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Histomorphology of excretory kidney of, Common carp, *Cyprinus carpio* during different salinity adaptation

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Abstract

In this research, excretory kidney of, Common carp, which is a very important Warm water fish species, has been studied under various environmental salinity changes. This research was conducted to find out how *C. carpio* can resist without changes in structure and damage of tissues, especially the cells involved in osmotic regulation of kidney against different salinity. For this purpose, 120 specimens of common carp were placed for 2 weeks in saline treatment with 4ppt, 8ppt, 12ppt and freshwater as a control treatment with three replications. For histomorphology studies, samples were taken at a maximum thickness of 0.5 cm. After fixation in 10% formalin buffer, the routine procedure of tissue preparation was done and sections of 4 -6 μm thickness stained with Hematoxylin and eosin. In microscopic studies excretory kidney including nephrons and glomerulus were seen scattered throughout of this organ. Urinary tubules were seen as, proximal and distal convoluted tubules and collecting ducts. In histometric studies, during the period of adaptation with different Salinities, the highest number and diameter of glomeruli were seen in 12ppt salinity and the lowest was seen in 8ppt and 4ppt. On the other hand, the highest number and diameter of collecting ducts were observed in the control treatment and the lowest was for 12ppt and 8ppt Salinities. Also, the maximum and minimum thickness of the muscle layer of collecting tubes belonged to salinity 4ppt and 8ppt. Therefore, according to the results, it can be concluded that the tolerance and adaptability of *C. carpio* are high to the salinity changes due to rapid changes in the tissue and physiological structure of the kidney for osmoregulation. So, the species has been able to adapt to the changes in its environmental salinity and to survive.

Keywords: Excretory kidney, *Cyprinus carpio*, Histomorphology, Salinity, Adaptation

Introduction

Common carp, was initially native to central Asia and has spread over the centuries to various parts of the world or has been transmitted by human beings. In a large-scale feeding system, conventional carp depends on natural nutrition in the pool that is propagated with organic fertilizer. In a semi-extensive breeding system, it receives a handful of foods that are mainly herbs. Also, in a dense breeding system, normal consumption of the common carp is entirely dependent on condensed food or balanced nutritional compounds (Evans 2010, Davidson 2011). The body of fish has slightly compressed from the sides and has different colors in different environments (Nebel et al. 2005). Based on our review studies, we founded that a normal kidney in the Osteichthyes has different parts, which respectively include glomerulus, proximal

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segment, distal and collecting tubule. Two organs kidney and gill play an important role in osmotic regulation (Grosell 2011, Polakof et al. 2011). In *Cyprinus carpio*, kidney is a mesonephric type that has a nephron with glomeruli. In marine fish, glomeruli are significantly reduced or eliminated. (Chew et al. 2010). In freshwater fishes, almost all nephrons have glomeruli, so that the blood flow to the renal arteries, such as gills, is discharged into the kidney in the aorta before entering the renal tubule (Elger et al. 2003). Due to the absence arch of Henle in fish, they are not able to excrete more concentrated urine than blood plasma. (Arjona et al. 2009). Also, in this group of fishes, the amount of glomerular filtration is very high, and as a result, urine is produced in a very hypotonic manner (Zhou et al. 2010, Fiess et al. 2007). Researchers believed that generally in Osteichthyes, the kidney is an organ with several functions. It does not only have the task of excretion and osmotic regulation but also have a large number of hematopoietic and phagocytic cells (Varsamos et al. 2005). The anterior or head area of kidney mainly consists of hematopoietic and lymphoid tissues, and the posterior part often consists of excretory tubules and glomeruli (Arjona et al. 2009, Drummond and Davidson 2010). Due to decreasing freshwater resources and increasing the degree of salinity of river, especially in Khuzestan province, which is the source of water supply for warm water fish farm, this study was conducted to how common carp can without altering the structure and damage of the tissues, especially the cells involved in the osmotic adjustment of the kidney against salinity.

The findings will be used in fisheries research centers, especially in the histology, physiology, pathology and immunology of this species, as well as in optimal progress.

Materials and methods

To do this research, 120 of common carp with mean length (16.5±0.5 cm) and mean weight (61.7±13.2 g) were obtained from Azadegan warm water fish farms in Khuzestan province and transferred to the aquatic lab of faculty of veterinary medicine, Shahid Chamran University of Ahvaz. To adaptation to urban tap water as a freshwater supply source, they were placed in five 100-liter Reservoirs containing dechlorinated water at a temperature of 22-26 °C and pH =7/8 for 96 hours. During the experimental period, fish feeding was done in twice using commercial food. The biochemical analysis of this food showed that it included 43% protein, 18% fat, 30% carbohydrate and 9% fibre. Third tanks water replacing was done daily after the completion of feeding to prevent an increase of ammonia and other metabolites (Grosell 2011). To carry out this research, twelve Hundred-liter tanks with three replicates were used for each treatment. Then salinity was obtained by dissolving salt in each tank. The fishes were gradually transferred to salinity levels of 4 ppt, 8 ppt, 12 ppt and freshwater as a control treatment. During the study period, the physicochemical factors of water, such as ammonia, nitrite, nitrate and pH, were measured. The physicochemical properties of water such as salinity, temperature, pH and dissolved oxygen were measured by the saline meter, oxygen meter and pH meter (A&Z, China). Samples were taken at the beginning of the experiment and on day 14. Fish after catching of tanks were immediately anaesthetized, to reduce the effects of stressors by 0.1% of 2-phenoxyethanol and their biometric measurements were performed. Sampling from kidney tissue was done and tissue samples placed in 10% formalin buffer solution. In the next step, routine histology procedure was done and sections of 4μ -6μ were cut by using a rotary microtome (Leica-Germany) and stained with hematoxylin-eosin (Rheubert et al. 2017, Herrera et al. 2009). In addition to the study of the microscopic structure of kidney and urinary tubules, the diameter and number of them were measured in different salinities and compared with each other. To view microscopic sections and histological studies, optical microscopy (Olympus, Japan) equipped with Dino-Lite digital lens and Dinocapture software were used in different magnifications (Dantzler 2003, Whittamore 2012). Data were analyzed with one-way ANOVA and Duncan's post-test, and $p<0.05$ was considered as a significant difference level . Data were presented as mean±SEM. All analyses were performed using SPSS (v. 22) software.

Results

Kidney in this species was reddish-pigmented, large, retroperitoneal and was attached to the spinal cord, above the swimming bladder. In the microscopic structure, the kidney was covered by a very delicate capsule of connective tissue in the external surface. The excretory part of kidney included nephrons, included of spherical structure, along with the collecting tubules. These structures were sporadic throughout the kidneys. Corpuscles of the kidney were consisted of large vascular glomeruli and bowman's capsules which had two vascular and urinary poles that were adjacent to each other. Glomeruli contain a network of capillaries and a large number of the nucleus that related to podocytes and endothelial cells. Bowman's capsule is around of corpuscles and bowman's space separated from it. The urinary tubules were included of proximal (PI & PII) convoluted and straight tubules with long columnar eosinophilic cells along with brush border. These two proximal tubules had columnar cells with microvillus and brush border in a free surface, but distal convoluted and straight tubules determined with columnar cells with no brush border with the large internal lumen. Collecting tubules that have eosinophilic cuboidal to short columnar cells with smooth muscle and connective tissue around it (Fig. 1).

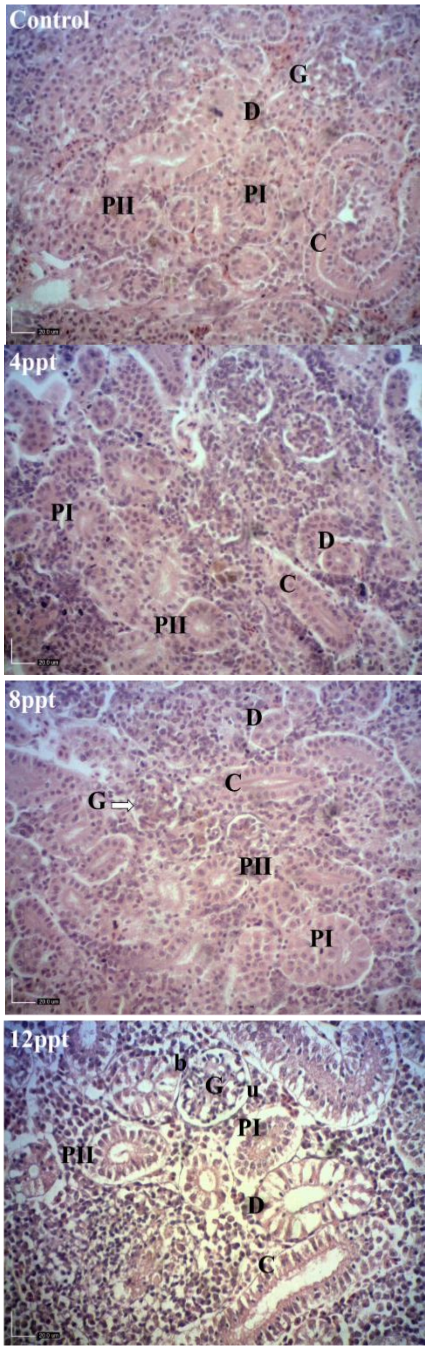

Fig 1. Microscopic view of the kidney of common carp in control and salinity group (4ppt, 8ppt and 12 ppt) (H & E, x1450).
Glumerule(G) with the vessels pole (b) and urinary pole (u), primary (PI) and secondary part of the proximal tubule (PII), distal tubule (D) and collecting tubule (C).

In histometric of microscopic observation, in high salinity (12 ppt) the number of collecting tubules reduced but did not show any significant changes in muscle thickness and lumen diameter. About glomeruli changes, the highest number was reported in the control group and 4 ppt salinity and the lowest number belonged to 12 ppt salinity. The smallest diameter of glomeruli belongs to 12 ppt salinity and the biggest diameter belongs to the control group ($p<0.05$) (Table 1).

Table 1. The means of number and diameter of glomeruli after transfer to different salinities, based on (mean±SEM).

Treatment	Control	4ppt	8ppt	12ppt
Number	6.61±1.11 [a]	6.22±1.17 [a]	5.26±1.14 [b]	7.23±1.12 [c]
Diameter(µ)	65.21±1.33 [d]	64.12±1.45 [b]	61.14±1.13 [b]	63.21±1.17 [a]

Different letters indicate a significant difference in each row ($p<0.05$).

During this period, the largest diameter of the lumen of the collecting tubules belonged to control and the smallest diameter lumen belongs to the 8 ppt salinity. The maximum thickness of the muscle layer of the collecting tubules was measured in 4 ppt and the lowest belongs to the 8 ppt salinity ($p<0.05$) (Table 2).

Table 2. The means of number, diameter and thickness of the muscle layers of the collecting tubules after transferring to different salinities (mean±SEM).

Treatment	Control	4ppt	8ppt	12ppt
Number	6.61±1.11 [a]	6.22±1.17 [a]	5.26±1.14 [b]	7.23±1.12 [c]
Diameter(µ)	65.21±1.33 [c]	64.12±1.45 [b]	61.14±1.13 [b]	63.21±1.17 [a]
Thickness(µ)	19.21±1.47 [a]	23.31±1.34 [b]	13.14±1.18 [c]	16.31±1.26 [d]

Different letters indicate a significant difference in each row ($p<0.05$).

Discussion

Researchers reported that kidney tissue, in addition to having an important role in maintaining homeostasis by altering glomeruli and renal tubes, is considered as the target organ for the recognition of the effects of salinity on freshwater fish and environmental pollutants. The changes of this organ are considered as an appropriate biochemical marker related to the stress associated factors (Diep et al. 2011). In aquatic animals, many studies have evaluated the effect of environmental pollutants on the structure of various tissues such as kidneys. In the present study, no mortalities were observed until the end of the trial period. The reasons for the survival of these fishes are probably due to a short period, low levels of salinity and also the ability of these species to regulate and stabilize blood serum ions and cortisol levels in these levels of salinity (Sardella et al. 2007). Researchers believe that further studies are needed to find common effects of adaptation and salinity methods to achieve all aspects of fish osmotic regulating organs. Based on the results of this study, the kidney of common carp was largely similar to other freshwater species in terms of appearance and histology. Glomeruli were scattered throughout in the kidney and the collecting ducts were the most widespread than the other ducts of this organ. In contrast to some fishes, such as Chondrichthyes and saltwater fishes, there have differences in tissue and morphology in this organ that due to reconciling with environmental characteristics or according to shape and body requirement (Gonzalez et al. 2005). In common carp, this organ was drawn from the front to the end of the body, retroperitoneal and adjacent the spinal cord. Microscopic studies in all carp showed that renal corpuscles were seen as scattered structures like high vertebrates and most other fishes' species. Collecting tubules differed for the location of the nucleus, staining and size. Kidney has an important role in osmotic regulation in fishes, it is effective in maintaining the state of homeostasis by changing the flow of urine and controlling the balance between the release and reuptake of ions in various environmental salinities (Watanabe et al. 2009). Scientists believe that kidney in freshwater species should have an effective function to be able to withstand the constant increase in water intake. Therefore, to properly balance the nephrons, extra salts need to be reabsorbed and excess water redirected out of the body (Taylor et al. 2010). In histometric studies of number and diameter of renal corpuscle indicated a different measurement in different salinity. The researchers also reported that the diameter and number of renal corpuscle are different in different species of fish, for example, in the grass carp, the diameter of renal corpuscles is big and similar to the freshwater fishes of stenohaline, while the diameter of the renal corpuscle in the stenohaline marine fishes is much smaller. The stenohaline freshwater fishes have the largest renal corpuscle and the stenohaline marine fishes have the smallest (Vargas-Chacoff et al. 2009). Glomeruli are found in a significant number in the bony fish kidney that determines the glomerular filtration capacity. Size of glomeruli of euryhaline species varies from one environment to another. As it was found, in fishes adapted with salinity 12 ppt, changes in glomeruli were observed, including some degree of destruction and an increase in their diameter. Because the morphology of the renal tubules has changed rapidly and surprisingly to adapt to hyperosmotic conditions due to structural and tissue changes under different osmotic conditions (Panfili et al. 2006, Oguz 2013). At glomerulus histomorphologyy studies, in all treatment and control group the number and diameter of renal corpuscle showed different performance in different salinity. In a study done on renal tissue changes in different salinities on euryhaline fish (*Sparus sarba*), it was found that mesangeal glomerular tissues were altered in lower salinities in comparison with higher salinities so in the hypotonic conditions, mesangeal tissue of glomeruli and capillaries were extensively exfoliated in the bowman's capsule and they were out of the ordinary condition (Hiroi and McCormick 2012). Also, on histometric study of the number collecting tubules, it was found changed significantly in response to different salinities, but there was no significant difference in thickness of muscle layer and lumen diameter. In the control treatment, the number and size of collecting tubules increased compared to the high salinities. Same results were reported on collecting tubules in different salinities (Gonzalez et al. 2005). Because the increase of muscular wall of the collecting tubules helps to increase urine flow into the end tracts of mesonephric ducts and facilitate urinary excretion. Therefore, the morphology of renal tubules has been modified to adapt to the conditions created by different environmental salinity, as in the recent study, changes in the structure of the tubules can be justified concerning the rate of urinary excretion under different osmotic conditions (Rheubert et al. 2017, McDonald 2007). Based on this study, it has been determined that this species can tolerate higher salinities to the living environment because of the changes that the animal can make to the kidney tissue structure.

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Conflict of interest

The authors of this article declare no conflict of interest.

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