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Biochemical and Histopathological Alterations Induced by Sublethal Concentrations of Silver Nanoparticles and Silver Nitrate in the Nile tilapia (*Oreochromis niloticus*)

Hala Elshahat Ghannam[1], Mohamed Y. M. Aly[1*], Radwa A. El-sayed[2] , Amal Said Mohamed[1]

[1]National Institute of Oceanography and Fisheries (NIOF), Cairo, Egypt
[2]Department of Zoology, faculty of Women for Arts, Science and Education, Ain Shams University
[*]**Corresponding Author: myahya120@yahoo.com**

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ABSTRACT

This study aimed to characterize the biochemical and histopathological alterations induced by sublethal concentrations of silver nanoparticles (AgNPs) and silver nitrate ($AgNO_3$) by determining their toxic effects on the Nile tilapia (*Oreochromis niloticus*). The investigation of growth performance revealed a decline in all measured parameters with increasing concentrations of AgNPs. Fish were exposed to non-lethal concentrations of AgNPs (10, 20, 50, and 100µg/ L) and to 100µg/ L of $AgNO_3$ for durations of 2, 4, and 6 weeks. Exposure to these sublethal concentrations resulted in a significant elevation ($P< 0.05$) in serum glucose, cholesterol, ALT, AST, uric acid, and urea levels compared to the control group. Conversely, total protein levels were significantly decreased ($P< 0.05$) in treated groups. The biochemical changes were more pronounced in fish exposed to AgNPs than in those exposed to silver nitrate, particularly at higher concentrations (50 and 100µg/ L) of AgNPs compared to the lowest concentration (10µg/ L). Histopathological examination revealed degeneration, necrosis, and hemosiderin accumulation in the epidermis, dermis, and muscle layers. Additional findings in the muscle layer included ballooning necrosis, covering cells, vascular infiltration, and edema. These pathological changes intensified with increasing concentrations and longer exposure durations.

INTRODUCTION

Synthesized nanoparticles (ENPs) have emerged as a significant area of interest in contemporary technological advancements, attracting significant research interest due to their exceptional potential across a diverse spectrum of applications. ENPs exhibit unique and superior properties arising from their nanoscale dimensions. These properties encompass a wide range of characteristics, including distinctive chemical reactivity, enhanced mechanical strength, and novel optical and electronic behaviors. These

characteristics make them applicable in several fields such as agriculture, medicine, environment, or biotechnology **(Dube & Okuthe 2023)**. Silver nanoparticles (AgNPs) are used in various applications including water purification, wastewater treatment and in the field of medicine. Moreover, they are used in medical implants, dressings and the manufacture of anti-odor textiles **(Zhang *et al.*, 2018)**. Various techniques have been used to synthesize AgNPs, such as electrochemical technology, chemical reduction, laser irradiation, and thermal decomposition **(Khan *et al.*, 2018)**.

Chemical reduction methods have emerged as a convenient method for synthesizing AgNPs due to their inherent advantages. Compared to other synthesis techniques, chemical reduction methods often require less complex equipment and can be carried out under milder conditions, making them more accessible and cost-effective. Some reports indicate that silver nanoparticles are acutely toxic to adult fishes such as *Cyprinus carpio* and *Perca fluviatilis* **(Bilberg *et al.*, 2011)**. Exposure to silver nanoparticles for 48 hours significantly impaired gill tissue health in adult zebrafish **(Griffitt *et al.*, 2009)**. Exposure of *P. fluviatilis* to a range of nanosilver concentrations for 24 hours resulted in an elevation of critical oxygen tension (Pcrit). Previous research has demonstrated that silver nanoparticles can induce concentration-dependent genotoxic and cytotoxic effects in medaka fish (*Oryzias latipes*) cell lines **(Wise *et al.*, 2010)**.

The literature extensively documents the silver toxicity and its adverse impacts on freshwater fishes **(Zhou, *et al.*, 2005)**. The heavy metals bioaccumulation can reflect the amount of toxicant ingested by the organism, the pattern by which metals are distributed through distinct tissues, and the degree of metal retention in organs. The majority of research has centered on the accumulation of within muscle tissue because this is the part ingested by humans **(Olojo *et al.*, 2005)**. The sensitivity of fish gills to water pollution arises from their direct and constant exposure to the aquatic environment. The accumulation, regulation, and excretion of metals within fish tissues are complex processes influenced by various factors, including the specific metal involved and the physiological characteristics of the fish species. These factors significantly impact the distribution and concentration of metals within different fish tissues **(Keskin, 2007)**. The toxicity of all silver ions is completely dose and formulation dependent. The powder form of silver nanoparticles is less toxic compared to the colloidal form because the powder form is suspended in water, while the colloidal form is soluble in water **(Asghari *et al.*, 2012)**.

A study was conducted to evaluate the toxicity of silver nanoparticles based on particle size. All particle sizes had strong toxic effect on cells at very low concentration. According to some studies, particle size less than 10nm was highly toxic and could cause very harmful effect to living organisms **(Zapor, 2016)**. Conversely, when nanoparticles are exposed to the ecosystem, they form aggregates, which reduce their toxicity **(Bae *et al.*, 2013)**. It is still unclear what quantity of nanoparticles will not be harmful to animals and the environment. Some studies have shown that concentrations below the expected

environmental concentrations can cause harmful effects in prokaryotes, invertebrates and fish **(Fabrega *et al.*, 2011)**. The L_{C50} of different forms of silver nanoparticles was determined for 96 hours and it was found that the nanoparticles were more toxic than silver ions **(Hedayati *et al.*, 2012)**. Different fish have different toxicity levels for these nanoparticles. Therefore, our study aimed to focus on the effect of various concentrations of $AgNO_3$ and AgNPs to study their adverse effect on biochemical and histological alterations in the Nile tilapia fish.

MATERIALS AND METHODS

Preparation of AgNPs

The chemicals used in this experiment were purchased from Sigma-Aldrich. Silver nanoparticles were synthesized using the chemical reduction method described by **Calderon-Jimenez *et al.* (2017)**. The nanoparticles were prepared in nanopowder form and characterized using X-ray diffraction (XRD) and transmission electron microscopy (TEM).

XRD measurements were performed using a Bruker D8 Advance powder diffractometer. The resulting XRD patterns confirmed the crystalline nature of the silver nanoparticles (Fig. 1). The crystal size, as calculated from the XRD data, ranged from 27.44 to 35.02nm, with an average size of 29.92nm.

Morphological characterization and particle size estimation were carried out using both TEM and high-resolution TEM (Tecnai G2 Super Twin, USA). Representative TEM images are shown in Fig. (1a, b).

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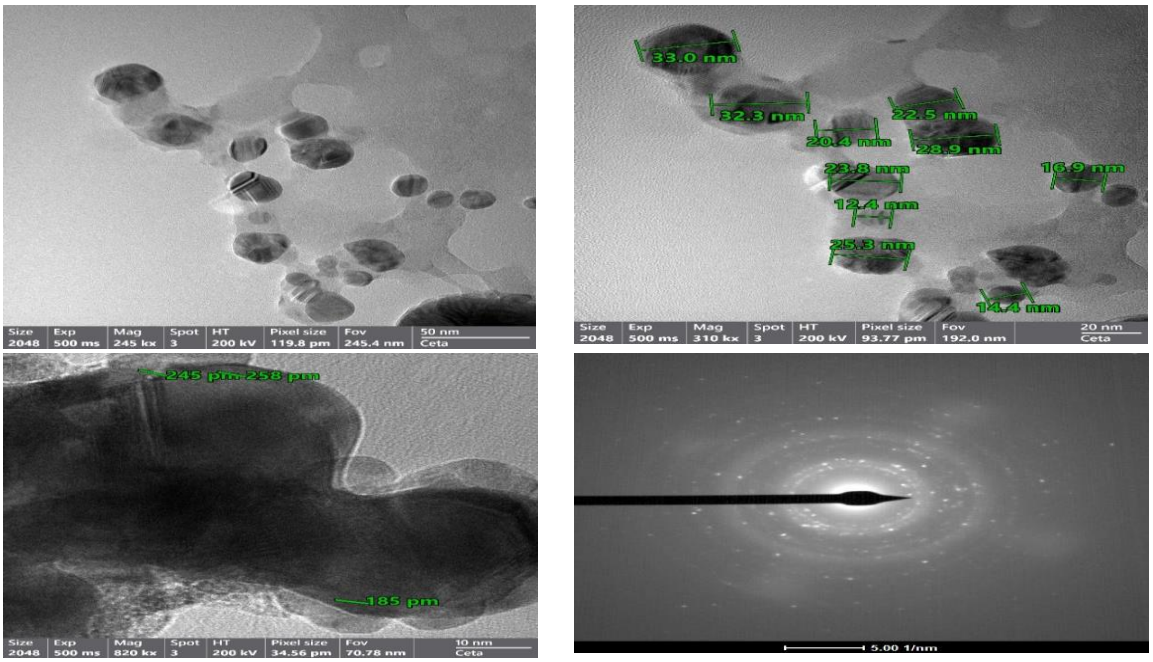

Fig. 1a, b. HR- TEM images of the prepared AgNPs

Experimental fish

Healthy Nile tilapia (*Oreochromis niloticus*) with a mean body weight of 20.2 ± 1.10g and a mean total length of 10.3 ± 0.5cm were obtained from the Qanater El-Khairia Fish Farm, Egypt. The fish were transported to the Pollution Laboratory at the National Institute of Oceanography and Fisheries and were acclimatized for two weeks under laboratory conditions in aerated tanks. Fish were distributed evenly at a density of 20 fish per tank. Water quality parameters were maintained according to **APHA (2005)** standards and were as follows: temperature 26 ± 1°C, pH 7.0 ± 0.20, ammonia concentration 0.53 ± 0.07mg/ L, dissolved oxygen 6 ± 0.5mg/ L, and a 12h light:12 h dark photoperiod. Water was renewed daily at a rate of 20%, and fish feces were removed to maintain cleanliness.

Toxicity bioassay and experimental design

A preliminary experiment was conducted to determine the 96-hour LC_{50} of silver nanoparticles (AgNPs) in the Nile tilapia using the method described by **Puerto *et al.* (2009)**. After acclimatization, fish were randomly assigned to experimental groups and were exposed to AgNP concentrations of 0, 5, 10, 15, 20, and 25mg/ L, including a control group. Exposure durations were 24, 48, 72, and 96 hours. Dead fish were immediately removed using a dip net, and feeding was suspended 24 hours prior to the experiment to minimize metabolic variation.

Growth performance parameters

The growth trial lasted for six weeks. Growth performance was evaluated using the following parameters:

- **Weight Gain (WG)** = Final body weight (g) – Initial body weight (g)
- **Specific Growth Rate (SGR, % day^{-1})** = 100 × [ln(Final weight) – ln(Initial weight)] / Number of days
- **Survival Rate (SR, %)** = 100 × (Final number of fish / Initial number of fish)
-

Biochemical analyses

Blood samples were collected from live fish in both control and exposed groups after 2, 4, and 6 weeks of exposure. Blood was allowed to clot at room temperature, then centrifuged at 3000 rpm for 15 minutes to obtain serum. The following biochemical parameters were analyzed:

- **Glucose**: **Burtis *et al.* (1996)**
- **AST and ALT**: **Thomas (1998)**
- **Urea**: **Kaplan (1984)**
- **Uric acid**: **Thomas (1998)**
- **Cholesterol**: Enzymatic photometric assay using the cholesterol oxidase–peroxidase method **(Artis & Zak, 1997)**

Histopathological examination

After dissection, muscle tissue samples were fixed in 10% formalin. Samples underwent standard histological processing: dehydration in ethanol, clearing in xylene, and embedding in paraffin wax. Sections of 5µm thickness were obtained using a rotary microtome. Slides were stained with hematoxylin and eosin according to standard procedures **(Mabrouk *et al.*, 2021)** and were examined under a light microscope for histopathological changes.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA), followed by Duncan-Waller's Multiple Range Test. A *P*-value of < 0.05 was considered statistically significant. Results are presented as mean ± standard error (SE).

RESULTS

Acute toxicity test

An analysis of the acute toxicity of silver nanoparticles (AgNPs) on the Nile tilapia (*Oreochromis niloticus*) revealed a dose-dependent increase in mortality rates over the 96-hour exposure period. No mortalities were observed in the control untreated group (0mg/ L) throughout the experiment. The results indicate that the Nile tilapia can tolerate AgNPs exposure up to 5mg/ L for 96 hours without significant mortality. On the other hand, increasing the concentrations from 10 to 25mg/ L of AgNPs led to an increase in mortality rate to 100% at 25mg/ l. Additionally, the test showed that the calculated 96h LC_{50} value of AgNPs for *O. niloticus* was 15.5mg/ l.

Table 1. Mortality rate of *Oreochromis niloticus* exposed to various concentrations of AgNPs (mg/l) for 96h

Concentrations of AgNPs (mg/l)	No. of exposed fish	No. of dead fish				Overall deaths within 96 h.	% Mortality			
		24 h.	48 h.	72 h.	96 h.			A	B	AxB
0	20	0	0	0	0	0	0	0	0	0
5	20	0	0	0	0	0	0	5	0	0
10	20	0	1	1	2	4	20	5	2	10
15	20	0	2	3	5	10	50	5	7	35
20	20	1	3	4	6	14	70	5	12	60
25	20	2	3	6	9	20	100	5	17	85
								∑ (A x B) =190		

A: differences between two consecutive doses

B: arithmetic mean of the mortality caused by two consecutive doses.

h $LC_{50} = LC_{100} - \sum (A \times B)/N = 25 - (190/20) = 15.5$ mg/l

Growth performance

The growth performance of the Nile tilapia exposed to $AgNO_3$ and different AgNPs concentrations (10, 20, 50 and 100µg/ L) are listed in Table (2). There were significant improvements in FBW, WG, and SGR in 10 and 20µg/ l groups exposed to AgNPs $L^{-1.}$. However, the lowest values were observed in the group exposed to 50 and 100µg/ l AgNPs $L^{-1.}$ compared to the control group. The SR increased with increasing AgNPs concentrations at 10 and 20 and the highest number of dead fish was recorded at the highest concentration of 100µg AgNPs $L^{-1.}$

Table 2. Growth performance parameters of the Nile tilapia (*Oreochromis niloticus*) exposed to AgNPs for six weeks

Parameter	control	10 µg/L AgNPs	20 µg/L AgNPs	50 µg/L AgNPs	100 µg/L AgNPs	$AgNo_3$ (100 µg/L)
I W (g)	20.23	20.47	20.53	20.60	20.67	20.57
SE	0.06	0.15	0.15	0.26	0.25	0.31
FW (g)	52.53	58.17	62.57	48.47	42.90	39.53
SE	0.51	0.38	0.31	1.06	1.68	0.55
WG (g)	32.3	37.70	42.04	27.87	22.23	18.96
SE	0.46	0.40	0.21	0.91	1.44	0.55
SGR%	2.38	2.46	2.77	2.1	1.73	1.61
SE	0.03	0.02	0.01	0.04	0.04	0.04
Survival rate (%)	90%	95%	95%	90%	85%	90%

Values were presented as mean ± standard error (SE). Initial body weight (IW), Final body weight (FW), weight gain (WG), specific growth rate (SGR)

Biochemical parameters of *O. niloticus* fish

The biochemical parameters of the Nile tilapia (*O. nilotiIcus*) were altered following exposure to sublethal concentrations (10, 20, 50 and 100µg/ L) of AgNPs and 100µg of $AgNO_3$ for the exposure periods (2, 4 and 6 weeks). Data were presented in Tables (3- 9).

Glucose and Cholesterol concentrations

The results revealed that glucose and cholesterol concentrations were significantly increased ($P \leq 0.05$) in groups exposed to various concentrations of AgNPs and 100µg/ L $AgNO_3$ compared to the control group after 2, 4 and 6 weeks. Moreover, glucose and cholesterol levels significantly increased ($P \leq 0.05$) by increasing AgNPs concentrations, where the groups of fish exposed to high concentrations (100µg/ L) have a highly significant increase in serum glucose than the group exposed to the lower concentrations (10µg/ L AgNPs), and the highest concentrations were observed after 6 weeks. In

addition, glucose and cholesterol levels were higher in fish groups that were exposed to AgNPs than those exposed to $AgNO_3$ at the studied exposure periods, except cholesterol concentration in the group exposed to 10µg/ L AgNPs after 2 weeks. The highest value was 101.66±0.57mg/ dl in case of glucose and 273.40±0.30mg/ dl for cholesterol which recorded in fish group that was exposed to high concentration (100µg/ L) of AgNPs at 6 weeks (Tables 3- 4).

Total protein concentration

A significant decrease ($P \leq 0.05$) in total protein levels was observed in *O. niloticus* exposed to sublethal concentrations of AgNPs or 100 µg/L of $AgNO_3$ compared to the control group at 2, 4, and 6 weeks of exposure. Total protein levels declined progressively with increasing concentrations of AgNPs and longer exposure durations. The lowest value (18.02 ± 0.09g/ dL) was recorded in the group exposed to the highest AgNP concentration (100µg/ L) at 6 weeks. Additionally, total protein levels were consistently lower in fish exposed to AgNPs compared to those exposed to $AgNO_3$ at all time points (Table 5).

ALT and AST activities

ALT and AST activities were significantly increased ($P \leq 0.05$) in fish groups at different concentrations of AgNPs and 100µg $AgNO_3$ compared to the control group after all exposure periods, except at 4 weeks in which ALT activity increased insignificantly with control at the exposure to 10µg/ L AgNPs100 µg $AgNO_3$. In addition, fish groups exposed to higher concentrations of AgNPs have a highly significant increase in AST and ALT activities than the lower concentrations. Notably, AST and ALT activities were higher in fish groups exposed to AgNPs than $AgNO_3$, except ALT activity in fish group exposed to 10µg/ L AgNPs. The maximum value was (34.73±0.20 U/l) in case of ALT activity and (107.49±0.06 U/l) for AST activity, recorded in fish group exposed to high concentration (100µg/ L) of AgNPs at 6 weeks (Tables 6- 7).

Urea and uric acid concentrations

When the fish groups were exposed to the concentrations of AgNPs and $AgNO_3$, their serum levels of urea and uric acid were increased significantly ($P \leq 0.05$) in comparison with control at 2, 4 and 6 weeks of the experiment. Urea and uric acid concentrations were higher in fish groups exposed to AgNPs than $AgNO_3$, except urea concentration in fish group exposed to 10µg/ L AgNPs after 2 and 4 weeks. It was noticed that, urea and uric acid concentrations increase with increasing concentrations of AgNPs and contact time. The maximum value was (74±1.52mg/ dl) of urea concentration and (13.49±.024mg/ dl) of uric acid recorded in fish group exposed to high concentration (100µg/ L) at 6 weeks (Tables 8- 9).

Table 3. Serum glucose levels in *O. niloticus* following exposure to sublethal concentrations of AgNPs and $AgNo_3$

Time	Control	$AgNo_3$ (100 µg/L)	10 µg/L AgNPs	20 µg/L AgNPs	50 µg/L AgNPs	100 µg/L AgNPs
2 week	71.42±0.69 [d]	73.75±0.50 [c]	74.64±0.29 [c]	75.08±0.38[c]	83.94±0.11[b]	88.03±0.19[a]
4 week	71.98±0.39 [e]	73.42±0.19 [d]	74.86±0.29 [c]	75.52±0.29 [c]	81.28±0.22 [b]	85.71±0.19 [a]
6 week	70.54±0.29 [e]	75.08±0.50 [d]	78.51±0.39 [c]	83.72±0.19 [b]	84.05±0.33 [b]	101.66±0.57 [a]

Data are expressed as mean ± standard error (SE) with n=3.
Different superscript letters within the same row indicate statistically significant differences ($P \leq 0.05$)

Table 4. Cholesterol mg/dl of *O. niloticus* exposed to sublethal concentrations of AgNPs and $AgNo_3$

Time	Control	$AgNo_3$ (100 µg/L)	10 µg/L AgNPs	20 µg/L AgNPs	50 µg/L AgNPs	100 µg/L AgNPs
2 week	129.07±0.77 [f]	153.01±0.46 [d]	142.55±0.61 [e]	177.65±0.81 [c]	246.80±0.61 [a]	214.89±0.53 [b]
4 week	120.21±0.81 [f]	151.77±0.63 [e]	158.33±0.46 [d]	166.13±0.63 [c]	226.59±1.22 [b]	243.79±0.46 [a]
6 week	130.67±0.93 [f]	142.55±0.30 [e]	177.65±0.81 [d]	252.65±0.61 [c]	257.44±0.30 [b]	273.40±0.30 [a]

Data are expressed as mean ± standard error (SE) with n=3.
Different superscript letters within the same row indicate statistically significant differences ($P \leq 0.05$).

Table 5. Total protein g/dl of *O. niloticus* exposed to sublethal concentrations of AgNPs and $AgNo_3$

Time	Control	$AgNo_3$ (100 µg/L)	10 µg/L AgNPs	20 µg/L AgNPs	50 µg/L AgNPs	100 µg/L AgNPs
2 week	37.06±0.02 [a]	31.46±0.07 [b]	29.04±0.04 [c]	24.16±0.04 [d]	21.81±0.07 [e]	19.52±0.04 [f]
4 week	36.24±0.04 [a]	31.76±0.09 [b]	26.08±0.09 [c]	22.05±0.09 [d]	20.24±0.04 [e]	18.05±0.09 [f]
6 week	37.38±0.07 [a]	25.97±0.17 [b]	24.26±0.07 [c]	19.62±0.02 [d]	18.08±0.12 [e]	18.02±0.09 [e]

Data are expressed as mean ± standard error (SE) with n=3.
Different superscript letters within the same row indicate statistically significant differences ($P \leq 0.05$).

Table 6. AST activity (U/l) of *O. niloticus* exposed to sublethal concentrations of AgNPs and $AgNo_3$

Time	Control	$AgNo_3$ (100 µg/L)	10 µg/L AgNPs	20 µg/L AgNPs	50 µg/L AgNPs	100 µg/L AgNPs
2 week	28.81±0.30 ^{f}	37.68±0.72 ^{e}	53.6±0.61 ^{c}	50.26±0.48 ^{d}	57.73±0.42 ^{b}	97.55±0.92 ^{a}
4 week	29.96±0.19 ^{f}	41.53±0.46 ^{e}	48.69±0.52 ^{d}	57.03±0.74 ^{c}	59.84±0.48 ^{b}	98.94±0.60 ^{a}
6 week	30.54±0.30 ^{f}	44.67±1.06 ^{e}	47.48±0.14 ^{d}	60.68±0.24 ^{c}	75.56±0.97 ^{b}	107.49±0.06 ^{a}

Data are expressed as mean ± standard error (SE) with n=3.
Different superscript letters within the same row indicate statistically significant differences ($P \leq 0.05$).

Table 7. ALT activity (U/l) of *O. niloticus* exposed to sublethal concentrations of AgNPs and $AgNo_3$

Time	Control	$AgNo_3$ (100 µg/L)	10 µg/L AgNPs	20 µg/L AgNPs	50 µg/L AgNPs	100 µg/L AgNPs
2 week	23.18±0.44 ^{e}	27.12±0.19 ^{c}	24.61±0.30 ^{d}	31.16±0.20 ^{b}	31.62±0.19 ^{b}	33.2±0.20 ^{a}
4 week	24.66±0.09 ^{c}	25.94±0.29 ^{c}	25.77±0.40 ^{c}	30.6±0.64 ^{b}	31.11±0.51 ^{b}	34.62±0.20 ^{a}
6 week	23.01±0.25 ^{e}	29.05±0.19 ^{d}	28.66±0.19 ^{d}	31.5±0.24 ^{c}	33.2±0.24 ^{b}	34.73±0.20 ^{a}

Data are expressed as mean ± standard error (SE) with n=3.
Different superscript letters within the same row indicate statistically significant differences ($P \leq 0.05$).

Table 8. Urea mg/dl of *O. niloticus* exposed to sublethal concentrations of AgNPs and $AgNo_3$

Time	Control	$AgNo_3$ (100 µg/L)	10 µg/L AgNPs	20 µg/L AgNPs	50 µg/L AgNPs	100 µg/L AgNPs
2 week	40.33±0.88 ^{d}	54±0.57 ^{c}	42.33±0.88 ^{d}	60.66±0.33 ^{b}	62±0.57 ^{b}	73±0.57 ^{a}
4 week	38.66±0.33 ^{c}	54.66±1.20 ^{b}	54±0.57 ^{b}	62.66±1.20 ^{a}	63±0.50 ^{a}	65±1.52 ^{a}
6 week	39.33±0.08 ^{d}	53.33±0.88 ^{c}	65±1.52 ^{b}	65.33±0.88 ^{b}	73.33±1.45 ^{a}	74±1.52 ^{a}

Data are expressed as mean ± standard error (SE) with n=3.
Different superscript letters within the same row indicate statistically significant differences ($P \leq 0.05$).

Table 9. Uric acid mg/dl of *O. niloticus* exposed to sublethal concentrations of AgNPs and $AgNo_3$

Time	Control	$AgNo_3$ (100 µg/L)	10 µg/L AgNPs	20 µg/L AgNPs	50 µg/L AgNPs	100 µg/L AgNPs
2 week	9.28±.009 [e]	11.31±.016 [d]	11.88±.032 [c]	11.93±.016 [c]	11.99±.033 [b]	12.36±.040 [a]
4 week	9.36±.024 [f]	10.29±.024 [e]	11.25±.033 [d]	11.39±.024 [c]	11.6±.032 [b]	12.43±.055 [a]
6 week	9.4±.016 [f]	12.06±.033 [e]	12.46±.016 [c]	12.37±.024 [d]	12.52±.042 [b]	13.49±.024 [a]

Data are expressed as mean ± standard error (SE) with n=3.
Different superscript letters within the same row indicate statistically significant differences ($P \leq 0.05$).

Histopathological alterations

Skeletal muscle tissue is composed of repeating structural units known as myomeres, which are distinct muscle segments with fibers aligned parallel to the body's longitudinal axis. Each myomere contains muscle fibers separated by connective tissue, as shown in the control group (Fig. 2a).

Histological examination of muscle sections after 15 days of exposure to $AgNO_3$ and silver nanoparticles at concentrations of 10, 20, 50, and 100µg/ L revealed various pathological changes, including muscle layer degeneration, edema, and necrosis. Additional findings included balloon necrosis and the presence of trophoblast-like cells (Fig. 2b– f). These histopathological alterations became more severe with increasing exposure time to both $AgNO_3$ and AgNPs.

After 30 days of exposure, pathological changes were more pronounced and included degeneration of the epidermis, dermis, and muscle layers. Necrosis, fatty degeneration, Kover cells, and hemosiderin accumulation were observed in the muscle tissue, along with edema and vascular infiltration (Fig. 3b–f).

By 45 days of exposure, histopathological changes included degeneration, necrosis, and hemosiderin staining in both dermal and muscle layers. Focal necrosis and persistent edema were also evident in the muscle tissue (Fig. 4b– f).

5.1-Histological changes in fish muscles during 15 days of the experiment period

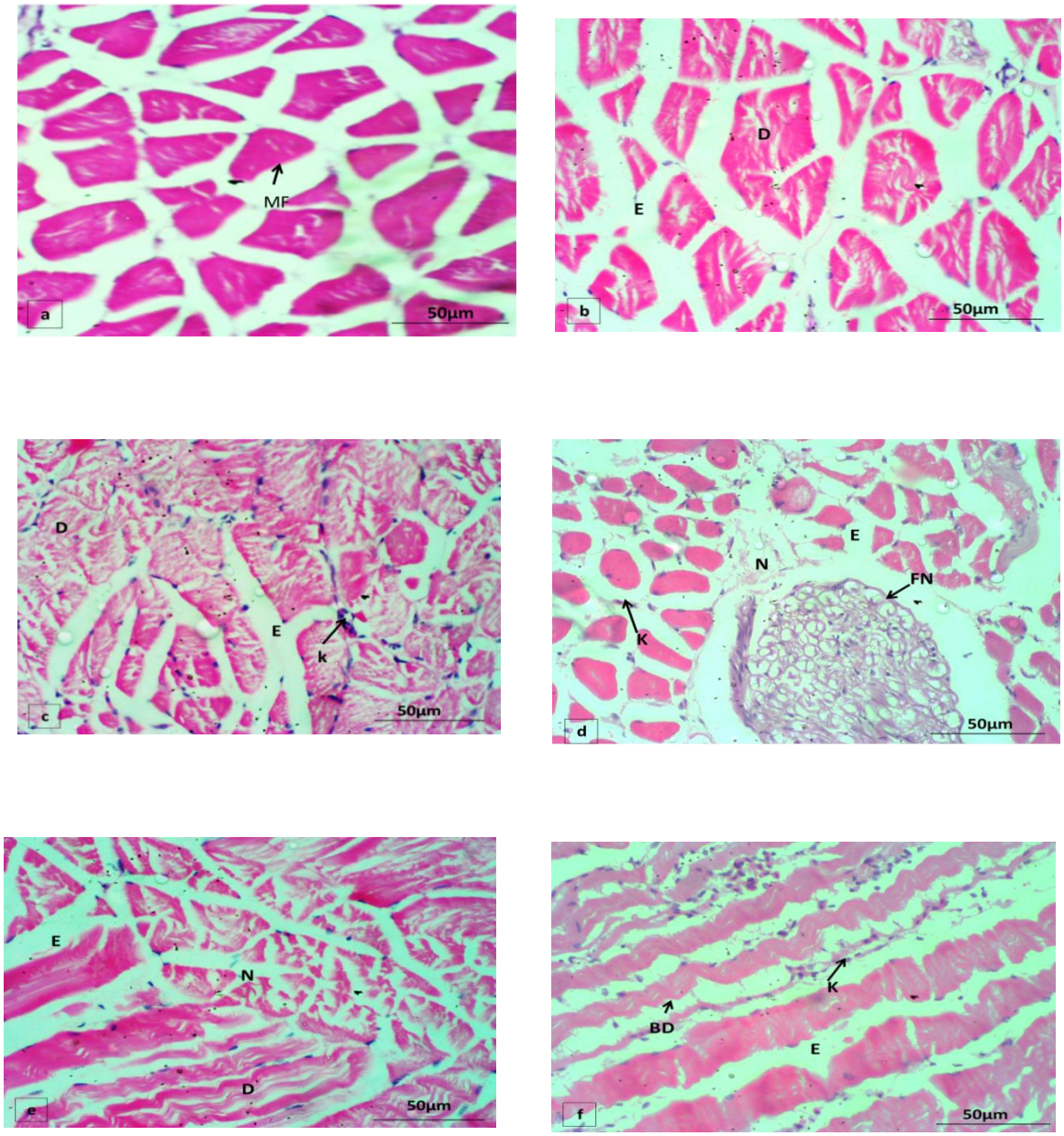

Fig. 2. Photomicrographs of skeletal muscle sections from *Oreochromis niloticus*. (a) Control fish showing normal muscle fibers with no pathological changes. (b–f) Muscle sections after 15 days of exposure to $AgNO_3$ (100 µg/L) and silver nanoparticles (AgNPs) at concentrations of 10, 20, 50, and 100 µg/L, respectively: (b) Degeneration (D) of muscle fibers and edema (E) in the muscle layer. (c) Degeneration (D), edema (E), and presence of Kupffer cells (K). (d) Necrosis (N), edema (E), focal necrosis, and Kupffer cells (K) in the muscle layer. (e) Edema (E), degeneration (D), and necrosis (N). (f) Balloon degeneration (BD), Kupffer cells (K), and edema (E) in the muscle layer. Scale bar = 50 µm. Magnification: H&E, ×400

5.2-Histological changes in fish muscles during 30 days of the experiment period

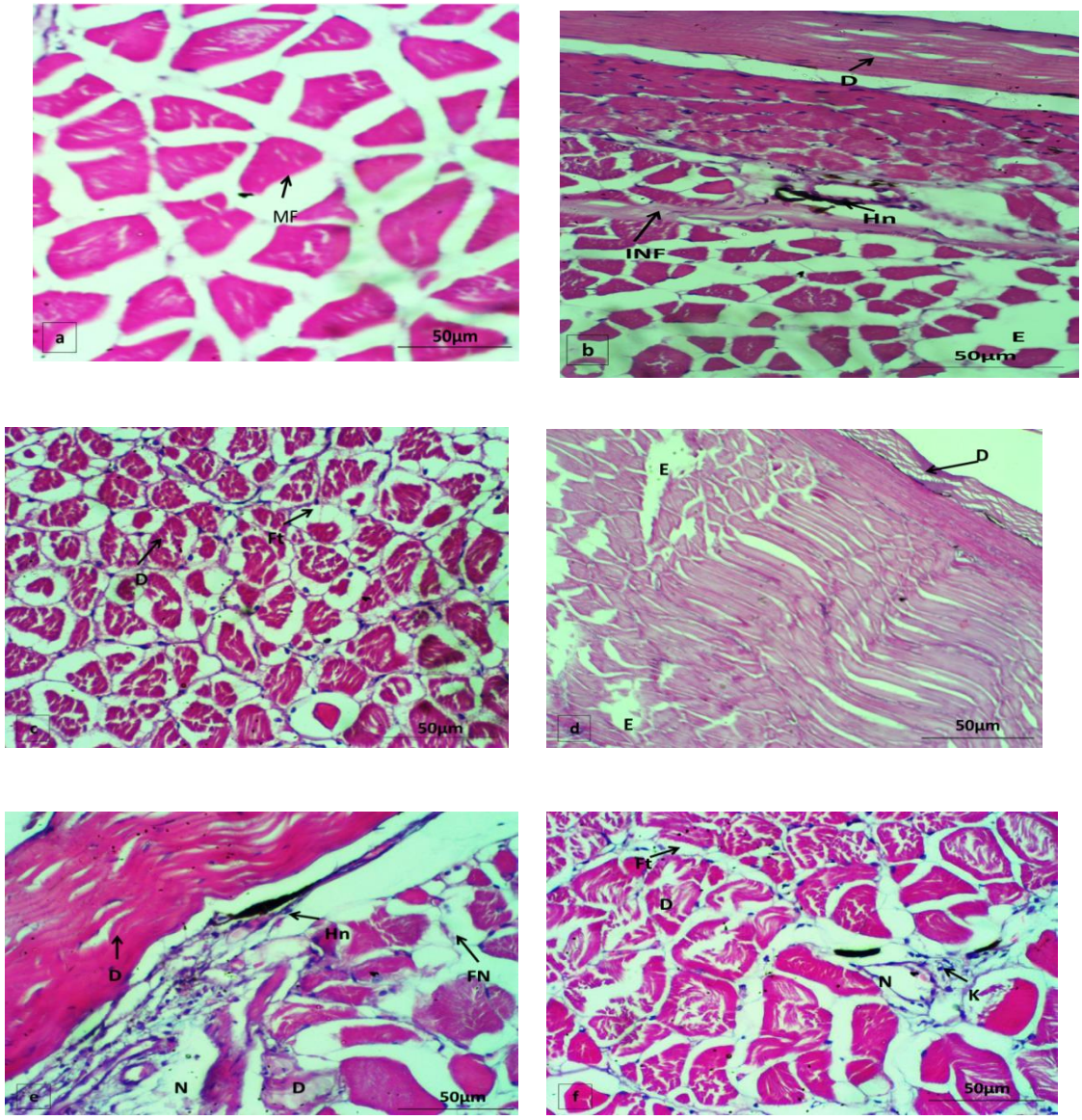

Fig. 3. Photomicrographs of skeletal muscle sections from *Oreochromis niloticus*. (a) Control fish showing normal muscle fibers with no histopathological alterations. (b–f) Muscle sections after 30 days of exposure to $AgNO_3$ (100 µg/L) and silver nanoparticles (AgNPs) at concentrations of 10, 20, 50, and 100 µg/L, respectively: (b) Degeneration in the dermal layer (D), edema (E), hemosiderin pigment (Hn), and blood vessel infiltration (INF) in the muscle layer. (c) Degeneration (D) and fatty degeneration (Fd) in the muscle layer. (d) Degeneration (D) in the epithelial layer and edema (E) in the muscle layer. (e) Degeneration (D) in the dermal and muscle layers, necrosis (N), hemosiderin pigment (Hn), and focal necrosis (F). (f) Degeneration (D), Kupffer cells (K), necrosis (N), and fatty degeneration (Fd) in the muscle layer. Scale bar = 50 µm. Magnification: H&E, ×400.

5.3- Histological changes in fish muscles during 45 days of the experiment period

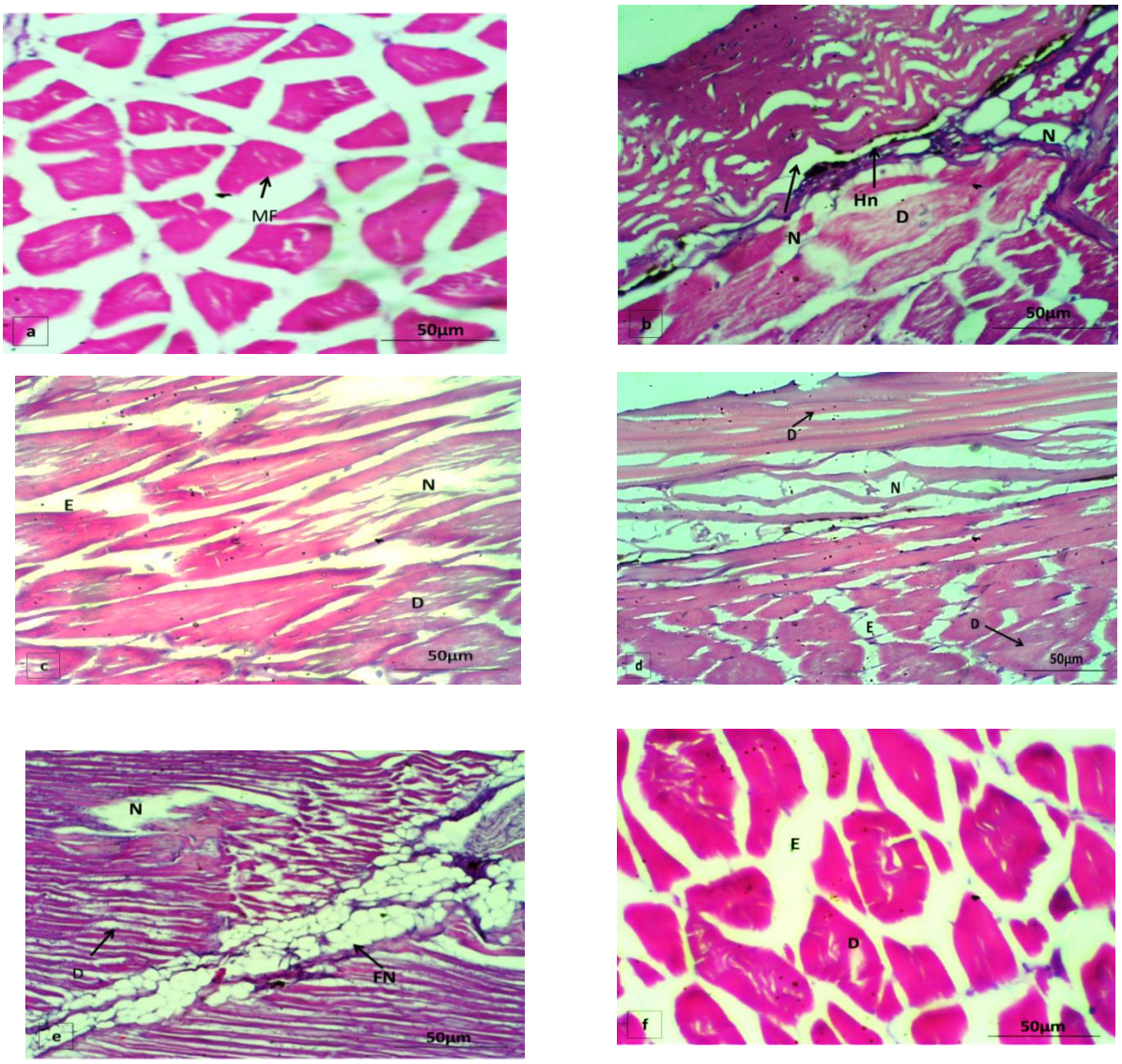

Fig. 4. Photomicrographs of skeletal muscle sections from *Oreochromis niloticus*. (a) Control fish showing normal muscle fibers with no histopathological alterations. (b–f) Muscle sections after 45 days of exposure to $AgNO_3$ (100 µg/L) and silver nanoparticles (AgNPs) at concentrations of 10, 20, 50, and 100µg/ L, respectively: (b) Degeneration in the muscle layer (D), hemosiderin pigment (Hn) in the dermal layer, and necrosis (N) in both dermal and muscle layers. (c) Degeneration (D), edema (E), and necrosis (N) in the muscle layer. (d) Degeneration (D) in the dermal and muscle layers, necrosis (N) in the dermal layer, and edema (E) in the muscle layer. (e) Degeneration (D), necrosis (N), and focal necrosis (F) in the muscle layer. (f) Edema (E) and degeneration (D) in the muscle layer. Scale bar = 50 µm. Magnification: H&E, ×400

DISCUSSION

The present investigation of the acute toxicity of *Oreochromis niloticus* under silver nanoparticle (AgNPs) exposure demonstrated a concentration-dependent rise in mortality rates over exposure periods of 24, 48, 72, and 96 hours. This pattern underscores the high toxicity of silver nanoparticles at elevated concentrations, as also noted by **Shalui *et al.* (2013)**. Furthermore, the 96-h LC50 of silver nanoparticles in *O. niloticus* was determined to be 15.5 mg/L, which closely aligns with the results of **Afifi *et al.* (2016)**, who reported a similar LC50 value of 19.5 mg/L for the same species. The trend of increasing mortality with higher silver nanoparticle concentrations further confirms the dose-dependent toxicity highlighted in the study of **Azadikhah *et al.* (2023)**.

The biochemical assessment revealed a significant elevation ($P \leq 0.05$) in serum glucose and cholesterol levels in *O. niloticus* exposed to varying concentrations of AgNPs (10, 20, 50, and 100µg/ L) and a comparable dose of $AgNO_3$ (100 µg/L) over exposure durations of 2, 4, and 6 weeks, compared to control groups. The observed increase in glucose levels could be attributed to heightened energy demands under stress conditions. Potential factors contributing to glucose fluctuations include hepatic or renal dysfunction, enhanced gluconeogenesis, nutritional inadequacies, and accelerated glycogen breakdown, which can ultimately disrupt carbohydrate metabolism, as supported by **Naguibb *et al.* (2020)**.

Notably, glucose concentrations were highest after six weeks of exposure and were more pronounced in fish exposed to AgNPs than those exposed to $AgNO_3$, echoing the observations of **Mohamed *et al.* (2021)**, who reported similar dose- and time-dependent increases in glucose levels in *O. niloticus* exposed to ZnONPs compared to ZnOBP-treated groups. Similarly, serum cholesterol levels showed significant elevation with rising AgNP concentrations, particularly after six weeks of exposure. This hypercholesterolemia may stem from hepatic tissue damage, leading to the release of volatile fatty acids into the bloodstream and impaired cholesterol metabolism due to liver stress **(Heeren & Scheja, 2021)**. Disruptions in cholesterol synthesis pathways could also contribute to the elevated levels **(Jaheed, 2021)**.

The trend of increased cholesterol with higher AgNP concentrations mirrored the pattern observed for glucose, with levels in AgNP-exposed fish surpassing those in $AgNO_3$-exposed groups. This pattern aligns with the findings of **Azadikhah *et al.* (2023)**, who noted significant cholesterol increases in *Capoeta capoeta* under prolonged AgNP exposure, and parallels the work of **Ghannam *et al.* (2022)**, who documented dose-dependent cholesterol elevation in fish exposed to both bulk and nano forms of ZnO.

Protein levels are fundamental biochemical indicators used to evaluate the overall health condition of fish. In this study, *Oreochromis niloticus* exposed to silver nanoparticles (AgNPs) and silver nitrate ($AgNO_3$) exhibited a significant reduction in total protein compared to the control group. This decline in protein concentration suggests an elevated energy demand triggered by silver nanoparticle exposure. The

reduction may also stem from damage to protein-synthesizing subcellular organelles and the inhibition of hepatic protein production, consistent with the findings of **Lavanya *et al.* (2011)**. Similar results were observed by **Shobana *et al.* (2021)**, who reported decreased protein content in *Labeo rohita* following exposure to sublethal doses of silver nitrate.

Serum enzyme activity analyses revealed significant elevations in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in fish exposed to varying concentrations of AgNPs and 100 µg/L $AgNO_3$. These increases are primarily attributed to the leakage of enzymes from damaged liver cells into the bloodstream, indicating hepatocellular injury. Such cellular lysis allows intracellular enzymes to diffuse into the serum, suggesting a hepatotoxic response. These findings align with those of **Naguib *et al.* (2020)**, who reported similar increases in AST and ALT levels following AgNP exposure.

Additionally, urea and uric acid concentrations were significantly elevated ($P \leq 0.05$) across all fish groups exposed to different concentrations of AgNPs and 100µg/ L $AgNO_3$ compared to the control group over the exposure period. This elevation is potentially linked to impaired glomerular filtration, leading to renal dysfunction. The increased serum urea levels may also result from gill damage, which disrupts the normal diffusion of urea between the bloodstream and surrounding water. Furthermore, elevated urea and uric acid concentrations may reflect underlying tubular damage and glomerular insufficiency **(Dawood *et al.*, 2020)**. These results are consistent with those of **Mohamed *et al.* (2021)**, who reported similar increases in urea and uric acid in Nile tilapia exposed to zinc oxide nanoparticles. Similarly, **El-Boshy *et al.* (2014)** demonstrated that cadmium exposure led to significant disruptions in nitrogenous waste metabolism in African catfish (*Clarias gariepinus*), as evidenced by increased urea and uric acid levels after three weeks of exposure.

The biochemical parameter analysis showed that the effects of AgNPs were more pronounced than those of $AgNO_3$, particularly at higher sublethal concentrations (50 and 100µg/ L) compared to lower concentrations (10µg/ L). This pattern is likely due to the accumulation of silver nanoparticles, which induces oxidative stress and activates biotransformation mechanisms, culminating in progressive cellular damage as the concentration of nanoparticles increases **(Sibiya *et al.*, 2022)**. This observation supports the findings of **Thummabancha (2016)**, who noted that while lower doses of AgNPs exhibit relatively mild toxicity in the Nile tilapia, higher concentrations can lead to significant hematological and immunological effects.

Although the direct impact of AgNPs on the muscle tissue of the Nile tilapia has been scarcely documented, the current study reveals a detrimental influence on both survival and growth rates. Numerous studies have corroborated the adverse effects of nanoparticles on aquatic organisms, with nanoparticle size being a key determinant of their toxicity. Moreover, changes in muscle fiber size distribution with increasing age in

fish were observed, primarily due to the hypertrophy and proliferation of larger muscle fibers **(Ostaszewska *et al.*, 2016)**. Experimental studies have demonstrated that fish muscle tissue exhibits heightened sensitivity to heavy metals and their salts compared to the muscle tissues of other vertebrate groups **(Girial *et al.*, 2015)**.

Impaired muscle growth in fish is typically accompanied by various histopathological alterations, often manifesting as atrophy and tissue degeneration. In this study, *Oreochromis niloticus* exposed to aqueous suspensions of silver nanoparticles (AgNPs) exhibited histopathological changes in muscle tissue consistent with findings from prior research on nanoparticle toxicity **(Al-Bairuty *et al.*, 2013)**. One notable alteration was the enlargement of intercellular spaces within the muscle fibers, a phenomenon similarly reported in studies investigating the effects of pesticides and heavy metals on fish **(Rajkumar & Thirumurugan, 2016)**. These findings are further supported by experiments on *Oreochromis mossambicus* and *O. niloticus* exposed to heavy metals and nanoparticles. Histopathological examination serves as a valuable tool for assessing tissue morphology and identifying toxicological impacts **(Kaur *et al.*, 2018)**.

In the present investigation, both treated groups exhibited distinct pathological alterations in muscle tissues, which can be attributed to the oxidative stress induced by silver nanoparticles. This oxidative stress may compromise cellular integrity, contributing to the observed structural damage. Consequently, histopathological changes in muscle tissue provide a reliable biomarker for evaluating the toxicity of AgNPs. Notably, muscle tissue—similar to the liver and gills—is highly susceptible to waterborne pollutants, underscoring its importance in environmental toxicity assessments.

CONCLUSION

The current findings revealed that both silver nanoparticles (AgNPs) and silver nitrate ($AgNO_3$) exerted adverse effects on the biochemical parameters of *Oreochromis niloticus*. Notably, the extent of alterations was more pronounced in fish exposed to AgNPs, particularly at higher sublethal concentrations (50 and 100µg/ L), indicating a higher potential toxicity associated with elevated exposure levels. In addition to biochemical disruptions, significant histopathological changes were observed in the muscle tissues of exposed fish compared to the control group, with the severity of damage escalating with increasing concentrations. These results highlight the necessity of exercising greater caution to prevent the excessive use and uncontrolled release of AgNPs into aquatic ecosystems, given their potential to cause substantial harm to aquatic organisms.

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