# Histopathological changes in various tissues of striped catfish, *Pangasianodon hypophthalmus*, fed on dietary nucleotides and exposed to water-borne silver nanoparticles or silver nitrate

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## Abstract

The objective of this paper focused on the ffects of dietary nucleotides (NT) on histopathological alternations of striped catfish *Pangasianodon hypophthalmus*, after exposure to water-borne silver nanoparticles (AgNPs) and silver nitrate ($AgNO_3$). Fish were fed with a diet containing nucleotide (0.75%) or control diet for 10 weeks and then divided into 3 experiments including control, 20 µg $L^{-1}$ of AgNPs or $AgNO_3$ for 10 days. At the end, histopathological changes in gill, liver and kidney were evaluated using haematoxylin-eosin technique. Water-borne AgNPs or $AgNO_3$ caused some distinctive histopathological alterations in both feeding groups. The most important damages were observed in epithelial cell hyperplasia, inflammation and necrotic epithelial cell in the gills, pigmentation, fiber cells and cytoplasmic vacuolization of hepatocytes in the liver and visualization of red blood cells and eosinophils, glomerular and tubular necrosis in the kidney. Based on organ index ($I_{org}$), the highest damages were observed in the tissues of the fish fed on the control diet and exposed to 20 µg $L^{-1}$ of AgNPs. No significant differences were observed in histopathological alterations between two feeding groups when compare the same organs with similar pollutant (kind and concentration). It could be concluded that dietary NT could not improve the fish ability against water-borne AgNPs or $AgNO_3$.

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**Keywords:** Nanotechnology, Gills, Kidney, Liver, Organ index, Food supplement.



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## Introduction

Nanotechnology has become an important element in the global economy. Nano-scale products will inevitably lead to an increase in sewage outflows of nanomaterials into the environment. Different kinds of nanoparticles, which their deposition and accumulation in aquatic environment are highly vulnerable (Ydollahi, Ahari & Anvar 2016). The nanomaterials can interact with living and non-living components, but their harmful and adverse effects are still not fully understood and this lack of knowledge leads to concern for human health and the environment (Scown, Santos, Johnston, Gaiser, Baalousha, Mitov, Lead, Stone, Fernandes, Jepson, Aerle & Tyler 2010). Silver nanoparticles (Ag-NPs) are applicable in various industries such as agriculture and animal husbandry, packaging, household appliances, cosmetics, medical and military field because of their special magnetic, electrical and catalytic properties (Choi, Deng, Kim, Ross, Surampalli & Hu 2008; Jayesh, Chatterjeec, Duttaguptab & Mukherji 2008). Ag-NPs are formed from silver materials in size of 10-100 nanometer and are very stable (Geho, Jones, Petricoin & Liotta 2006). The increased production and widespread use of Ag-NPs have raised the possibility of their release into aquatic environments. Various research groups have recently demonstrated that Ag-NPs can constantly release in to the water systems, therefore the toxicity of Ag-NPs to aquatic organisms has given reasons for concern recently (Blaise, Gagne, Ferard & Eullaffroy 2008; Yuan & Zhou 2013). Recently, the negative effects of different concentrations of silver nano-particles were studied of haematological and histological indices of different fish species such as Rainbow catfish, *Pangasianodon hypophthalmus* (Razmara, Paykan Heyrati & Dorafshan, 2014a; Razmara, Dorafshan, Paykan Heyrati, Talebi & Ranjbar, 2014b) and Zayandehrud Chub, *Petroleuciscus esfahani* (Raki, Paykan Heyrati & Dorafshan, 2015). Different methods can be used in order to reduce the effects of stress in aquatic organisms (Bahrami Babaheydari S., Dorafshan S., Paykan Heyrati F. & Mahboobi Soofiani N. 2014a; Bahrami Babaheydari, Dorafshan, Paykan Heyrati & Mahboobi Soofiani 2014b; Bahrami Babaheydari, Paykan Heyrati, Dorafshan, Mahboobi Soofiani & Vahabi 2015). One of them is food supplements such as nucleotides (NTs). NTs are low molecular biochemical compounds that have numerous essential physiological and biochemical functions, including chemical energy transference, building monomeric units of nucleic acids, biosynthetic pathways, coenzyme components and biological regulators (Li & Gatlin III 2006). Initial efforts to evaluate dietary supplementation of NTs for fish can be traced back to the early 1970s, which focused on diet palatability and feeding behaviour for supplementation of NTs in fish diets (Gatlin III & Li 2007). Although NTs are synthesized endogenously, supplementing it in diet may have beneficial effects on fish growth performance, hematology, immune response, intestinal morphology and microbiota



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(Yaghobi, Dorafshan, Paykan Heyrati, & Mahmoudi 2014a; Yaghobi, Paykan Heyrati, Akhlaghi, Dorafshan & Mahmoudi 2014b; Yaghobi, Paykan Heyrati, Dorafshan & Mahmoudi 2015a) and even reproduction performances (Gonzalez-Vecino, Cutts, Batty, Mazorra & Burrells 2003). More recently, Pournori, Dorafshan & Paykan Heyrati (2017) indicated that feeding fish with dietary nucleotide could reduce silver bioaccumulation in different tissues especially gills where subjected to water-borne silver nanoparticles or silver nitrate. The aim of this study was to investigate the effects of dietary NT on histopathological changes in different tissues of striped catfish (*Pangasianodon hypophthalmus*), under water-borne Ag-NPs or silver ions (silver nitrate) stress.

## Materials and Methods

### Fish and feeding trial

One hundred and eighty (180) striped catfish fry (Pangasianodon hypophthalmus), were obtained from a commercial supplier (Sepanta Mahianab Aria Pars, Isfahan, Iran). The fish were acclimatized to laboratory conditions for 2 weeks prior to the experiment and afterwards, they were subjected to a 1% saline bath for 30 min for disinfection (Post, 1987). Subsequently, they were measured for their initial weight (1.52 ± 0.11 g (mean ± SD)) and length (6.05 ± 0.32 cm). The animals were randomly deployed in 6 aerated aquaria (total volume of 150 L). Water temperature (30 ± 2 °C), dissolved oxygen (6.5 ± 0.4 mg $L^{-1}$), and pH (7.8 ± 0.2) were monitored on a daily basis. A practical iso-caloric diet (14.25 KJ $g^{-1}$ diets) containing 39% crude protein, 14% fat, 21.7% carbohydrate, 3% fiber, and <10% moisture (National Research Council, 1973) was formulated with fishmeal, soybean oil, soybean meal, corn, corn gluten, and fish oil and supplemented with the commercial NT mixture 'Optimum' (Chemoforma, Augst, Switzerland) to give 0 and 7.5 g of mixed NTs/kg diets. Fish were fed about 3% of their body weight three times a day (at 10:00, 13:00, and 16:00) for 70 days in triplicate for each NT supplement level.

### Chemicals and particle characterization

The colloidal Ag-NPs (under the commercial name of Nanocid; United States Patent Application No.: 20090013825) at a nominal concentration of 4000 mg $L^{-1}$ were purchased from Nano Nasb Pars Co. (Tehran, Iran). The specifications of the nonparticles are summarized in Table 1 based on previous analysis (Salari Joo, Kalbassi, Yu, Lee & Johari 2013). The nanoparticles were dispersed by sonicating the Ag-NPs stock in an ultrasonic bath (Micro $10^{+}$ sonic, Iran Electronic Industries) prior to each dosing. The silver nitrate dosing stock (Merck Co., Germany) was prepared with ultrapure water (100 mg $L^{-1}$) and sonicated simultaneous with the nanosilver stock.

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**Table 1.** Some measured specifications of colloidal silver nanoparticles (Salari Joo et al. 2013; Katuli et al. 2014)

| Parameter | Evaluation method | Metabolites | Explanations |
|---|---|---|---|
| mg $L^{-1}$ concentration | ICP-AES | 3980 | Concentration with little difference from the manufacturing plant. |
| Shape | TEM | Globular | ------- |
| Particle size (hydrodynamic diameter) (nm) | Zetasizer | 3.9-163.5 | 54.1% of particles have less than 100 nm hydrodynamic diameters. |
| The average of the hydrodynamic diameter (nm) | Zetasizer | 54.8 | ------- |
| The maximum diameter (nm) | TEM | 129 | 65.14% of the particles have a diameter between nm 1-13. |
| Purity | EDX | | Only silver element is in colloids of silver nanoparticles |

**Experimental design and exposure**

After the feeding period, a hundred and fifty fish (average weight: 16.12 ± 0.21g, and average length: 12.97 ± 0.46cm) were randomly captured from the previously treated shoals. The group fed on the dietary NT were distinguished from the one fed on the regular diet by cutting the upper part of the caudal fins of the former and the lower part of the caudal fins of the latter. Then, the fish were housed in 3 aerated aquaria filled with a total volume of 100 L of dechlorinated tap water (each aquarium containing 50 fish, 25 from each dietary group). The experimental aquaria were dosed 24 h prior to the exposure period (adding fish), drained again, and redosed once the fish were added to prevent any likely dose reduction due to the adhesion of silver forms to the glass or air stones. The experiment was carried out in a completely randomized design with the following 3 different concentrations as treatments: 20µg $L^{-1}$ of silver nitrate ($20AgNO_3$), 20µg $L^{-1}$ of Ag-NPs (20AgNPs), and blank or plain water as control. In each treatment fish were exposed to the corresponding level of Ag-NPs and $AgNO_3$ without feeding for 10 days (Scown et al. 2010). Silver nitrate was also used to distinguish the toxicity of Ag-NPs from that of silver ions. Water (50% of the total volume) was changed and the solutions were re-dosed at 48-hour intervals. The physiochemical properties of the solutions including temperature, dissolved oxygen, pH, electric conductivity, total hardness, and total phosphate were monitored on a daily basis and maintained within the ranges of 29.8−31.2 °C, 6.1−7.2 mg $L^{-1}$, 7.8−8.2, 482.2−484.4 µs $cm^{-1}$, 179−182 mg $L^{-1}$ $CaCO_3$, and 0.07−0.09 ppm, respectively. The actual concentrations of silver in the aquaria



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were measured 5 times throughout the exposure period (24 h after redosing) by sampling 50 ml of the central water column. Atomic absorption spectroscopy (Perkin Elmer Analyst Model A Analyst 700) was used to determine silver concentrations based on the NIOSH 7300 (1999) protocol; the actual concentrations of silver in $20AgNO_3$ and 20AgNPs, were measured to be $19.5 \pm 0.05$ and $20.5 \pm 0.07$ µg $L^{-1}$, respectively. Finally, no silver concentration was detected in the control plain water.

**Experimental sampling**

At the end of the exposure period (10 days), the fish were anesthetised with $MS_{222}$ (200 mg $L^{-1}$). Gill tissue, liver and kidney tissues of at least 10 fish from each treatments were collected and fixed in 10% buffered formalin (pH = 7.4) for further analysis.

**Histological analyses**

After fixation, the segments of the tissues were rinsed in water, dehydrated in graded ethanol solutions, cleared in xylene and embedded in paraffin. De-waxed sections (4-5 µm) were stained for histological purpose by haematoxylin and eosin (H & E). The slides were examined under a light microscope (Olympus, BX60, Tokyo, Japan) to check for any signs of tissue damage. The modified version of the method described (Bernet, Schmidt, Meier, Burkhardt-Holm & Wahli 1999) was used to quantify the histological changes in different tissues. Briefly, the histopathologic changes of tissues are classified into five response patterns including circulatory disorders, reversible changes, progressive changes, inflammation and tumour. Each response pattern has two factors (a: score value and w: importance factor). Score value (a) was between 1-6 based on the severity of the histopathological changes; where score grade 0 belongs to the tissue without any sign of histopathological changes and 6 to the highest alterations. Importance factor (IF) was evaluated between 1-3 where 1 and 3 were used for changes with the lowest and highest importance for the fish survival. e.g. haemorrhage alteration gets IF = 1 while IF for tumour or necrosis will be 3. The organ index, $I_{org}$ was calculated as follow:

$$I_{org}=\sum_{rp}\sum_{alt}(a_{org\ rp\ alt}\times w_{org\ rp\ alt})$$

Where $I_{org}$: organ index, rp: reaction pattern eg. Inflammation or neoplasm, alt: kind of alteration, a: score value and w: importance factor. The study was performed under ethics code of Iran's Veterinary Organization and Iran's Fisheries Organization.

**Statistical analysis**

The data ($I_{org}$) was reported as means ± standard error of mean (S.E.M) and subsequently subjected to one-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) to find out significant differences among the treatments (the same tissue in different treatments or different context in same treatment) at $P<0.05$. Statistical analysis was performed using SPSS 22.0. To assess significant differences between two groups of fish fed on diet contacting dietary NT or control diet, Pairwise comparison of means, an independent t-test was used.



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## Results

### Histopathological changes in the gill tissue

Histopathological changes in the gill tissues in various treatments have been shown in Fig. 1. In control treatment, an epithelial cell hyperplasia complication with the loss of gill lamellae as well as swelling and fluid in the secondary blade was observed. Exposure to other treatments brought up complications such as necrosis of epithelial cells, blood accumulates in the bottom of the blade gill, swelling and fluid in the secondary blades, increased secretion of eosinophilic and hyperplasia with lift in epithelial cells. According to a ranking table (Table 2) as well as quantitative analysis of gill tissue damage, the most dramatic change compared to the control group was observed in fish exposed to the 20AgNPs. There was no significant difference between the two feeding groups regarding intensity of injuries (neither in control plan water nor in water containing $AgNO_3$ or Ag-NPs) (Fig. 2).

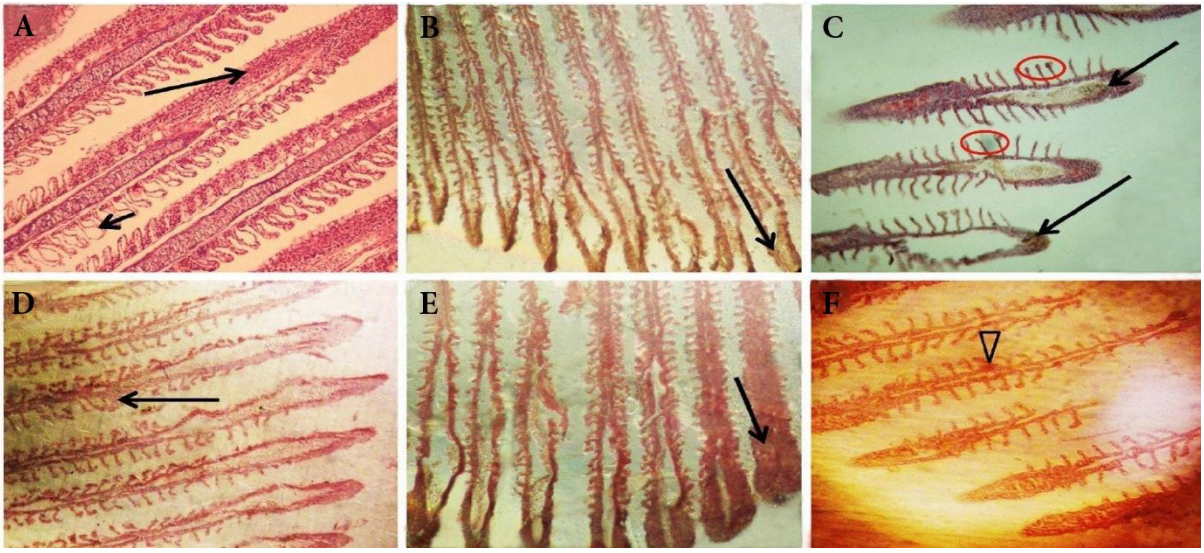


**Figure 1.** Gill histopathological changes of the fish in the fish after 10-days exposure to 20 µg $L^{-1}$ of silver nanoparticles or silver nitrate. A) epithelial cell hyperplasia with loss of gill lamellae (long arrow), swelling and fluid in the secondary blades (short arrow) (× 600), B) epithelial cell necrosis (× 400), C) blood accumulation in the gill lamellae (red circle) (× 400), D) swelling and fluid in the secondary blade (× 400), E) increased eosinophilic secretions (× 400), F) slight hyperplasia with raise in epithelial cells (arrow heads) (× 400).

### Histopathological changes in the liver tissue

Histopathological changes in the liver tissues in various treatments have been shown in Fig. 3. Liver tissue of control was natural and healthy with quite regular liver cells. In other treatments, complications such as bleeding and pigmentation, fibrosis of hepatocyte cells, vacuolization of cytoplasm, necrosis of hepatocytes area, dual-core, the pyknotic nuclei, nuclear degeneration and hypertrophy were observed. According to the quantitative grading method, in both control groups fed on supplemented NTs or without-NTs only two major regional necrosis of hepatocytes and dual-core were observed. According to the quantitative grading method, in both control groups fed on supplemented NTs or without-NTs only two major regional necrosis of hepatocytes and dual-core were observed.



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Treating fish with 20AgNPs caused fibrosis of hepatocyte cells and pyknotic nuclei although no significant difference was observed between two dietary groups. Some other histophatological changes such as regional necrosis of hepatocytes tissue were observed in the groups of fish exposed to 20AgNPs, while fish fed on dietary NT showed lower changes in comparison to the fish fed on the control diet (Table 3). According to the quantitative analysis of the histopathological changes (Iorg), treating fish with water-borne AgNO3 or Ag-NPs could cause significant increase in Iorg, While, statistical analysis of $I_{org}$ between two feeding groups at each chemical exposure did not show any significant changes, indicating that feeding fish with dietary NTs could not affect fish resistance to water pollution (Fig. 4).

**Table 2.** Semi-quantitative analysis of gill tissue damages in different groups of fish fed on diet enriched with nucleotide or without nucleotides exposed to plan water (control) or water polluted with 20 µg $L^{-1}$ of silver nitrate or nanoparticles

| Treatment Phenomenon | Control | Silver nitrate | Silver nanoparticles | Silver nitrate + nucleotide | Silver nanoparticles + nucleotide |
|---|---|---|---|---|---|
| Accumulation of blood | 0 | +++ | +++ | ++ | +++ |
| Epithelial cell hyperplasia | + | +++ | ++++ | +++ | ++++ |
| Raise in epithelial | 0 | + | ++ | + | ++ |
| Eosinophilic secretions | 0 | ++ | ++ | ++ | ++ |
| Swelling and fluid in the secondary blades | + | + | +++ | + | ++ |
| Necrosis | 0 | 0 | + | 0 | + |

Value score: very mild: 0, mild: +, light: ++, medium (to the extreme): +++, high: ++++.

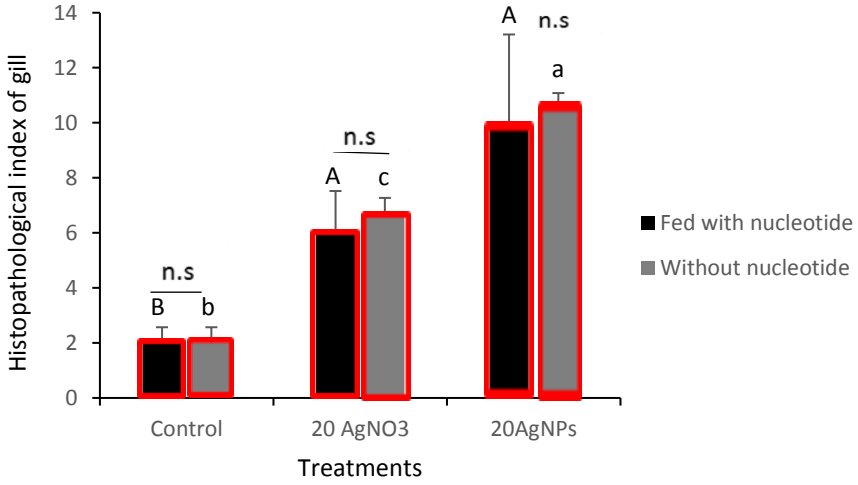


**Figure 2.** The $I_{org}$ changes (mean ± SE) in the gill tissues of the fish fed on supplemented nucleotide or free-nucleotide diet after exposing to clean water (control), 20 µg $L^{-1}$ of silver nanoparticles or silver nitrate as water pollution. Significant differences between different kinds of water pollution in the same dietary group are indicated by unlike upper or lower case letters for fish fed on supplemented nucleotide or free nucleotide diet respectively ($P<0.05$; DMRT); while no significant differences between two groups (with or without supplemented diet) at the same water pollution treatment ($P>0.05$; t-test) are indicated (n.s.).



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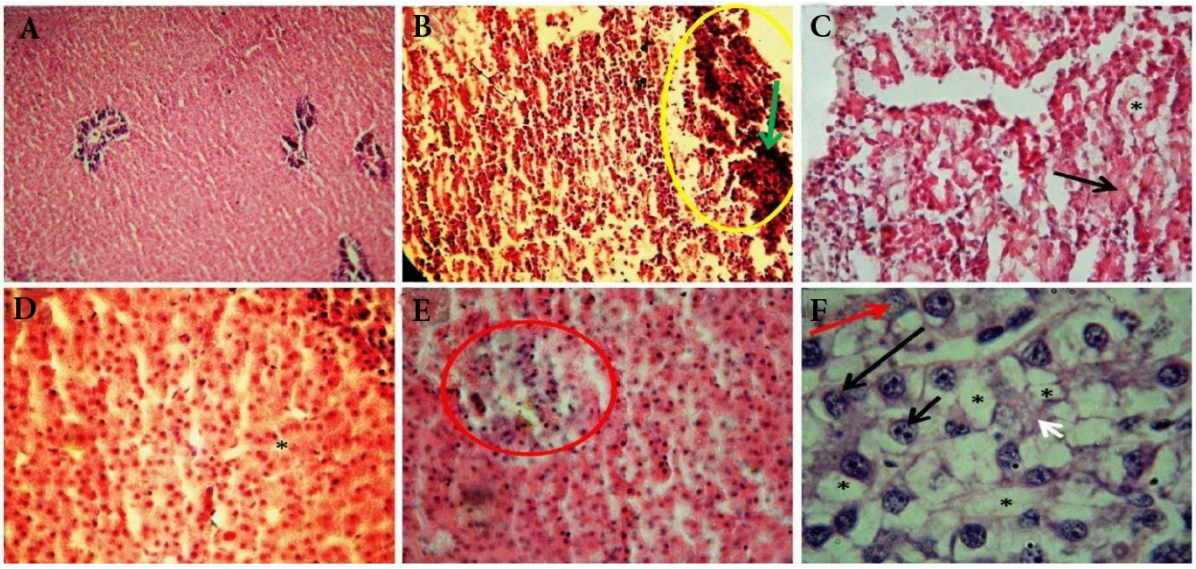


**Figure 3.** Liver histopathological changes of the fish in the fish after 10-days exposure to clean water and/or 20 µg $L^{-1}$ of silver nanoparticles or silver nitrate. A) healthy liver tissue with quite regular liver cells (× 100), B) bleeding in the liver tissue (the area inside the yellow oval), pigmentation (green arrow) (× 100), C) fibrosis of hepatocytes and liver cells, vacuolization of cytoplasm (*) (× 400), D) vacuolization (*) (× 400), E) area tissue necrosis of hepatocytes (the area within the red circle), vacuolization of cytoplasm (*) (× 400), F) dual-core (short black arrow), pyknotic nuclei (long black arrow), degeneration of the nucleus (red arrow), vacuolization of cytoplasm (*), hypertrophy (white arrows) (× 1000).

**Table 3.** Semi-quantitative analysis of liver tissue damages in different groups of fish fed on diet enriched with nucleotide or without nucleotides exposed to plan water (control) or water polluted with 20 µg $L^{-1}$ of silver nitrate or nanoparticles

| Treatment<br>Phenomenon | Control | Silver nanoparticles | Silver nitrate | Nano Silver + nucleotides | Silver nitrate + nucleotides |
|---|---|---|---|---|---|
| Regional necrosis of hepatocytes tissue | + | ++++ | +++ | +++ | ++ |
| Bleeding | 0 | ++ | + | + | 0 |
| Pyknotic nuclei | 0 | + | + | + | + |
| Pigmentation | 0 | ++ | ++ | + | + |
| Nuclear degeneration | 0 | ++++ | ++ | +++ | ++ |
| Vacuolization of cytoplasm | 0 | +++ | ++ | ++ | ++ |
| Division into two nuclei | + | +++ | +++ | ++ | + |
| Fibrosis of hepatocyte cells | 0 | + | ++ | + | + |
| Hypertrophy | 0 | + | ++ | 0 | + |

Value score: very mild: 0, mild: +, light: ++, medium (to the extreme): +++, high: ++++.



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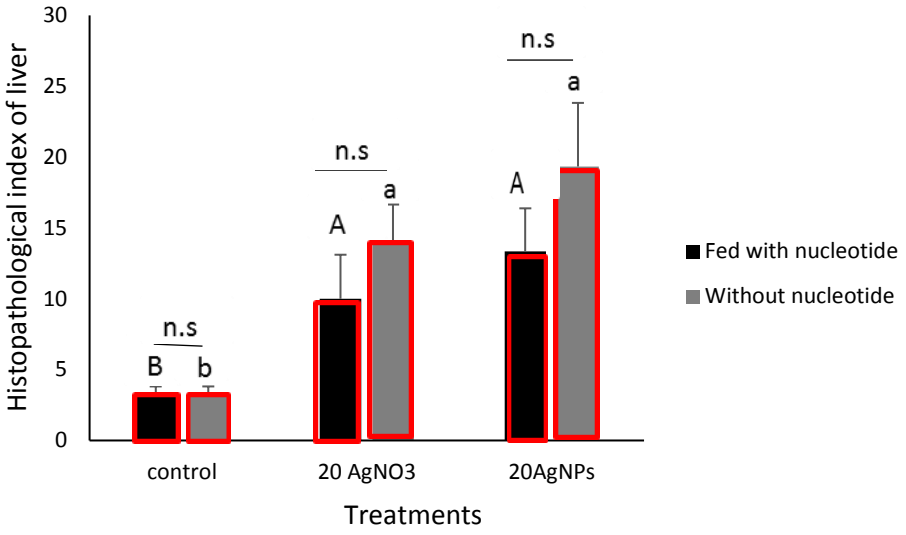


**Figure 4.** The $I_{org}$ changes (mean ± SE) in the liver tissues of the fish fed on supplemented nucleotide or free-nucleotide diet after exposing to clean water (control), 20 µg $L^{-1}$ of silver nanoparticles or silver nitrate as water pollution. Significant differences between different kinds of water pollution in the same dietary group are indicated by unlike upper or lower case letters for fish fed on supplemented nucleotide or free nucleotide diet respectively ($P<0.05$; DMRT); while no significant differences between two groups (with or without supplemented diet) at the same water pollution treatment ($P>0.05$; t-test) are indicated (n.s.).

**Histopathological changes in kidney tissue**

Histopathological changes in the kidney tissues of the fish in different treatments have been shown in Fig. 5. In the control group, kidney was perfectly healthy consisting of glomeruli, tubules, hematopoietic tissue and a variable number of visible melanocytes. In other groups of fish exposed to Ag-NPs or $AgNO_3$, histopathological alterations such as bleeding in hematopoietic tissue, loss of hematopoietic tissue in the excretory renal (kidney posterior part), absence of dark inflation degeneration and of Bowman's space, destruction of Bowman's space, inflammatory exudate, glomerular necrosis, tubular necrosis, increased presence of red blood cells and eosinophils or eosinophilic accumulation were detected. Table 4 show semi quantitative tissue alterations, generally, it could be concluded that only some narrow changes in histopathological alterations between two silver chemicals, Ag-NPs and $AgNO_3$ were observed. For example, hematopoietic bleeding and dark inflation degeneration were two histopathological changes which might be affected by dietary NT (Table 4). $I_{org}$ was ranged between 0-12 in fish exposed to the clean or contaminated water with Ag-NPs, showing a significant increase in organ index changes after exposing fish to the Ag chemicals (Fig. 6). Statistical analysis between two feeding groups (diet supplemented with NTs or without NTs), when exposed to water-born $AgNO_3$ or Ag-NPs, showed no significant differences. Therefore, it could be concluded that dietary NTs could not affect histopathological changes caused by water-born Ag-NPs or $AgNO_3$ (Fig. 6).



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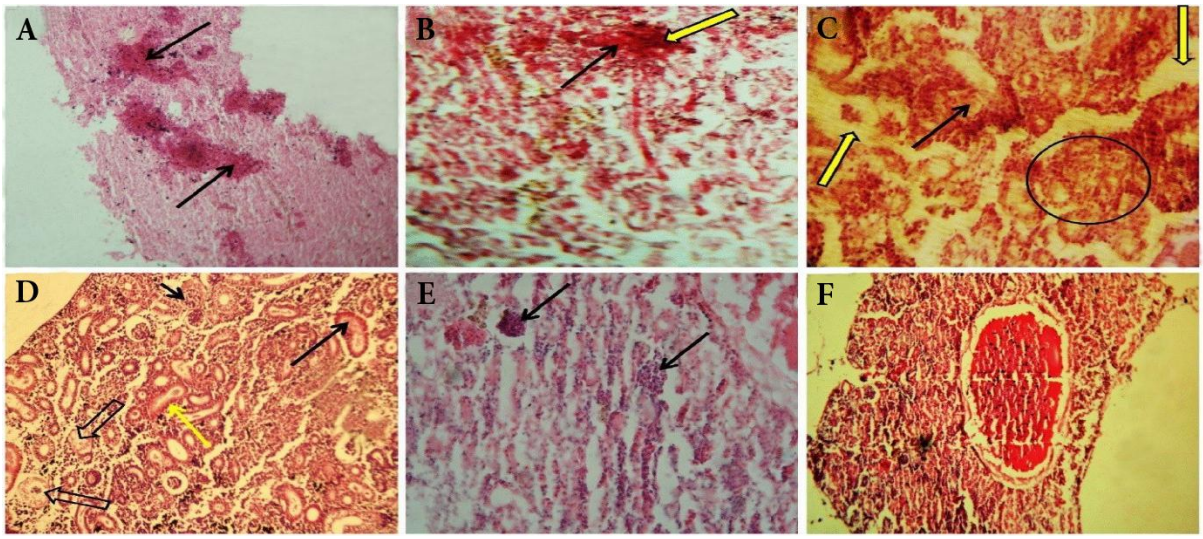


**Figure 5.** Kidney histopathological changes in the fish after 10-days exposure to 20 µg $L^{-1}$ of silver nanoparticles or silver nitrate. A) bleeding in the hematopoietic (× 400), B) bleeding associated with hematopoietic tissue necrosis in the kidney (× 400), C) the loss of hematopoietic tissue in the excretory renal (kidney posterior part) (The yellow arrow), absence of Bowman's space (inside the circle), Inflation degeneration dark (black arrows) (× 400), D) the loss of Bowman's space (short black arrow), Inflammatory exudate (long black arrow), glomerular necrosis (arrow hollow), Tubular necrosis (yellow arrow) (× 1000), E) increase the presence of red blood cells and eosinophils (in the whole picture), Accumulation of eosinophils (black arrows) (× 400), F) the normal controls group kidney and consists of glomeruli, tubules, hematopoietic tissue and a variable number of melanocytes (× 400).

**Table 4.** Semi-quantitative analysis of kidney tissue damages in different groups of fish fed on diet enriched with nucleotide or without nucleotides exposed to plan water (control) or water polluted with 20 µg $L^{-1}$ of silver nitrate or nanoparticles

| Treatment<br>Phenomenon | Control | Silver nanoparticles | Silver nitrate | Nano Silver + nucleotides | Silver nitrate + nucleotides |
|---|---|---|---|---|---|
| Hematopoietic necrosis | 0 | + + | + | + + | + |
| Bleeding in the hematopoietic | 0 | + + + | + + + | + + | + + |
| Inflammatory exudates | 0 | + | 0 | + | 0 |
| Absence of Bowman's space | 0 | + | + | + | + |
| Dark inflation degeneration | 0 | + | + + | 0 | + + |
| Increased presence of red blood cells and eosinophils | 0 | + + | + + | + + | + |
| Glomerular necrosis | 0 | + + + | + + | + + + | + + |
| Tubular necrosis | 0 | + | + | + | + |

Value score: very mild: 0, mild: +, light: ++, medium (to the extreme): +++, high: ++++.



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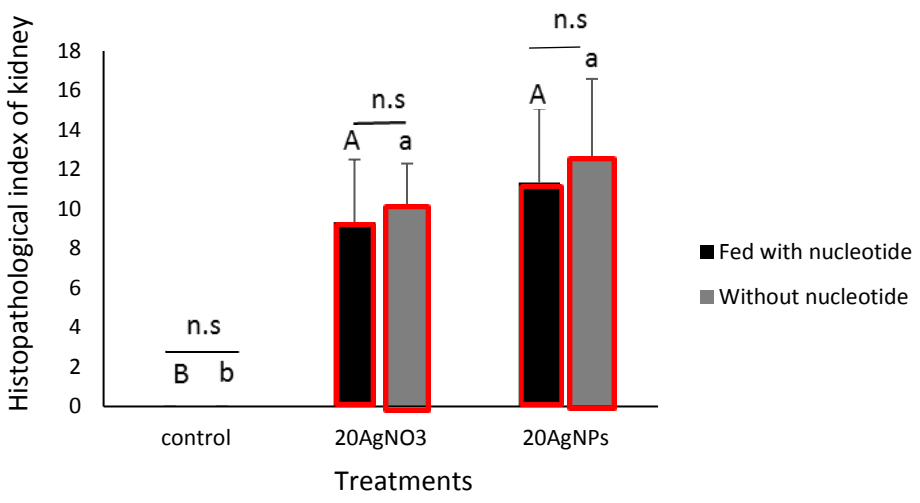


**Figure. 6.** The $I_{org}$ changes (mean ± SE) in the kidney tissues of the fish fed on supplemented nucleotide or free-nucleotide diet after exposing to clean water (control), 20 µg $L^{-1}$ of silver nanoparticles or silver nitrate as water pollution. Significant differences between different kinds of water pollution in the same dietary group are indicated by unlike upper or lower case letters for fish fed on supplemented nucleotide or free nucleotide diet respectively ($P<0.05$; DMRT); while no significant differences between two groups (with or without supplemented diet) at the same water pollution treatment ($P>0.05$; t-test) are indicated (n.s.).

## Discussion and conclusion

Due to the increasing importance of histology in comparison to other toxicological parameters such as mortality and behavioral studies in recent years, more attention has been paid on histological studies as one of the basic methods in investigating aquatic toxicity and it provides useful information for a better understanding of the damage caused by chemical contaminants (Yuan et al. 2013). In this study, histopathological alterations in different tissues (gill, liver and kidney) of the fish fed on two diets, supplemented with or without NT, were investigated after exposing fish to the water-borne Ag-NPs or $AgNO_3$ at 20 µg $L^{-1}$. Results showed that all tissues were affected by water contamination and generally there was no significant difference between groups of fish fed on dietary NTs or NTs free-diet. On the other hand, it seemed that silver nanoparticles showed higher effects on tissues in comparison to the silver ions derived from $AgNO_3$. The toxicity of nanoparticles is associated with the small size and high surface area of these particles. Theoretically, nanomaterials are expected to have a higher level of toxicity due to their larger reaction surface as well as their ability to penetrate and accumulate in the cells of organisms. There are some evidences that may suggest silver ions released by the silver nanoparticles can be the primary cause of the toxicity of silver nanoparticles. Due to the low solubility of silver nanoparticles in an experimental environment, the results may suggest particular cellular mechanisms which



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could lead to toxic effects of silver nanoparticles (Volker, Kamoken, Boedicker & Oetken 2015). However, the exact mechanisms of nanoparticles on aquatic organisms are not clear yet and it is possible that different environmental factors can affect metal solubility and its toxicity. It was reported that copper ions may be released from the copper nanoparticles thus the fish are exposed to both copper nanoparticles and ions in a same time which caused higher histopathological changes in comparison to the groups of fish exposed only to copper ions (Shaw & Handy 2011). Similar results were reported on zinc oxide nanoparticles (Araujo, Dubourguier, Kasemets & Kahru 2009). It is also documented that nanoscale particles of titanium dioxide had more adverse effects than larger particle size in zebra fish, *Danio rerio* (Xiong, Fang, Yu, Sima & Zhu 2011). After exposing rainbow trout, *Oncorhynchus mykiss* (Walbaum) to several dosages of copper sulphate or nanoparticles (average diameter 50 nm), histopathological changes on the gill, liver, kidney, intestine, muscle and brain tissues showed that copper nanoparticles had higher adverse effects on the tissues in comparison to the copper sulphate (Al-Bairuty, Shaw, Handy & Henry 2013). It seems that nanoparticles had significantly higher adverse effects on fish tissues when compared to the similar dosage of ions.

Semi-quantitative analysis showed that fish fed on dietary NT had lower histopathological changes in comparison to the fish fed on the NT-free diet, but statistical analysis did not show any significant changes between two feeding groups. There is no information on the effects of dietary NTs on fish resistance against water-borne contamination respecting histopathological changes, but it is well-documented that dietary NTs can affect resistance to variety of stressors such as salinity in the Atlantic salmon (Burrells, William, Southage & Wadsworth 2001) and Caspian brown trout (Abedian Kenari, Mahmoudi, Soltani & Abedian Kenari 2012) as well as handling and crowding stress in beluga sturgeon (Yousefi, Abtahi & Abedian Kenari 2012) and rainbow trout (Tahmasebi-Kohyani, Keyvan Shokooh, Nematollahi, Mahmoudi & Pasa-zanoosi 2012). In contrast to these finding, red drum, *Sciaenops ocellatus* (Li et al. 2007), barramundi (Glencross & Rutherford 2010), Nile tilapia (Barros, Giumaraes, Pezzato, Orsi, Junior, Teixeira, Fleuri & Padovani 2013) and the catfish (Yaghobi, Dorafshan, Akhlaghi, Paykan Heyrati & Mahmoudi 2015b) did not show such a positive effect of NT inclusion when fish exposed to the stressful condition. A possible description for such unlike results among different fish species could be caused by variation among individual fish (Li et al. 2006). It is also reported that high levels of NTs or its long duration administration may cause undesirable effects even on growth performance (Matsuo & Miyazano 1993; Adamek, Hamackova, Kouril, Vachta & Stibranyiova 1996). At the end, as a definite conclusion it can be stated briefly that exposure to the chronic form of colloidal silver nanoparticles or silver nitrate caused various microscopic structure changes in the tissues of the catfish and the dietary NTs has very limited positive effects on the catfish welfare after exposing to these chemicals, which may indicate the inappropriate



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usage of NTs on tissue resistance to water contamination.

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## Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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# تغییرات آسیب‌شناسی بافت‌های مختلف گربه ماهی رنگین‌کمان *Pangasianodon hypophthalmus* تغذیه شده با جیره حاوی نوکلئوتید تحت تأثیر نانوذرات نقره یا نیترات نقره محلول در آب

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**چکیده**

اثر نوکلئوتید جیره بر تغییرات بافتی گربه‌ماهی رنگین‌کمان *Pangasianodon hypophthalmus* پس از مواجهه با نانوذرات نقره (AgNPs) و نیترات نقره ($AgNO_3$) محلول در آب بررسی شد. ماهیان به مدت ۱۰ هفته با جیره حاوی نوکلئوتید (۰/۷۵٪) یا شاهد تغذیه و سپس به مدت ۱۰ روز در معرض سه گروه آزمایشی شامل تیمار شاهد (۱ و ۲۰ $\mu g\ L^{-1}$ AgNPs یا $AgNO_3$ قرار گرفتند. در پایان، تغییرات آسیب‌شناسی بافت‌های آبشش، کبد و کلیه با روش هماتوکسلین-ائوزین ارزیابی شد. افزودن AgNPs یا $AgNO_3$ به آب در هر دو گروه، تغییرات آسیب‌شناسی واضحی را پدید آورد. مهم‌ترین آسیب‌های بافتی شامل هایپرپلازی سلول‌های اپیتلیال، تورم و نکروز سلول‌های اپیتلیال در آبشش، تجمع رنگدانه، واکوئله‌شدن سیتوپلاسم و فیبری‌شدن سلول‌های هپاتوسیت در کبد، افزایش گلبول‌های قرمز و ائوزینوفیل‌ها، نکروزیس گلومرول و نکروزیس توبولار در کلیه بود. شاخص ارگان ($I_{org}$) بیشترین شدت آسیب را در بافت‌های ماهیان تغذیه شده با جیره شاهد در مواجهه با غلظت ۲۰ $\mu g\ L^{-1}$ AgNPs نشان داد. تفاوت معناداری در اندامهای مشابه هنگام مواجهه با یک نوع ماده آلاینده در غلظت مشابه بین دو گروه تغذیه شده با نوکلئوتید یا شاهد مشاهده نشد. جیره حاوی نوکلئوتید نمی‌تواند توانایی ماهی را در برابر مواجهه با نانوذرات نقره و نیترات نقره محلول در آب بهبود بخشد.

**کلمات کلیدی:** نانو فن‌آوری، کلیه، کبد، شاخص ارگان، مکمل غذایی.

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