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Anatomical and Histological Study of the Excretory System in the Bosc's Fringe-Toed Lizard (*Acanthodactylus boskianus*)

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Abstract: The lizard, *Acanthodactylus boskianus* has recently been reported from the West of Iran. The purpose of this study was to examine the morphology and histological structure of excretory system in this species. To perform this task, a number of 12 lizards (male and female) were collected from their natural habitat and transferred to the laboratory where their kidneys and excretory ducts were dissected out. The dissected samples were then processed to be prepared for histological examinations. Microscopical observations showed that in this taxon there is no evident boundary between the cortical and medullar regions of kidneys. It also demonstrated that each kidney consists of very few nephrons which do not show any loop of Henle. Each nephron is composed of a glomerulus surrounded by a doubled-walled Bowman's capsule, the other segments are proximal tubule, distal tubule, connecting tubule and collecting duct. The epithelial tissues lining the lumen of these segments were simple cubiodal cells, cubiodal cells, columnar and tall columnar cells, respectively. A ureter is located on the ventral surface of each kidney. The ureters open into the urodaeal cavity of the cloaca with a common duct or tube. In general, this lizard as other reptiles live in such areas display few glumeruli in the kidneys and highly likely produce the urine that is hyposmotic with blood.

Key words: *Acanthodactylus boskianus*, excretory system, nephron segments, tubular epithelium, Iran

INTRODUCTION

The morphology and physiology of reptilian kidneys has previously been investigated by some researchers (Roberts and Schmidt-Nielsen, 1966; Davis and Schmidt-Nielsen, 1967). These investigations have revealed that the morphology of the tubular cells is similar to what seen in avian kidneys but shows considerable differences from that of mammalian and amphibian kidneys. Like other vertebrates, the functional units of kidneys are called nephrons (Bradshaw and Bradshaw, 2002). Unlike the human and other mammals whose kidneys consist of approximately 2 million nephrons, reptiles have a relatively few nephrons. In reptiles, each nephron is comprised of several segments including, renal corpuscule (Bowman's capsule plus glomerulus), the proximal tubule, distal tubule which connects to the collecting duct (Jacobson, 2007; Davis *et al*., 1976). However, there are variations and differences in the structure and functioning of various reptilian kidneys that adapt them to the environment in which the animals live. These include variations in the number, size, length and structural complexity of nephrons. To illustrate, it has been reported that some reptiles have no glumeruli at all. Moreover, threre is a report that snakes and lizards have many aglomerular nephrons. The glumeruli of snakes and lizards are small and few in number whereas those of aquatic turtles are much larger and more abundant (Edward, 1998; Mcnab, 2002). The Bosc's fringe-toed lizard, *Acanthodactylus boskianus* has recently been collected and reported from West of Iran (Rastegar-Pouyani, 2000). This taxon is usually found in semi-arid areas and live on the sandy hills covered by various species of *Astragalus* sp. Considering its harsh habitat, this species was chosen to determine whether there is any specific morphological and histological characters which are not found in related species found in more humid regions.

MATERIALS AND METHODS

Specimens of *A. boskianus* were collected from the vicinity of Harsin (34°17'N, 47°24'E), a town in Kermanshah province, West of Iran in Spring and Summer, 2010. A sample of 12 females and males was examined in this research. Snout-vent and tail lengths (to the nearest 0.5 mm) were measured for each lizard. The largest specimen used for this research had a 60.5 mm snout-vent length and 115 mm tail length. Specimens were then euthanized with ether within 2 days of collection. The gender of each animal was determined according to morphological features and testified after dissection. The specimens were dissected and the excretory system (kidney, ureter and cloaca) was removed from each

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individual. The excretory system was immediately fixed in 4% formalin saline fixative. Tissues subsequently were dehydrated in a graded series of ethanol, cleared in xylene and embedded in paraffin. About 7 micron sections were prepared by a rotary microtome and the sections were then stained with routine Haematoxylin-Eosin protocol. The sections were finally observed under a Nikon microscope equipped with a camera for photography.

RESULTS

Morphologically, the excretory organs consist of two kidneys, two ureters and a cloaca, all of which contribute to the final composition of the urine. The two kidneys seem flatten and the symmetrical organs are located on each side of the cloaca. They lie dorsal to the peritoneum against the body wall. They present a convex laterodorsal surface and a more flattened medioventral surface containing the renal vessels. The firm, light-reddish kidneys are covered by a connective tissue capsule. Each kidney consists of seven to eight lobules. A ureter is located on the ventral surface of each kidney. Ureters are opened into the ureodeum of the cloaca (Fig. 1).

In terms of histology, the transverse sections of the kidney showed that it is surrounded by a very thin capsule, composed of reticular fibers and smooth muscles (Fig. 2). There is no distinct border between the cortex and medulla (Fig. 1). Few of nephrons and urinary tubules all were connected by connective tissues (Fig. 2). The examinations revealed that in this taxon glomeruli consist of a simpler system of capillary loops with connective tissues, surrounded by a doubled-layered Bowman's capsule including an outer (Parietal) and an inner (Visceral) layer. The results also clarified that glomeruli have two poles, urinary and vascular (Fig. 3a). Four types of tubular segments were found in each nephron. The first proximal tubule is lined by a layer of cuboidal cells. These cells display a dense cytoplasm and moderately dense nuclei located near to the cell base. The cells in the proximal tubules contain microvilli (Fig. 3b). The next tubule is the distal tubule and lined by cuboidal cells which are recognized by their rare cytoplasm and dense nuclei which are centrally or basally located (Fig. 3c). The distal tubule is followed by connecting tubule which is lined by low columner cells. There are two types of cells in the connecting tubule. The pink cells, believed to be mucosa cells are distributed between the non mucous cells (cells having a fairly dense cytoplasm and dense nuclei which are basally located). The mucous cells are taller than the proximal or distal tubular cells and contain basally located nuclei. In many instances, the apical

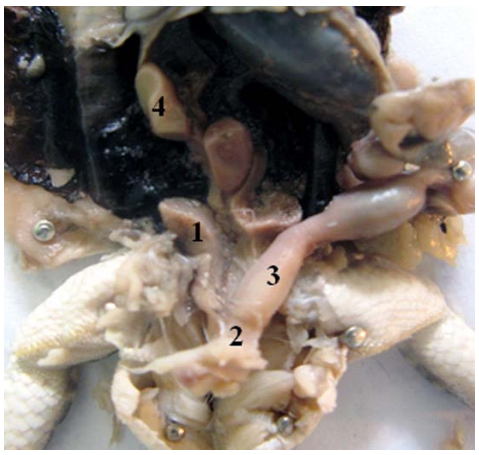

Fig. 1: Displaying the position of kidneys and other organ in the lower region of the body cavity: 1) Kidney; 2) Cloaca; 3) Rectum; 4) testis; ×2

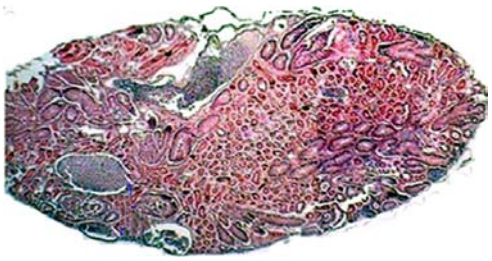

Fig. 2: A transverse section of the kidney showing the organ is surrounded by thin capsule and tubular segments are observed in stroma; ×100; H&E

regions of the cytoplasm bulge into the lumen as though in an active process of excreting their contents into the tubular lumen. The non mucous cells are about the same size as mucous cells (Fig. 3c).

The collecting duct shows a larger diameter than the previous segment. The tall columnar cells in the collecting duct represent the largest cell type found in the entire renal tubule. The nuclei of these cells are basally located and are surrounded by a moderately dense, thin fringe of cytoplasm. The apical two-thirds of the cells, however are slightly stained and contain vacuoles and granular materials. In most cases, the plasma membranes between the cells are clearly defined (Fig. 3d).

The ureters have been descended to lie dorsally. They are embedded dorsolaterally in an urodaeum and lined with a simple columnar epithelium (Fig. 4a). The ureters are very close to each other between which there is a common duct. At the end, the common duct joins with the urodaeal cavity. Each of the ureters are lined with a stratified epithelium (transitional, mostly cuboidal), involves several layers in thickness (Fig. 4b).

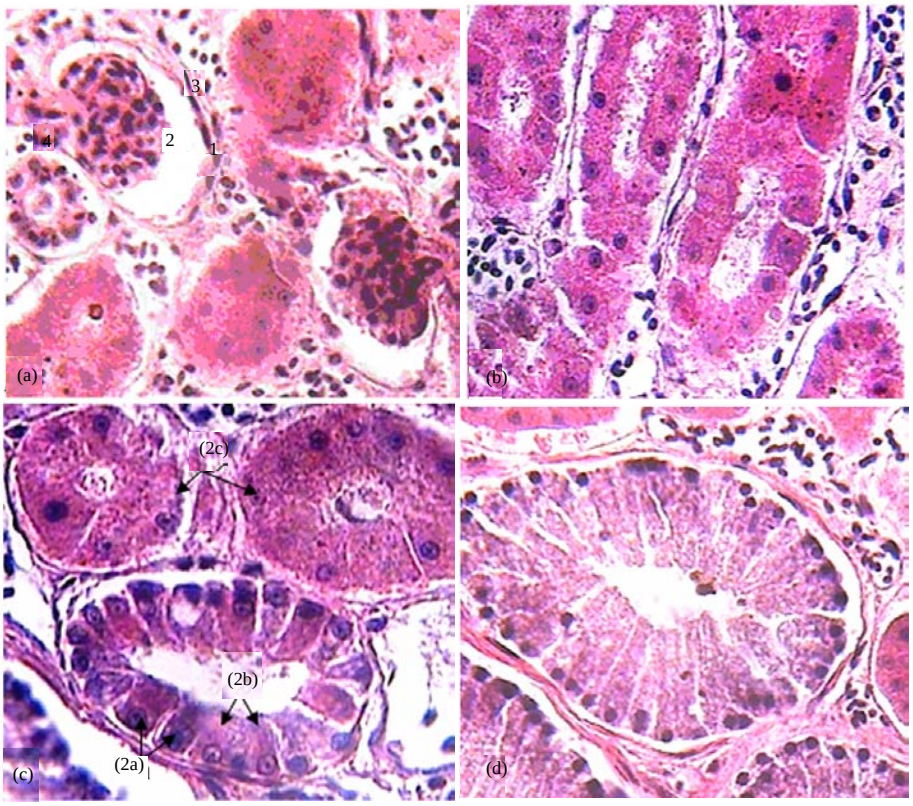

Fig. 3: Micrographs showing cross sections of different kidney's tubular segments: a) Histology of glomeruli and Bowman's capsule revealing the parietal (1) and visceral (2) layers, urinary pole (3) and vascular pole (4); b) A cross section of proximal tubules with its cuboidal cells; c) Distal tubules are lined by a layer of cuboidal cells (1) while two types of cells line the connecting tubule, non mucous cells (2a) and the mucous cells (2b); d) A histological section through a collecting duct; the tall columnar cells in the collecting duct represent the largest cell type in the entire renal tubule; ×400; H&E

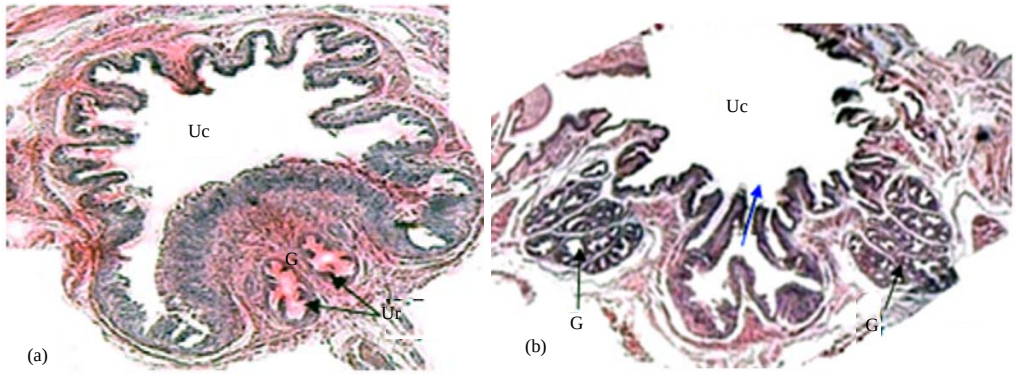

Fig. 4: Micrographs taken from ureters and the common duct: a) The Ureters (Ur) are embedded dorsolaterally in the (Uc) Urodaeum cavity; b) The common duct between ureters is opening to the Urodeum cavity (Uc) (colored arrow denotes merging point); Glands (G); ×400; H&E

DISCUSSION

It is thought that the number of glumeruli in kidney is related to the habitat of the animal, since lower glumerular filtration rate is associated with a smaller output of urine. The lizard *A. boskianus* inhabits a semi-desert environment in the west of Iran and need to conserve water due to a limit water supply. The results

presented here demonstrated that in agreement with other reports performed on lizards inhabiting arid environment, *A. boskianus* also displays a small number of glumuli in the kidney. Even though, the real number of glumeuli was not counted here but according to the observations made on serial sections it was possible to make a rough estimation. It was estimated that this number is around 1500 for each kidney. In the lizard, *Sceloporus cyanogenys* inhabiting an arid environment in the South-Western part of the United State a number of 2000 glumeluli has been counted in two kidneys (Davis *et al*., 1976). It has also been reported that in the lizard *Uromastyx microlopis* collected from Iraq, neighboring to Iran and not very far from where *A. boskianus* was collected, kidneys display a small number of glomeuli (Khalef and Ata, 2010). According to Oshea and Bradshaw (1993), this number is 400-6000 per kidney in the lizard, *Ctenophorus ornatus*. There are also other studies that support the belief about existence of a low number of renal renal glumeli in lizards (Gabri and Butler, 1984). Moreover, the present study showed that in comparison with other vertebrate the cortical and medullary regions of kidney are not separated by a distinct border. It was also revealed that there were very few non-Henle nephrons in the kidney while all the urinary tubules were connected by connective tissues. In this respect, it is in agreement with observations previously made by others (Mcnab, 2002; Edward, 1998). The results elucidated that the kidney's glomeruli of this species is composed of a simpler system of capillary loops with mesangium and this testify the results provided by other researchers (Mcnab, 2002; Gabri and Butler, 1984). The observation that glomeruli are surrounded by doubled-walled Bowman's capsule is in agreement with Jacobson (2007).

Moreover, the finding that four types of segments are observed in nephron tubules have been also reported in different reptiles (Davis *et al*., 1976; Jacobson, 2007). Here, it was reported that in *A. boskianus*, the ureters form a common duct which fuses with the dorsal surface of the urodaeal cavity. However in contrast to the finding, there are reports that in other species (*Cnemidophorus*, *Gerrhonotus* and *Elgaria multicarinata*) the ureters are joined to the oviduct (Blackburn, 1998; Fox, 1977). In most lizards and snakes, the ureters open into the urodaeum (Blackburn, 1998; Kuchel and Franklin, 2000), yet in the families of Polychrotidae, Tropiduridae, Xantusiise, Teiidae, Anguidae, Phrynosomatidae, Scincidae and Liolemidae, it has also been found in the proctodaeum (Sanchez-Martinez *et al*., 2007). It has been documented that ureters show a simple columnar epithelium and in some species they are composed of a few layers of stratified columnar epithelium (Sanchez-Martinez *et al*., 2007).

CONCLUSION

The present study showed that unlike desert mammals which have abundant long and complicated nephrons in their kidneys, arid-dwelling lizards display few small, short and simple nephrons which seems to produce the hyposmotic urine compared to the blood. This is possible because of their lower metabolic rate compared with mammals. The general morphology and histology of nephron in this taxon resembles of the nephrons reported from other species. However because of the lack of references, it was not possible to compare the kidney and nephron parameters in this species with those lizards living in more humid regions.

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