and *H. peronii*) in Australia. Veterinary Journal 204 (3): 275-281.

An, H., Kang, H., Han, H., 2014. Isolation and characterization of 26 novel poly-nucleotide microsatellites from short barbeled grunter (*Hapalogenys nitens*) for genetic analysis. Conservation Genetics Resources 6: 669–672.

Cakici, O., Akat, E., 2013. Some histomorphological and histochemical characteristics of the digestive tract of the snake-eyed lizard, *Ophisopselegans menetries*, 1832 (Squamata: Lacertidae). North-Western Journal of Zoology 9: 257-263.

Close, M., Cundall, D. 2014. Snake lower jaw skin: extension and recovery of a hyperextensible keratinized integument. Journal of Experimental Zoology 321(2): 78–97.

Damotharan, P., Arumugam, M., Vijayalakshmi, S., Balasubramanian, T. 2010. Diversity, biology and ecology of sea snakes (*Hydrophiidae*) distributed along The Parangipettai coast, southeast coast of India. International Journal of Current Research 4: 62-69.

De Oliveira, L., Jared, C., da Costa Prudente, A.L., Zaher, H., Antoniazzi, M.M. 2008. Oral glands in dipsadine ‘‘*goo-eater*’’ snakes: morphology and

histochemistry of the infralabial glands *in Atractus reticulatus*, *Dipsas indica*, and *Sibynomorphus mikanii*. Toxicon 51(5): 898–913.

Edwards, MA., Frazer, TK., Jacoby, CA., 2013. Age and growth of invasive lion fish (*Pterois* spp.) in the Caribbean Sea, with implications for management. Bulletin of Marine Science 90: 953–966.

El- Bakry, AM., Abdeen, AM., Abo- Eleneen, RE., 2012. Anatomical and morphometrical study of the alimentary canal of the lizard *Scincus scincus* and the snake *Natrix tessellate*. Life Science Journal. 9: 1010-1022.

Elliott, JR., 2007. Overview of Reptile Biology, Anatomy, and Histology. Infectious Diseases and Pathology of Reptiles. Edited by Elliott JR, Brooklyn, New York, Taylor & Francis Group: 1-25.

Ferri, D., Liquori, GE., 1994. Immunohistochemical investigations on the pyloric glands of the ruin lizard (Podarcis sicula campestris de betta). Acta Histochemica 96: 96-103.

Firmiano, EM., Cardoso, NN., Vieira, DA., Sales, A., Santos, MA., 2011. Histological study of the liver of the lizard, *Tropidurus torquatus* Wied 1820, (Squamata: Tropiduridae). Journal of Morphological Science 28: 165-170.

Gasperetti, J., 1988. The snakes of Saudi Arabia. In: Fauna of Saudi Arabia, Riyadh, Saudi Arabia, National Commission of Wildlife Conservation and Development (NCWCD). 19: 169–450.

Hamdi, H., El-Ghareeb, AW., Zaher, M., Essa, A., Lahsik, S., 2014. Anatomical, histological and histochemical adaptations of the reptilian alimentary canal to their food habits: II-*Chamaeleona fricanus*. World Applied Sciences Journal 30: 1306-1316.

Hewitson, TD., Darby, IA., 2010. Histology Protocols. 1thED. New York. Humana Press Inc 325P

Holmberg, A., Kaim, J., Persson, A., Jensen, J., 2002b. Effects of digestive status on the reptilian gut. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 133: 499-518.

Nardini, G., Leopardi, S., Bielli, M., 2013. Clinical Hematology in Reptilian Species. Veterinary Clinics of North America: Exotic Animal Practice 16: 1–30.

Putterill, JF., Soley, JT., 2003. General morphology of the oral cavity of the Nile crocodile, *Crocodylus niloticus* (Laurenti, 1768). I. Palate and gingivae. Onderstepoort. Journal of veterinary research 70: 281-97.

Rezaie-Atagholipour, R., Riyahi-Bakhtiari, A., Sajjadi, M., Kong, C., 2012. Metal concentrations in selected tissues and main prey species of the annulated sea snake (*Hydrophis cyanocinctus*) in the Hara Protected Area, northeastern coast of the Persian Gulf, Iran. Marine Pollution Bulletin 64: 416-421.

Secor, SM., Diamond, J., 1998. A vertebrate model of extreme physiological regulation. Nature 395: 659-662.

Unver, S., Saraydın, SU., 2012. Macroscopical and histological analysis of gonadal development of *Squalius cephalus* (L., 1758) in Tödürge Lake, Turkey. Pakistan Veterinary Journal. 32(1): 55-59.

Vasaruchapong, T., Disarapong, P., Chulasugandha, P., 2014. Comparative studies on hematological and plasma biochemical parameters in different types of venomous snakes in Thailand. Comparative Clinical Pathology 23: 955-959.