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HISTOLOGY AND STUDY OF SOME BLOOD BIOCHEMICAL PARAMETERS OF SEA SNAKE *HYDROPHIS CYANOCINCTUS* (DAUDIN, 1803) IN COLD SEASON IN MINAB, OMAN SEA- IRAN

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ABSTRACT

Study of blood biochemistry provides low cost tools to assess the health condition in live reptiles. Even it is useful to identify the changes of visceral organ function prior to the sign of the clinical abnormality. This research has investigated natural values of some most important blood biochemical parameters of sea snake *H. cyanocinctus* in Minab city of the Oman sea. For this purpose blood was collected from 10 adult male and female snakes from ventral tail vein in winter. Then samples were taken to the central laboratory of Minab in standard conditions. Parameters including sodium, potassium, chloride, calcium, magnesium, glucose, albumin, urea, total protein and creatinine measured after separation of serum and obtained results were compared with male and female snakes by t-test. Comparative studies of male and female samples showed significant statistical differences of some parameters including calcium, glucose and albumin. In comparison the values of this parameters in females was more than males. But, in other parameters no significant differences was detected. Also magnesium was the lowest amount and was seen in females while sodium was the highest amount and was seen in males. Results obtained in these species have differences and similarities to the other species that have been examined in the present study.

Keywords: Hydrophis cyanocinctus, sea snake, biochemical parameters, Minab

INTRODUCTION

Sea snakes are the largest and most complete group of marine reptiles. They constitute 86 percent of total marine reptiles. They inhabit in the tropical and sub tropical Indo-Pacific coastal habitat (Rasmussen et al., 2011). These species can be found in the Malay Archipelago, Indonesia, India, China and parts of Australia. They inhabit in shallow waters alongside the coast, around the islands and mouth of the river. They can dive into depths as great as 100 meters and have an important role in the food chains of these communities (Ineich, 1996). Sea snake, H. cyanocinctus has the highest distribution in the Persian Gulf and western areas of the Oman Sea (Gasperetti, 1988). It has dark transverse lines and olive green colors. The combination of blood parameters required for detection of physiological stress and clinical evaluations in reptiles (Campbell et al., 2007; Vasaruchapong et al., 2014). Researchers believe that the plasma biochemical parameters are useful to assess and management of physiological status. Their levels are useful to identify the changes of visceral organ function prior to the sign of the clinical abnormality (Vasaruchapong et al., 2014). Plasma biochemical parameters activity show seasonality, phase of reproductive cycle, age, sex, geophysical conditions of the habitat, acclimation temperature and photo period (Lillywhite et al., 1984). In addition, different physiologic conditions and adaptations to the environment in reptiles cause changes at the levels of these values. For this reason obtaining a reference value in hematology that studies on reptiles is difficult (Parida et al., 2013). Also physiological and clinical studies of reptiles such as snakes is challenging for researchers, because they are poisonous and dangerous. According to our research, there are few studies in this field about sea snakes. But more studies were about terrestrial snakes. For this purpose, this study has investigated important plasma biochemical parameters of sea snake *H. cyanocinctus*, in the Minab beaches, located in the Oman Sea.

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MATERIALS AND METHODS

Ten adult male and female sea snakes *H. cyanocinctus* were collected from Minab beaches, located in the Oman Sea, in winter. At first, samples examined for healthy population, so their body observed carefully for abnormal signs such as infection, bleeding and skin parasite. Before blood collection, snakes were measured in weight and length. The (Mean \pm SE) of weight for males was $552\pm73/3$ gr whereas for females was $555\pm33/4$ gr. The (Mean \pm SE) of length for males was $122\pm23/2$ cm whereas for females was $121\pm05/3$ cm. The place of blood collection cleaned aseptically. Blood was collected from each snake's ventral tail vein, as described by Tosunoglu *et al.*, (2011). A needle 22 to 25 gauge was inserted at an angle of 45° to 60° between the scales on ventral midline. Blood of each snake was collected in a sterile tube containing lithium heparin, as an anticoagulant (Abou-Madi and Jacobson, 2003). Then snakes were transported to their habitat and released. Samples were immediately placed at nitrogen tanks and quickly moved to the central laboratory of Minab city. After centrifugation and separation of serum, samples were examined and for assess levels off actors performed required tests. The results were analyzed with SPSS software and values were determined as minimum, maximum, mean, range, standard deviation, total range and total standard deviation. For comparison between the two sexes were used the t-test data confidence level of p<0.05 percent.

RESULTS

In tissue studies of red blood cells, it was determined that some had an oval and some had a relatively round nucleus. This cells were also relatively large, with a length of 24 ± 1 and a width of 18 ± 1 microns (fig 1). The results of blood biochemical parameters in sea snake *H. cyanocinctus* in winter were compared according to sex and parameters based on the chemical composition type, were summarized in tables (1) and (2). According to (1) there was no significant difference in data values for sodium, chloride and potassium in both males and females (p<0.05). Also based on this table the measured value for calcium in both males and females, was12.28 \pm 0.42 and 13.21 \pm 0.41 mmol / 1 which based on the t-test a significant difference between male and female samples observed (p<0.05). Magnesium and phosphorus values in both males and females were determined and there was no significant difference between them.



Figure 1: In this image, the oval nucleus with horizontal arrow and the circular nucleus with vertical arrow are shown (Gimsa, x100).

Table1:	(Mean	±	SD)	biochemical	parameters	of	blood	samples	of	male	and	female	sea	snake	Н.
cyanocin	ctus														

Parameters	Sex	Min.	Max.	Mean±SD	Mean±SD (total)
Sodium(mmol/L)	Male	158	165	160.67 ± 2.78	
	Female	153	162	157.33±3.65	159 ±3.55
Chloride (mmol/L)	Male	134	143	139.25 ± 3.62	
	Female	132	141	136.92±3.49	138±3.61
Potassium(mmol/L)	Male	4.5	8.5	6.25 ± 1.36	
	Female	3.8	8.5	5.65 ± 1.56	5.95±1.34

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Calcium [*]	Male	11.90	12.80	12.28 ± 0.42	
(mmol/L)	Female	12.70	13.80	13.21 ± 0.41	12.75±0.64
Magnesium(mmol/L)	Male	1.90	2.80	2.25 ± 0.39	
	Female	1.70	2.90	2.20 ± 0.48	2.22±0.46
Phosphorus(mmol/L)	Male	3.20	5.60	4.11 ± 0.87	
	Female	4	5.30	4.58 ± 0.44	4.35±0.70

* indicates a different value significantly (P<0.05). Min: minimum, Max: maximum, SD: standard deviation

Based on table (2) the values of albumin and glucose in both males and females were determined as 3.14 ± 0.11 , $3.50 \pm 1.05 \text{ mg} / \text{dL}$, $68.04 \pm 3.18 \text{ and} 72.4 \pm 5.08 \text{ mg} / \text{dL}$ and t-test showed a significant difference between them (p<0.05). In comparison, there was no significant difference between values of uric acid, creatin and total protein (p<0.05).

Table 2: (Mean \pm SD) biochemical parameters of blood samples of male and female in sea snake *H*. *cyanocinctus*

Parameters	Sex	Min.	Max.	Mean±SD	Mean±SD(total)
Albumin [*]	Male	2.7	3.4	3.14 ± 0.11	
(mg/dL)	Female	2.8	4.2	3.50 ± 1.05	3.32±0.58
Uric acid	Male	9.8	11.8	10.96 ± 1.23	
(mg/dL)	Female	9	10.8	10.07 ± 1.49	10.51±1.36
Creatinine (mg/dL)	Male	0.31	0.88	0.58 ± 0.02	
	Female	0.28	0.78	0.51 ± 0.01	0.54±0.015
Glucose [*]	Male	63	75	68.04 ± 3.18	
(mg/dL)	Female	62	82	72.4 ± 5.08	70.40±4.13
Total protein	Male	4.3	5.8	5.02 ± 0.87	
(g/dL)	Female	3.9	5.8	4.92 ± 1.11	4.97±0.94

* indicates a value significantly different (P<0.05).

Min: minimum, Max: maximum, SD: standard deviation.

In this study among biochemical parameters in both males and females significant differences observed between calcium, albumin and glucose values (P<0.05). So that this values have been achieved higher in females rather than males. Also Phosphorus levels were measured higher in females than males, but there were no significant difference between them. Other parameters were higher in males than females but there were no significant difference between them.

DISCUSSION

In relation to blood biochemical parameters of sea snakes *H. cyanocinctus*, there is no published comprehensive work, also in other marine species there are a few publications. Evaluation of blood biochemical parameters is a common approach for clinical diagnosis to give essential information on sea snake's physiological conditions (Martinez *et al.*, 2004). It has been confirmed that any change in these factors is due to water quality, infectious disease and toxic contamination. Physiological effects of environmental stressors might change some biochemical parameters (Vasaruchapong *et al.*, 2014). Researchers believe that, it is important to establish reference ranges of hematological and biochemical parameters of blood for disease diagnosis, health monitoring, and detection of any ecological and geographical differences in sea snakes (Tosunoglu *et al.*, 2011). About 80% of blood osmolarity in marine is due to sodium and chloride ions (Banfalvi, 1991). On the other hand marine reptiles, especially present snake, live in an osmotically challenging environment where the concentration of salt in their surroundings is approximately three times greater than their internal environment (Reina and Cooper, 2000). Therefore, plasma concentration of sodium can be directly reflecting the results of osmotic challenges in

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the sea snakes. The Mean±SD of sodium values for both sexes of sea snake H. cyanocinctus was obtained 159 ±3.55 mmol/L. In this regard, Dessauer (1970) during the study on plasma sodium for physiological and evolutionary aspects of sea snake, Laticaudasemi fasciata reported values of 159 mmol/L. Despite the presence of this snakes in hyperosmoticall environment, researchers reported that the values of sodium, chloride and potassium are similar to terrestrial snakes values (Vasaruchapong et al., 2014; Nardini et al., 2013). Reported values of sodium, potassium and chloride in the snake Siamese cobra are 153.3±12.2, 5.0±1.5 and 134.1±12.2 mmol / 1. Also in species such as Pseudonaja Nuchalis, Notechis scutatus, Pseudechis prophyriacus and Austerelaps superbus were reported similar to our findings (Pal et al., 2008). Potassium is the major item that present within cells, so minor changes in blood levels may have fatal cardioarrhythmic effects, so maintaining homeostasis is extremely important for survival (Eatwell et al., 2014). According to this study, blood plasma calcium in both sexes had a significant difference as in females was more than males. Similar studies showed that in the reptiles this case occurs when female is pregnant. For instance Knotkova et al (2005) reported high levels of blood calcium in females and has been reported cause it the metabolism and activity during pregnancy for females. Also Eatwell et al (2014) in their study reported during the pregnancy, the calcium level increases naturally in female reptiles. Also, a number of researchers have reported that stress is one of the factors that may lead to high levels of calcium until 200 mmol/L (Hidalgo et al., 2007). In this study the values of phosphorus were measured in females more than males, but no significant difference was detected between them. Researchers also reported that the phosphorus levels are generally related to calcium because of the effects of parathyroid and calcitonin hormones (Eatwell et al., 2014). Calcium to phosphorus proportion is a sensitive parameter for the detection of early stages of kidney disease in reptiles (Kolle and Hoffmann, 2001). Phosphorus is usually excreted through the kidneys, so any reductions in renal blood flow often result in hyper phosphataemia (Parida et al., 2013; Eatwell et al., 2014). The values of magnesium in present snake obtained 2.22 mmol / l which is similar to other studies published and reported that its levels highly increase in stress state (Coz-Rakovac et al., 2011; Dessauer, 1970). As was observed such as calcium, glucose and albumin levels in both males and females had significant difference. Glucose levels in reptiles can be influenced by internal and external factors. It is increased often in stress conditions and water temperature changes (Eatwell et al., 2014). In this study, albumin was obtained 3.32 mg/dL that is more of the reported values in other sea snakes (Gillett et al., 2015). Albumin is an important protein that is synthesized in the liver. It plays a key role in providing colloidal oncotic pressure (Eatwell et al., 2014). Albumin increase in female snakes further relation to water loss and pregnancy (Stahl et al., 2006; Rezaie-Atagholipour et al., 2012). In this snakes the pregnancy is more likely. Reptiles are uricotelic and the end product of catabolic of nitrogen removal is uric acid (Arikan et al., 2009). The nutrition, dehydration and kidney diseases associated with hype ruricemia (Gillett et al., 2015). The value of uric acid in present snake was measured more than other snakes. For instance uric acid were reported in three species of sea snake, Hydrophis curtus, Hydrophis elegans and Hydrophis peronii, about 8.06 mg / dL (Stahl et al., 2006). The value of creatinine in this snake was determined 0.54 mg/dL that is nearby to the reported values of other sea snakes species and mentioned this amount is normal for any sea snakes that have normal activities (Stahl et al., 2006). The values of total protein in present sea snake was 4.97 g/dL that slightly lower than reported values of other snakes. The value of total protein in Najaka outhia and Ophiophagus hannah was 5.8 g /dL and 6.0 g / dL. The reason for this difference can be justified based on the ambient temperature (Amber et al., 2015). This report, unlike our work has been obtained for the warm season. It should be noted that the value biochemical characteristics described in this study can be considered as the standard source for sea snake H. cyanocinctus and other similar species.

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