

Full Length Research Paper

Histomorphological and anatomical study of kidney in berzem (*Barbus pectoralis*)

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The kidney of the vertebrate is the main organ which balances the body fluid homeostasis. *Barbus pectoralis* (Cyprinidae) inhabit Karoon River (Southwest Iran). There was no available report on anatomy and histology analysis of *B. pectoralis*. So in this study, the morphologic and microscopic features of kidney of *B. pectoralis* were recognized. A total number of 10 young fish freshly prepared from Karoon River in Khouzestan was used. After removing the kidney, it was immediately fixed in Bouin's solution. Then, 5 to 6 μ m sections were made using paraffin embedding techniques and stained with Haematoxylin and Eosin and Periodic Acid Schiff. The results reveal that the kidney of *B. pectoralis* consisted of two lobes that are slender and long. Microscopic studies showed that the kidneys consisted of excretory portion and hematopoietic-lymphoid tissues. Excretory portion of kidney parenchyma consisted of renal corpuscle and urinary tubules. The neck segment consists of a single layer of ciliated cuboidal or low columnar epithelial cells. The proximal tubules consisted of tall columnar cells with well-developed brush border. Two proximal tubule segments were distinguished by the different height of the cells and brush border. The distal tubules had pale columnar epithelial cells and oval nuclei situated in basal area without brush border. The collecting tubules lined with high cuboidal epithelium. The result shows that although the kidney structure of *B. pectoralis* has many similarities to other fish species kidney like *Cetenopharyngodon idella* and *Barbus sharpeyi*, it also has considerable structural differences.

Key words: Histology, anatomy, *Barbus pectoralis*, kidney, hematopoietic, renal corpuscle.

INTRODUCTION

As in all vertebrates, the kidney in fish is located retroperitoneal, exterior to the dorsal wall of the body cavity. The kidney is a paired organ that has been described as having various anatomical and functional compartments (Morovvati et al., 2006). The kidney of all vertebrates is made up of nephrons which are the

functional, structural and morphological unit (Mobjerg et al., 2004). The kidney of the vertebrate is the main organ which balances the body fluid homeostasis (Ojeda et al., 2003). The kidney of fish receives majority of postbranchial blood and renal lesions may be expected to be good indicators of environmental stress (Kurtovic et al., 2008). The head of kidney contains endocrine elements, the chromaffin cells and interregal tissue which are located around the blood vessels. The posterior kidney contains the nephrons with variable quantities of hemopoietic and lymphoid tissue in the interstitium. In vertebrates, three kind of kidney are found: the pronephros,

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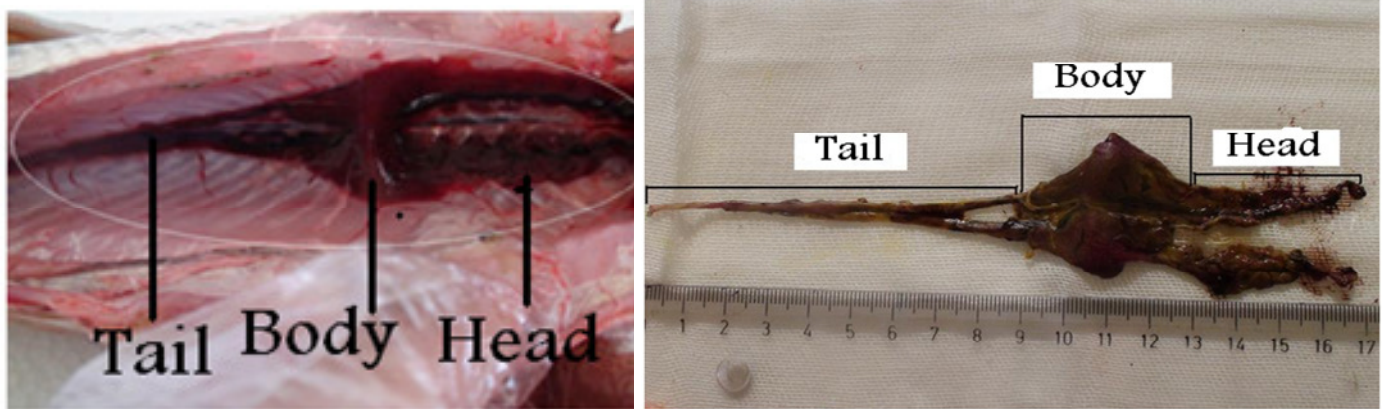


Figure 1. Kidney of *Barbus pectoralis* (cyprinidae). The kidney is divided into three parts: A head, body and caudal (tail) part.

mesonephros and metanephros (Mobjerg et al., 2004).

Barbus pectoralis (Cyprinidae) inhabit Karoon River (Southwest Iran). The biology of *Barbus* has been widely investigated worldwide (Coad, 2008; Kerdegari et al., 2009). There is no available report on anatomy and histology analysis of *B. pectoralis*. Therefore, an attempt has been made to study the macroscopic and microscopic of kidney of *B. pectoralis* and is the first to present complete reports of kidney based on observation by macroscopic and light microscopic. Therefore, the aim of the present study was to investigate the anatomy and histology differences of the kidney in the *B. pectoralis* and to compare it to other fishes.

MATERIALS AND METHODS

A total number of 10 young fish freshly from both sex (mean std. total length \pm SD = 31 cm \pm 2.87, mean std. standard length 26.75 cm \pm 2.64, mean body mass 258.5 g \pm 75.83) prepared from Karoon River was used. Total and standard length of fishes was measured accurately (1 mm) and their weight was recorded in accuracy of 0.01 g (Nikpey, 1996). Fishes were anesthetized with overdose of MS222 (Merck, Germany) and then by an incision on the abdominal wall, the kidney was removed and the samples were collected with maximum 0.5 cm thickness from head, body and caudal part and were fixed in Bouin's solution. Fixed samples were dehydrated in aggraded series of alcohols, cleared in xylene, embedded in paraffin and cut with microtome at 5 to 6 μ m. Sections were mounted on glass slides, deparaffinized and stained by Haematoxylin and Eosin (H and E) and Periodic Acid Schiff (PAS) (Bancroft and Gamble, 2002). The studies were observed under light microscope.

RESULTS

The kidney of the *B. pectoralis* is a large organ and has two lobes that are slender and long. So, the kidney is divided into three parts: A head, body and caudal part (Figure 1). Microscopic studies showed that the kidneys consisted of excretory portion and hematopoietic-lymphoid

tissues (Figures 2 and 11). Cells with melanin pigment were seen in hematopoietic tissue; these cells are called melanomacrophages which are organized in melanomacrophage centers (Figures 2 and 5). The head of the kidney composed exclusively of hematopoietic tissue and islet of interregional tissue devoid of renal tubules and glomerulus. This structure changes in the body, where the hematopoietic tissue is gradually decreased, but the numbers of tubules and glomeruli are increased. In the caudal part of the kidney dispersion of hematopoietic tissue is completely reduced and substituted by numerous glomeruli and convoluted tubules. The hematopoietic tissue fills among the nephrons. Each nephron consists of glomerulus that is enclosed by Bowman's capsule, the proximal, distal and collecting cells. Urinary tubules consisted of neck segment, proximal tubule (first and second segment), intermediate segment and distal segment.

B. pectoralis kidney surface is covered by a delicate and thin capsule of a loose connective tissue that is composed of layer of squamous cells (Figure 3). The renal corpuscles of *B. pectoralis* consist of a glomerulus and a glomerular (Bowman's) capsule. The glomerulus is a globular network of dense packed anastomosing capillaries that invaginates Bowman's capsule. The relatively wide diameter afferent arteriole enters Bowman's capsule at the vascular pole of the renal corpuscle and then forms the network of glomerular capillaries. The efferent arteriole drains the glomerulus and leaves the capsule at the vascular pole which is usually situated opposite the entrance to the renal tubule, the urinary pole. Bowman's capsule consists of a single layer of flattened cells resting on a basement membrane; it forms the distended, blind end of the renal tubule.

Bowman's capsule has two layers: visceral and parietal layers. The internal or visceral layer of the glomerular capsule surrounds the glomerular capillaries with modified epithelial cells, called podocyte. At the vascular pole of the renal corpuscle, the epithelium of the visceral

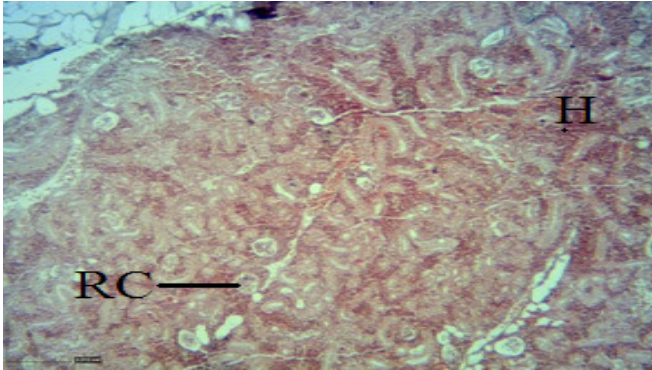


Figure 2. Histological picture of renal corpuscles (RC) in body of kidney in *B. pectoralis* (H and E, 40×) showing hematopoietic-lymphoid tissues (H) between urinary tubules.

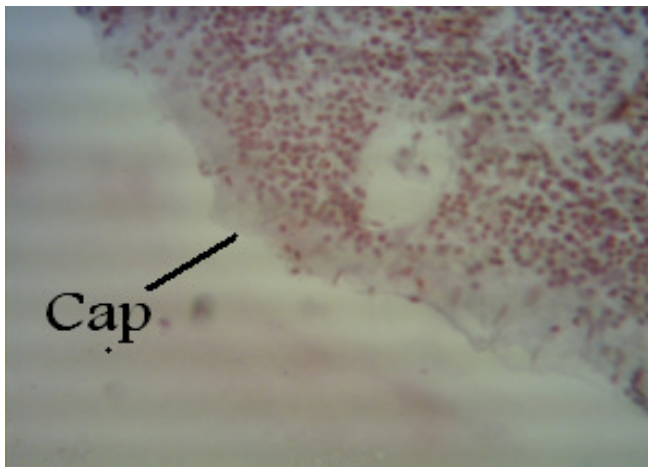


Figure 3. Histological picture of kidney in *B. pectoralis* (H and E, 40×) showing capsule (Cap) (arrowhead).

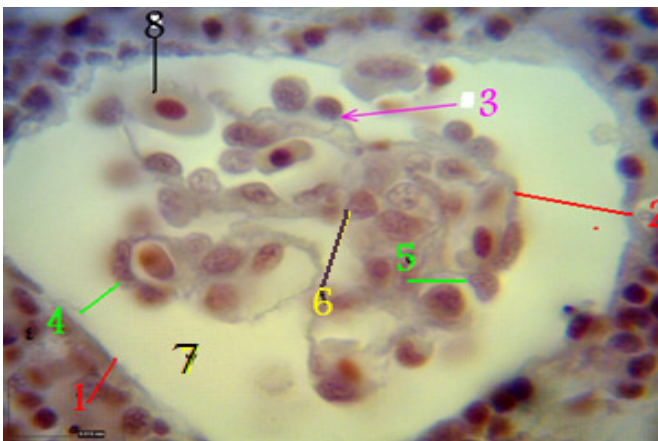


Figure 4. Histological picture of renal corpuscles in body of kidney in *B. pectoralis* (H and E, 40×). (1) parietal layer of Bowman's capsule; (2) visceral layer of Bowman's capsule; (3) podocyte; (4) capillary; (5) endothelial cell; (6) mesangial cell; (7) urinary space; (8) nucleated red blood cells.

layer reflected to form the simple squamous parietal layer of the glomerular capsule. The space between the visceral layer and parietal layer of the renal corpuscle is called the capsular (urine) space. There are numerous nuclei in the glomerulus that bare capillary endothelial cells, mesangial cells and podocyte. In *B. pectoralis*, two to four glomeruli were observed within each cluster (Figure 4). Microscopic study of urinary tubules showed that neck segment exists and consists of a single layer of ciliated cuboidal or low columnar epithelial cells. Cytoplasm of these cells stained slightly basophilic, and round or oval nuclei of them are situated in the basal region of the cell (Figure 6).

The proximal convoluted tubule in *B. pectoralis* is the longest and most developed segment of the nephrons. This tubule is lined by eosinophilic-granular simple columnar cells with a well development brush border. In these cells, the nuclei are mainly spherical and situated in lower part of the cells. There were two proximal tubule segments: the epithelium of first proximal segment consisted of tall columnar epithelial cells with basal nuclei with a slightly eosinophilic cytoplasm and an apical brush border (Figure 7A). The apical cytoplasm of these cells is lighter than basal cytoplasm and brush border were seen clear in PAS staining (Figure 7B), but, the second proximal segment has a taller columnar epithelium with more centrally located nuclei and less well-developed brush border. The cytoplasm of these cells was stained intensely eosinophilic (Figure 8).

Intermediate segment, well developed in the *B. pectoralis* is surrounded by cuboidal or short columnar epithelial cells with inconspicuous brush border. The cytoplasm of these cells was stained intensely eosinophilic (Figure 9). The distal convoluted tubules in *B. pectoralis* are short and not encountered as frequently in sections as proximal tubules. Their luminal diameters are greater than in proximal tubules. They have pale columnar epithelial cells and their cytoplasm stains less acidophilic than proximal tubules. The distal segment has oval nuclei situated in basal area. The brush border was absent in this segment (Figure 10).

The initial collecting duct in *B. pectoralis* is larger than preceding distal segment. Columnar epithelium is slightly eosinophilic with basal nuclei and no brush border. Subsequent collecting tubule segment increase in diameter and their epithelium change to pseudostratified columnar. The smooth muscle and connective tissues are seen in this portion. The smooth muscle and connective tissues are increased in larger collecting duct. Collecting tubules are larger than proximal and distal tubules (Figure 12).

DISCUSSION

The results show that the structure of kidney in this species is similar to the other fishes and even mammals; however, there are also considerable structural

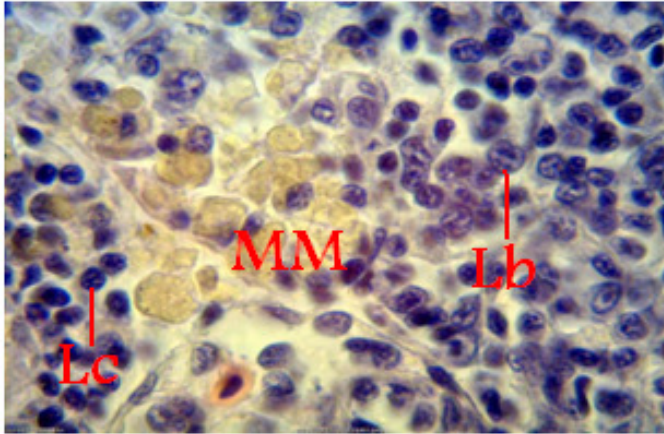


Figure 5. Histological picture of lymphatic tissue in kidney of *B. pectoralis* (H and E, 40×). Lb, Lymphoblast; Lc, lymphocyte; MM, melanomacrophage.

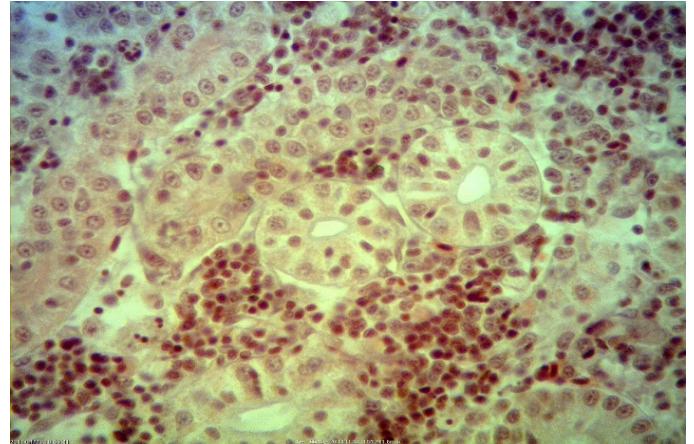


Figure 8. Histological picture of second proximal tubules in *B. pectoralis* (H and E, 20×).

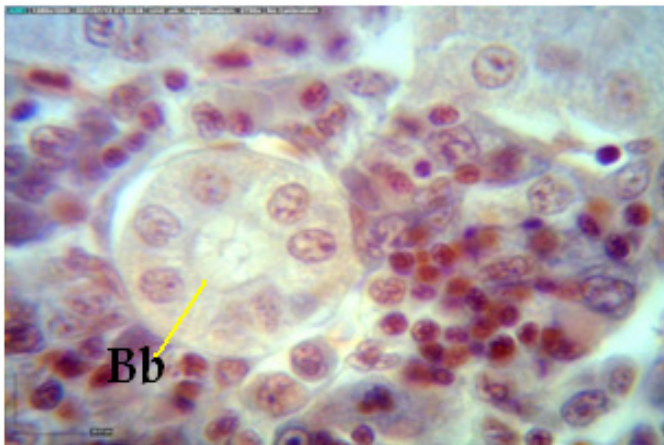


Figure 6. Histological picture neck segment in kidney of *B. pectoralis* (H and E, 40×): showing that the neck segment consists of a single layer of cuboidal or low columnar epithelial cells. Bb, Brush border.

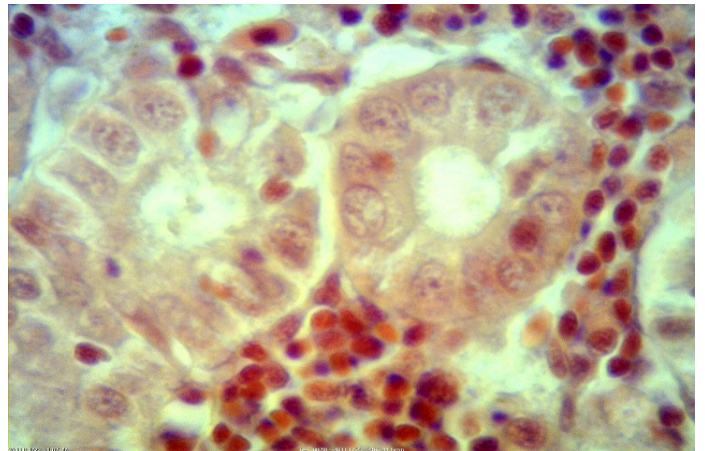


Figure 9. Histological picture of intermediate segment in *B. pectoralis* (H and E, 40×) surrounded by cuboidal or short columnar epithelial cells with inconspicuous brush border.

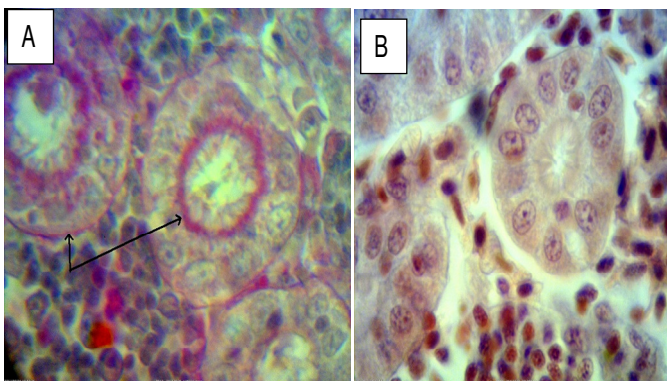


Figure 7. Histological picture of first proximal convoluted tubules (A) (H and E, 40×) and urinary tubules in *B. pectoralis* (PAS, 40×) showing the base membrane and brush border.

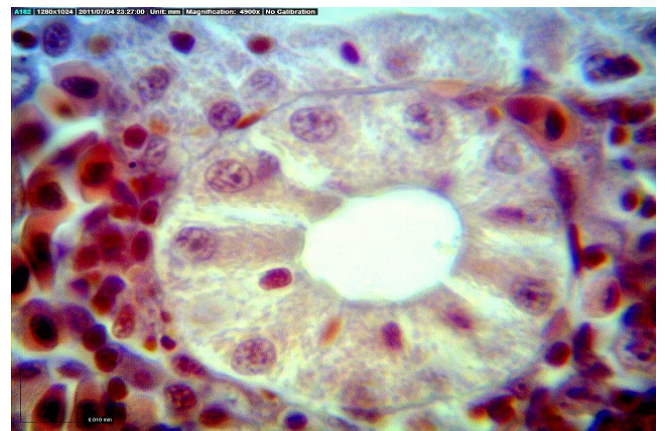


Figure 10. Histological picture of distal tubule in *B. pectoralis* (H and E, 40×) showing the oval nuclei situated in basal area. The brush border was absent in this segment.

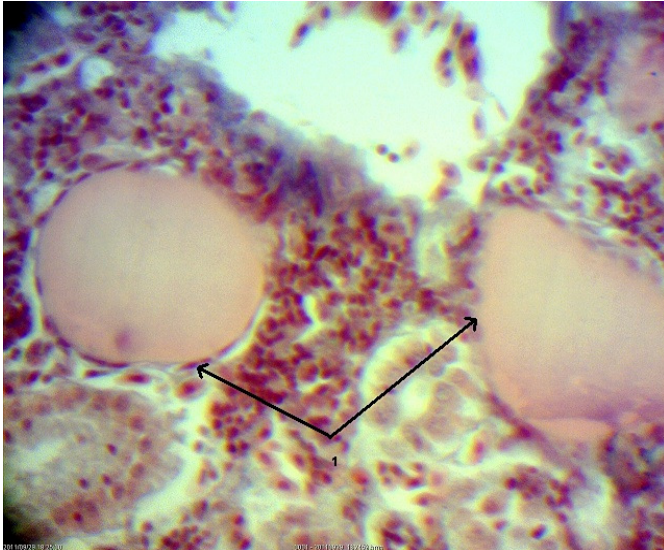


Figure 11. Histological picture of body of kidney in *B. pectoralis* (H and E, 40×) showing the lymphoid tissue in kidney.

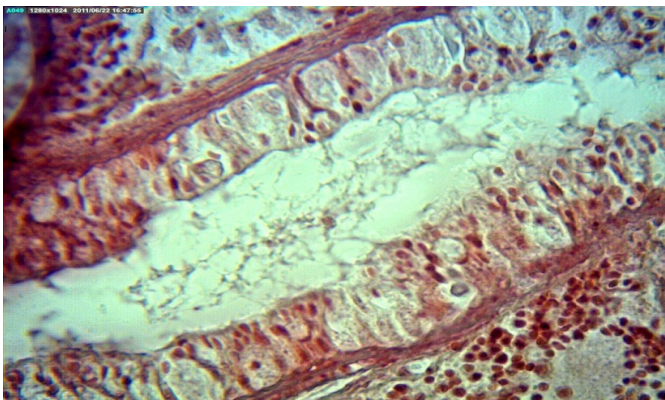


Figure 12. Histological picture of collecting tubule in caudal part of kidney in *B. pectoralis* (H and E, 40×) showing columnar epithelium is slightly eosinophilic with basal nuclei and no brush border. The smooth muscle and connective tissues were seen in this portion.

differences. The morphology of kidney in fishes varies (Elger and Hentschel, 1981). In *B. pectoralis*, it is a long couple with the massive body which resembles trout (Ogawa, 1962), but in Silver sea bream (*Sparus saba*) (Wong and Woo, 2006), Killfish (*Fundulus heteroclitus*) (Tsuneki et al., 1984), *Huso huso* and *Acipenser persicus* (Charmi et al., 2010), it is elongated from the anterior to caudal part along the spinal cord. The kidney of *B. pectoralis* is Y-shaped like that of *B. sharpyei* (Moradi, 2005) and *Ctenopharyngodon idella* (Morovvati et al., 2011). Like *B. sharpyei* (Moradi, 2005), *C. idella* (Morovvati et al., 2011), *Huso huso* and *A. persicus* (Charmi et al., 2010), the kidney of *B. pectoralis* is divided into three parts: the head, body and caudal part.

B. pectoralis kidney surface was covered by a delicate and thin capsule of a loose connective tissue that was composed of layer of squamous cells. In this case, it was similar to that of *C. idella* (Morovvati et al., 2011) and *B. sharpyei* (Moradi, 2005). The head of the kidney in *B. pectoralis* mainly consists of hematopoietic tissue with malpighian body or nephrons tubules. Fange (1986) showed that the head of kidney in sturgeon is a universal hematopoietic organ, which is in agreement with the results of the present study. The head of the kidney in Salmoniformes, such as *Salmo trutta fario*, *C. idella* (Morovvati et al., 2011), *B. sharpyei* (Moradi, 2005), *A. persicus* and *H. huso* (Charmi et al., 2010) consists of lymphoid, endocrine and hematopoietic tissue with isolated malpighian bodies and nephrons (Krayushkina et al., 1996b). Also, Krayushkina et al. (1996a) and Krayushkina (1998) believed that most part of caudal tissue of the kidney contains the highest number of nephrons and the caudal kidney is composed of nephrons surrounded by hematopoietic tissue dispersed throughout the organ. The anterior of kidney in *B. pectoralis* such as teleosts consists of lymphoid and hematopoietic tissue (Stoskopf, 1993). Indeed, Charmi et al. (2009) reported that in head of the kidney of *Huso huso* there are not any nephrons cells, and only hematopoietic tissue and interregional cells are distributed in this part.

Moreover, the results show that the hematopoietic tissue covers the head of the kidney and it is similar to Hoar and Randall's (1983) reports which shows that the variable amounts of hematopoietic tissue are distributed among the tubules and vascular spaces in the body of the kidney in teleosts.

The glomerulus is a tuft of capillary which occupies the Bowman's space. Glomeruli were grouped as clusters in *B. pectoralis* kidney like *C. idella* (Morovvati et al., 2011), *B. sharpyei* (Moradi, 2005), *H. huso* and *A. persicus* (Charmi et al., 2010). Cataldi et al. (1995) stated that the glomerulus in *Acipenser naccarii* has multilobed glomerulus. The development of diameter and number of glomeruli clusters is strongly age-dependent. As described by Wong and Woo (2006), the glomeruli were grouped as small clusters in the peripheral regions of the kidney in Silver sea bream (*Sparus sarba*). Unlike the previous-mentioned findings, many teleostean kidneys consist of dispersed glomeruli (Anderson and Lowen, 1975). The renal corpuscles are globular and small, relatively larger than proximal tubules. Hematopoietic tissue surrounds the renal corpuscles (Ojeda et al., 2003). Mobjerg et al. (2004) reported renal corpuscles in amphibian have two structures; Glomerulus and Bowman's capsule. The Bowman's capsule consists of a parietal layer and a visceral layer which surrounds the glomeruli capillaries. So, this structure is similar to glomerulus of *A. naccarii* (Ojeda et al., 2003), amphibians (Mobjerg et al., 2004).

In young *H. huso* and *A. persicus*, the proximal tubules

have a tall epithelium with the brush border on the apical surface with the basal nuclei (Krayushkina et al., 1996a). In *A. naccarii*, these cells are composed of squamous and slightly rounded cells (Ojeda et al., 2003). The proximal tubules of *H. huso* and *A. persicus* like *A. naccarii* (Cataldi et al., 1995), *Acipenser oxyrhynchus* (Krayushkina, 1998), *Euryhaline teleostean* (Hickman, 1968), *C. idella* (Morovvati et al., 2011) and *B. sharpie* (Moradi, 2005) have two types, namely the first and the second proximal tubule. The first proximal tubules were also reported in lamprey by Hoar and Randall (1983), but the *Poyodon spathula* has just one type proximal tubule and the second proximal tubule was not observed (Krayushkina et al., 1996b). The proximal tubular epithelium of columnar cells has a prominent brush border (Ferguson, 1995). As described by Cataldi et al. (1995), two proximal tubule segments were identified by different heights of cells with different sparse of brush border.

The distal tubules have lower epithelium than proximal tubules and the nuclei are not basal and spherical (Krayushkina et al., 1996b). As a result of this study, like Elasmobranchs (Hoar and Randall, 1983), distal tubules in *B. pectoralis* have cuboidal epithelium without brush border. In general, proximal and distal tubules characteristics in *Barbus* are similar to those of other teleosts (Hickman and Trump, 1969; Ogawa, 1962)

The collecting tubules in *Barbus* due to their regular feature and columnar epithelium differ from other nephrons cells. Similar to our results, the collecting tubules in Silver sea bream (*S. sarba*) have a circular lumen without any curvature or folding (Wong and Woo, 2006). While in Perciformes, the collecting tubules have high dispersion in the caudal part of kidney (Milano et al., 1997). In line with Cataldi et al. (1995) issue, there are reports that these cells in tilapia are recognized by the high columnar cells and the connective layer around them. In addition, they noted that in tilapia, the collecting tubule has basally located nuclei. While in *B. pectoralis*, the cells are high cuboidal with centrally located nuclei.

Conclusion

The important morphologic characteristics of the kidney studied in the *B. pectoralis* for the first time showed some similarities and some differences between kidney of this species and mammals. Histomorphology of kidney showed that although the kidney structure of *B. pectoralis* has many similarities to other fish's species kidney like *C. idella* and *B. sharpyei*, it has also considerable structural differences.

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