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Original Article

The Effect of different concentrations of ammonia on histomorphometry of kidney and some blood factors of Nile tilapia, *Oreochromis niloticus*

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Abstract

In the present study, *Oreochromis niloticus* juveniles were exposed to 10, 20, and 30% (96h LC50) of ammonia for two weeks. After this period, the fish were anesthetized and blood samples were taken from the caudal peduncle with a heparin-coated syringe to evaluate blood parameters. The 0.5 cm samples of the kidney tissue were taken, fixed in 10% formalin buffer, and after dehydration with alcohol, clarification with xylol, blocking with paraffin and cutting 4-6 microns thick with a microtome. Finally, the stained slides were studied with a light microscope. The results showed an enlarged Bowman's capsule, hyaline accumulation, epithelial detachment, increased melanomacrophage centers, necrotic glomeruli, and tubules in the kidneys after ammonia exposure. In the studies of blood serum factors with the increase of ammonia, cholesterol and BUN compared to the control and other groups. As the ammonia concentration increased, the severity of the lesions also increased. Therefore, ammonia causes changes in the structure and activity of some factors of the kidneys, which must be controlled by creating the appropriate ammonia and management conditions in the aquatic environment.

Keywords: Histomorphometry, Ammonia, *Oreochromis niloticus*, Kidney

Introduction

The genus Tilapia is a member of the cichlid family and belongs to the Perciformes order, which has a rectangular body (Becke et al, 2019). Most tilapias are microphytes, but some prefer more organic plants and use organic plants in places where other species of farmed fish feed on plankton (Hoseini et al, 2019). Today, one of the most important human concerns is the increased concentration of contaminants in the environment, especially water, which contaminates world waters in the form of sewage, oil spills, effluents of organic and mineral materials of factories, various chemicals including metals and quasi-metals, etc (Saha and Kaviraj 2003; Peebua et al, 2008). One of the most important contaminants is nitrogen compounds, the most dangerous of which is ammonia. Ammonia is widely recognized as a common contaminant in aquatic environments. Ammonia (NH3) consists of nitrogen and hydrogen (Gomes et al, 2015). It is a common nitrogenous waste,

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especially among aquatic organisms, and significantly contributes to the nutritional needs of terrestrial organisms by acting as a precursor in fertilizers (Pérez et al, 2018). Ammonia poisoning is one of the leading causes of fish mortality in aquaculture environments. Ammonia is mainly produced by fish through the catabolism of proteins. It is very toxic to fish, so they need to get rid of this waste product by releasing it into the water. Ammonia damages the kidney of other tissues and causes a variety of symptoms. According to research, compounds such as ammonia can easily enter the circulatory system of fish. Because fish are permanently immersed in such polluted environments and excretory organs such as the liver and kidney are directly exposed to ammonia for a long period (Zeitoun et al, 2016; Metwally and Wafeek, 2014). As a result, damage to these tissues disrupts factors such as cholesterol, urea, and total protein in the kidney. After facing stress, fish maintain their body homeostasis in different ways. The most common ammonia-related renal complications in fish include damage to the tubules and glomeruli and reduced lumen space. The kidney is very sensitive to contamination and is rapidly affected by contaminants and its function is negatively affected. On the other hand, the kidney is considered a defense organ in fish that helps in the detoxification and excretion of contaminants (Dastan et al, 2017). Considering the role of ammonia in different forms of water contamination and its possible entry into water sources, the present study aimed to investigate its effect on *Oreochromis niloticus* as well as kidney and some blood factors.

Materials and Methods

In the present study, a total of 120 *Oreochromis niloticus* with an average weight (of 35 ±1g) were prepared, transferred to the laboratory, and kept in a 500-liter tank for two weeks to adapt to the new conditions. They had access to proper aeration, feeding by plate (0.02 body weight/day). During the experiment, the mean temperature of the water, oxygen concentration, and water hardness were 27 ± 90 °C, 6.2 ± 1 mg / L, and 269 ± 3 mg / L respectively. After sorting, the fish adapted to the new environmental conditions. Since the lethal concentration (96h LC50) of ammonia is not known, a range-finding test was performed to find the lethal range of ammonia on *O. niloticus*. A static acute toxicity test was performed on *O. niloticus* for 96 hours according to standard instructions (Latif et al, 2014). Ammonia (Merck, Germany) was prepared as an ammonium chloride solution. Feeding of juveniles was stopped 24 hours before the toxicity test. Effective physicochemical parameters of water including pH, dissolved oxygen, and temperature was recorded daily. After determining the lethal range, the final acute ammonia toxicity test was performed in five control treatments for three replications. In each treatment, ten fish were placed in pre-aerated 15-liter aquariums. The dead fish were collected from the aquarium environment immediately and the number of fish losses at 24, 48 , 72, and 96 hours were calculated and recorded. Data obtained from the acute toxicity test was also analyzed by probit analysis with a 95% confidence interval. For sub-lethal toxicity studies, 120 *O. niloticus* juveniles were assigned into four treatments, three groups based on different percentages of LC50 96h (10, 20, and 30% LC50 96h) and one control group exposed to ammonia for 14 days at constant temperature and pH. Ten juvenile *O. niloticus* were randomly distributed in 100-liter aquariums and experiments were performed semi-statically according to the standard O.E.C.D method (20% of the aquarium water was changed on a daily basis). The food residues were removed from the aquariums when changing water. In order to keep the ammonia concentration constant, the same water content, which was removed from the aquarium, was added to the aquarium. Tissue sampling was

performed on five fish randomly on days 0 and 14 after exposure. At the end of the experimental period, the fish were randomly caught from each treatment. To evaluate the pathological kidney impacts of ammonia, kidney tissues of each of the treatments were sampled and fixed in 10% formalin buffer solution for 24 hours. Then the fixed samples were transferred to 70% ethanol, and dehydration was performed with a series of 70, 80, 90, and 100% ethanol concentrations. Afterward, clarification was performed by xylene. Kidney tissue samples were then deparaffinized and molded using molten paraffin. All these steps were performed by the tissue passage device under a pre-defined program. The tissues were then molded and paraffinized on the Tissue-Tek Mold System at a melting point of 56-58° C. A microtome was used to prepare 4-6 μm thick sections from paraffin molds. After being placed on the slide, molds were placed in an oven at 60°C for half an hour to remove excess paraffin from the tissue. After deparaffinization and replacing it with xylene, the samples were restained by reducing ethanol solutions series (Morovvati et al. 2017; Morovvati et al. 2012). The prepared tissues were transferred back to the oven to dry. Five sections were prepared from each sample and stained and investigated using hematoxylin and eosin and specific stain. The prepared slides were investigated under a light microscope that was connected to a Dinolit lens and a computer system equipped with Dinocapture software (Basir and Peyghan, 2016). Then five fields of view from each kidney slide were considered.

To study blood factors, the fish were anesthetized using powdered dianthus; blood was then taken from the tail vein using a 2 cc heparin syringe. Finally, the blood was centrifuged at 3000 rpm for 10 minutes and the plasma was transferred to newly labeled microtubes and stored at -80 °C and was used to measure some blood biochemical factors (Zimmer and Wood, 2016; Jeney et al, 1992).

Since we have quantitative and continuous data in the present study, the number of independent groups was evaluated. Considering the normal data distribution, the Kolmogorov-Smirnov test was used (Savari et al, 2013). Data analysis was also carried out using SPSS ver. 19 (SPSS Inc, version 19.00, California, USA), and all the results were expressed based on the mean ± standard deviation. One-way analysis of variance was used for inter-group comparison and then Tukey's multiple comparison test was used. $P<0.05$ was considered as the level of significance.

Results

The results of the microscopic studies of *O. niloticus* kidney showed that the kidney was dark red in the vicinity of the spine and extraperitoneal above the swim bladder and stretched along the spine from the head to the posterior end of the body, and like other real bony fish, had the head and body. This tissue was covered on outer surface by a very thin capsule of loose connective tissue and a row of mesothelial cells. This capsule was observed on the entire outer surface of the kidney (Figure 1). Exposing *O. niloticus* to ammonia resulted in tissue changes including Bowman's capsule dilation (Figure 2), hyperemia (Figure 3), hyaline accumulation (Figure 4), epithelial detachment (Figure 5), increased melanomacrophage centers (Figure 6), and necrotic glomeruli and tubules (Figure 7). The severity of the lesions as necrosis (Figure 8), epithelial lifting (Figure 9) and dilation of bowman space (Figure 10) increased with increasing ammonia concentration (Table 1).

Serum biochemical analysis showed that compared to the control, A and B, groups, cholesterol and BUN increased but total protein decreased in group C. But this difference was significant only in the value of BUN. (Table 2).

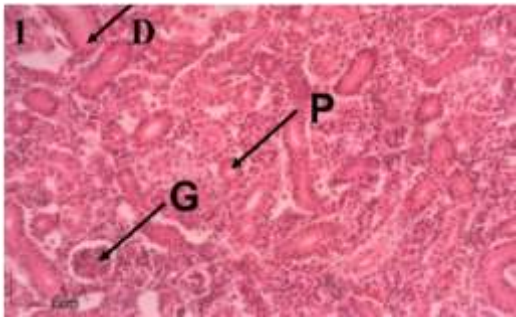

Figure 1. Control group kidney. Distal tubule (D), Proximal tubule (P), and glomerulus (G) were shown. H-E x400.

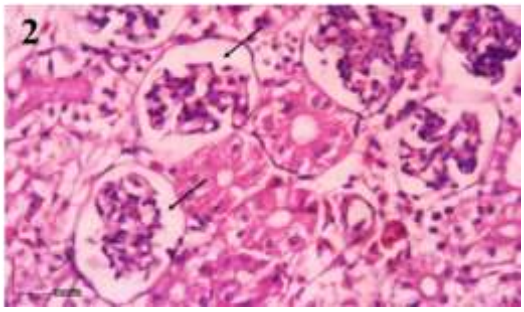

Figure 2. Group exposed to 2.7 mg/l ammonia after two weeks. Dilation of Bowman's capsule (arrow), (H-E, x400).

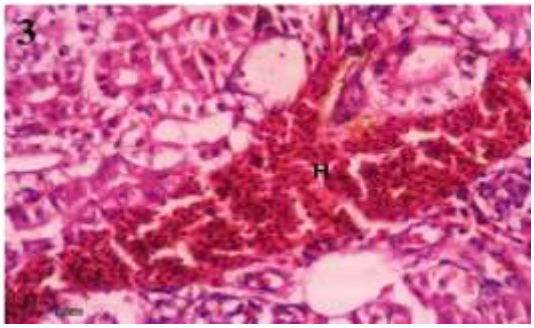

Figure 3. Group exposed to 2.7 mg/l ammonia after two weeks. Hypermia (H), (H-E, x400).

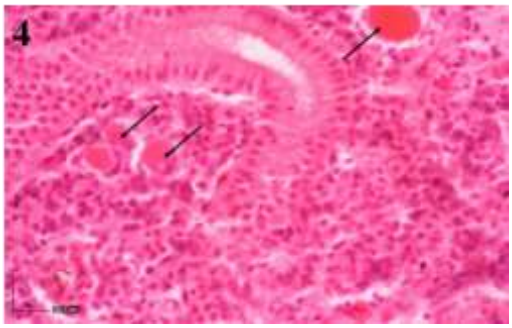

Figure 4. Group exposed to 2.7 mg/l ammonia after two weeks. Hyalen accumulation (arrows), (H-E, x400).

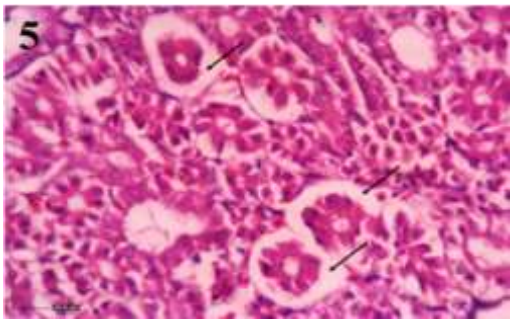

Figure 5. Group exposed to 2.7 mg/l ammonia after two weeks. Epithelial lifting (arrows), (H-E, x400).

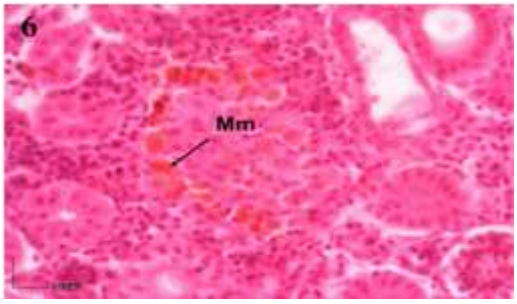

Figure 6. Group exposed to 2.7 mg/l ammonia after two weeks. Melanomacrophages aggregates (Mm), (H-E, x400).

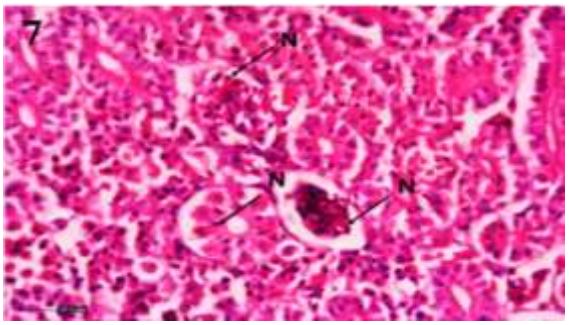

Figure 7. Group exposed to 2.7 mg/l ammonia after two weeks. Necrosis of glomeruli and tubules (N), (H-E, x400).

Table 1: Morphometric analysis of the kidney of tilapia after 2 weeks of exposure to ammonia

Changes	Control	NH3 0.9 mg/l	NH3 1.8 mg/l	NH3 2.7 mg/l
Melanomacrophages aggregates	-	+	++	+++
Hypermia	-	+	++	+++
Inflammation of interstitial tissue	-	+	++	+++

Note: absent (-); rare (+); frequency (++); very frequency (+++); Number fish per group =10

Table 2: Serum blood factors of tilapia after 2 weeks of exposure to ammonia

Factor	Control	A	B	C
Cholesterol (mg/dl)	156.71±13.66 [a]	167.31±15.72 [a]	179.73±18.67 [a]	187.21±18.33 [a]
BUN (mg/dl)	5.44±0.27 [a]	6.21±0.69 [a]	7.18±0.25 [a]	9.41±1.17 [b]
Total protein (g/dl)	2.47±0.33 [a]	2.15±0.52 [a]	1.84±0.62 [a]	1.23±0.21 [a]

Note: Different letters within each row indicate a significant difference in level of ($p \leq 0.05$).

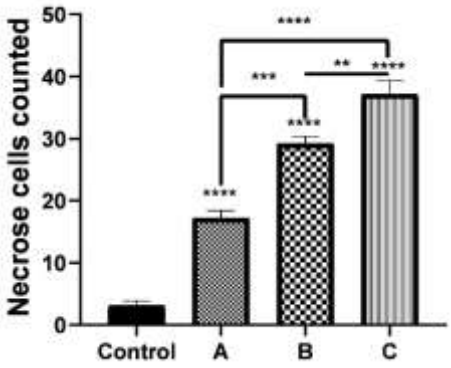

Figure 8. Control and Groups exposed to 0.9 mg/l (A), 1.8 mg/l (B), and 2.7 mg/l (C) ammonia after two weeks. Increased number of cell necrosis, The asterisks indicate a significant difference at the level of (P<0.05), N=10.

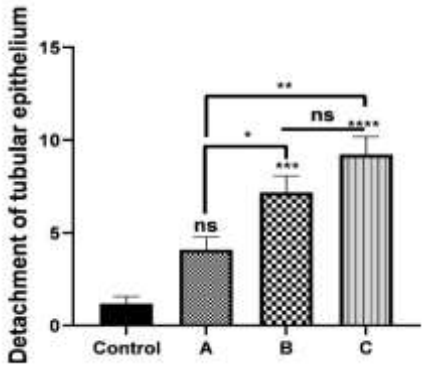

Figure 9. Control and Groups exposed to 0.9 mg/l (A), 1.8 mg/l (B), and 2.7 mg/l (C) ammonia after two weeks. Epithelial lifting of tubules, The asterisks indicate a significant difference at the level of (P<0.05), N=10.

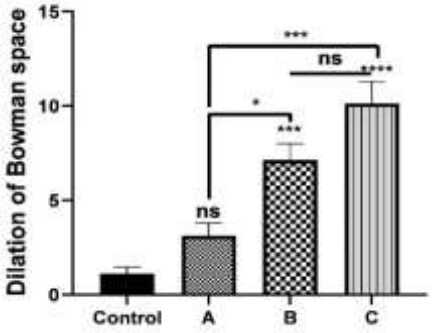

Figure 10. Control and Groups exposed to 0.9 mg/l (A), 1.8 mg/l (B), and 2.7 mg/l (C) ammonia after two weeks. Dilation of bowman space, The asterisks indicate a significant difference at the level of (P<0.05), N=10.

Discussion

Ammonia is one of the serious problems in the aquaculture industry and causes a series of changes in the tissue indices of the liver, kidney, and some blood biochemical factors in different fish (Au, 2004). One of the fundamental problems is to obtain a standard ammonia concentration that is not dangerous and harmful to aquatic life and to create an environment in which this water toxin causes the least harm to the fish (Kim et al, 2019; El-Sayed et al, 2008). Juvenile fish show higher sensitivity to ammonia and *o. niloticus* was used in the current study. Ammonia concentrations are strongly influenced by temperature, pH, and other factors such as dissolved oxygen levels, salinity, animal species, age, and fish size (Khalil et al, 2016). When the NH3 concentration in water increases, it enters the cells because it can be infiltrated into the cell membrane, and plasma ammonia levels

increase as its excretion from the fish body decreases. Elevated blood ammonia levels increase oxygen consumption and reduce the blood's ability to transport oxygen in the body. Kidney damage also occurs due to lesions of the glomeruli and renal tubules that include a decrease in the oxygen-carrying ability of the blood, a decrease in the entry of oxygen into the fish body due to gill damage, and a disturbance in the metabolism of brain cells (Ferreira and Martins, 2008). These damages are attributed to the dirty option of the electrochemical properties of nerve cell membranes and the effect of ammonia on neurotransmitters and biochemical activity in the brain. Fish of different species do not have the same ability to convert ammonia to urea. This can be considered as one of the reasons for the difference in sensitivity to ammonia. Since there is a need for a special device for measuring blood ammonia, like other blood gases, there is a high possibility of an error during this process, it is possible to indirectly detect ammonia poisoning by measuring urea. Because aminotransferase is located in the mitochondria and is a biologically beneficial marker for cell damage, changes in blood aminotransferase levels can be associated with mitochondrial disorders and tissue damage. So, there was an increasing trend in the severity of these complications with increasing ammonia concentration. The kidney is an important target organ for the toxic effects of a large number of environmental contaminants. Histological changes occur in the glomeruli and renal tubules of fish following exposure to toxins and contaminants (Magouz et al, 2021; Gammerdinger et al, 2016). Microscopic kidney examination showed several pathological lesions such as dilatation of Bowman's capsule, hyperemia, hyaline accumulation, epithelial detachment, increased melanomacrophage centers, and necrosis of glomeruli and tubules (Lim et al, 2015; Isani et al, 2013). In conclusion, the ammonia susceptibility of fish species in a family is not the same. In the face of chronic ammonia poisoning, growth rate and survival rate decrease and the fish become more susceptible to infectious agents (Abdelghany, 2020). These conditions are usually associated with gill, liver, and kidney lesions. In general, it can be concluded that the presence of nitrite and ammonia in water can cause microscopic and macroscopic damage in different tissues and changes in some blood factors in N. tilapia.

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Conflict of interest

The authors declare no conflict of interest.

Funding

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اثر غلظت‌های مختلف آمونیاک بر روی هیستومورفومتری کلیه و برخی فاکتورهای خونی ماهی تیلاپیای نیل

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چکیده

در مطالعه حاضر، ماهیان تیلاپیای جوان به مدت دو هفته در معرض ۱۰، ۲۰ و ۳۰ درصد (۹۶ ساعت LC50) آمونیاک قرار گرفته که به ترتیب معادل ۰/۹، ۱/۸ و ۲/۷ میلی‌گرم در لیتر است. پس از این مدت، ماهی‌ها بیهوش و از ساقه دمی با سرنگ هپارینه برای ارزیابی شاخص‌های خونی نمونه خون گرفته شد. نمونه‌های بافتی به اندازه ۰/۵ سانتی‌متر از کلیه جدا و در فرمالین بافر ۱۰ درصد تثبیت و پس از آب‌گیری با الکل، شفاف‌سازی با زایلول، بلوک گیری با پارافین و برش به ضخامت ۶-۴ میکرون با میکروتوم انجام گرفت. در نهایت اسلایدهای رنگ‌آمیزی شده توسط میکروسکوپ نوری مورد مطالعه قرار گرفتند. نتایج حاکی از بزرگ شدن کپسول بومن، تجمع هیالین، کنده شدن اپیتلیوم، افزایش مراکز ملانومکروفاژ و گلومرول‌ها و لوله‌های نکروزه در کلیه‌ها پس از قرار گرفتن در معرض آمونیاک شد. در بررسی فاکتورهای سرم خون با افزایش آمونیاک، کلسترول و BUN نسبت به گروه کنترل و سایر گروه‌ها افزایش یافته بود. همچنین با افزایش غلظت آمونیاک، شدت ضایعات نیز افزایش یافت. بنابراین آمونیاک باعث تغییراتی در ساختار و فعالیت برخی از عوامل کلیوی شده که باید با ایجاد شرایط مدیریتی مناسب در خصوص مقدار آمونیاک در محیط آبی کنترل شود.

کلمات کلیدی: هیستومورفومتری، آمونیاک، تیلاپیای نیل، کلیه

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