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Protective Role of Vitamins (E + C) on Cholesterol and Albumin in Oreochrmis niloticus subjected to Zinc Oxide Bulk and Nanoparticles

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Abstract:

The present work investigated the addition effect of vitamins (E + C) to two types of zinc oxide bulk and nano (ZnOBPs, ZnONPs) on cholesterol and albumin levels in *Oreochrmis niloticus*. The studied fish groups were subjected to 1/8, 1/4 and 1/2 sublethal concentrations (LC50) under different exposure period. The results of serum albumin give a significant decreasing (P↑0.05) when compared with the control group. On the other side, when under study fish groups were subjected to the same concentration of ZnO (bulk or nano) supplemented with vitamin (E and C) a significant increasing (P↑0.05) in serum albumin level were observed. The maximum value of fish exposed to ZnOBPs and ZnONPs plus vitamin (E and C) were 2.77±0.09 and 2.68±0.10 g/dl, respectively recorded at 1/8 LC_{50} concentration after 7 days. In case of cholesterol concentration, fish groups subjected to sublethal concentrations of ZnO bulk or nano, showed significant increased ($p \leq 0.05$). However, the addition of vitamins (E and C) leads to a significant decreasing (P (0.05) in cholesterol concentration when compared to the group that have no vitamins.

Keywords: Fish, ZnONPs, Cholesterol, Albumin, Vitamins

1- Introduction

Nanotechnology has made a great revolution in many sectors in recent decades. The aquatic life is in danger of nanoparticles exposure but, there is a lake in data about their toxicological potency and their effect on aquatic bodies **(Rundle et al., 2016).** Particle with 100 nm dimension is classed as nanoparticle, these materials have unique properties due to many reasons, including the high surface-to-volume ratio, as well as physicochemical properties such as color, solubility, magnetism and toxicity; the properties of these materials are changed with their bulk particles **(Auffan et al., 2009).** The main relation between nanoparticles and their bulk equivalents are the physicochemical and electrical properties. Furthermore, the environment in which the nanoparticles present effect on their behavior, reactivity, and toxicity (**Bian *et al.*, 2011**).

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The importance of the behavior for ZnO-NPs in water systems have been investigated in the laboratory. According to the data, the lowest dissolution percentage occurred at the highest ZnO concentrations (**Adam *et al.*, 2015**). ZnONPs dissolve in water and have species that are both soluble and particulate in aquatic systems, (**Heinlaan *et al.*, 2008**).

Fish is considered a cheap source of food in Egypt compared to other foods of animal origin. The health of fish is an important indicator of the quality of the aquatic environment so, the pollution have a great effect on the value of blood cholesterol in addition to diseases, nutritional status and seasons, year of fish species. Also, triglycerides have low water content and high calorific value and are found in the presence of more than one type of fatty acid compound, so the storage of energy as triglyceride in the fat deposits is very active because of their proprieties. Plasma triglycerides changed under different condition like age, sex, and diets (**Çelik and Bilgin, 2007**). Albumin is agraet serum protein for transportation of steroid hormones, also, the physiological index Albumin: Globulins ratio (A/G) is widely used (**Nakagawa, 1978**; **Shahsavani *et al.*, 2017**).

Antioxidative vitamins like E and C that found in food products have a strong effect on heavy metals toxicity (**Asaikkutti *et al.*, 2016; Sahiti et al., 2018**). Although, the role of vitamins against toxic effects in fish is relatively well documented, there is a lake of information available on the effect of vitamins (E + C) on cholesterol and albumin concentrations. So, this work aims to study the role of the addition of vitamins (E + C) to ZnONPs , ZnOBPs on cholesterol and albumin in *O. niloticus* at different exposure periods.

2- Materials and Methods

Experimental design:

Nanoparticles of zinc oxide were obtained from the Faculty of Postgraduate Studies for Advanced Sciences, Beni Suef University, Egypt, while samples of ZnO-BPs were purchased from El-Nasr pharmaceutical chemicals Co. Egypt.

Live fish (*O. niloticus*) were collected from fish farm at Fayoum governorate, Egypt. A total 150 fish samples with weighting110 ±5 g and body length 20±4 cm were kept in plastic bags supplied with oxygenated water and transported to pollution laboratory, (NIOF). Fish were acclimated to the laboratory conditions for 2 weeks before the experiment started. The water quality parameters were within the recommended range for the culture of Nile tilapia. Fish were fed twice daily with commercial pellet food 30% protein (control diet) while the second diet was supplemented with 250 mg of each vitamin (E + C).

After two weeks of acclimatization fish were divided into 13 experimental groups (G1, control), (G2,3,4 ZnOBPs), (G5,6,7 ZnOBPs+ vitamins), (G8,9,10 ZnONPs) and (G11,12,13 ZnONPs+ vitamins) Table (1). The experimental periods were 7, 14, 21, and 28 days for all groups.

Biochemical Analysis:

Blood samples were prepared for biochemical analyses according to the method described by **Burtis et al. (1996).** Serum cholesterol concentration (mg/dl) was estimated using assay kits supplied by (Diagnostic Systems (DiaSys), Egypt) by enzymatic photometric test (CHOD-PAP) method described by (**Artiss and Zak 1997**). Serum albumin was measured by colorimetric method using Bio Med diagnostic kit (Egypt) described by **Doumas and Biggs (1976**).

Table (1). The types and fish distribution in the different experimental groups

Experimental groups	Type	Fish distribution
G1	Control	Each group consisted of 30 fish triplicate (10 fish/ aquarium
G2, G3 and G4	Fish groups exposed to three sublethal concentrations (1/8, 1/4 and 1/2) LC_{50} of ZnOBPs	
G5, G6, and G7	Fish groups subjected to (1/8, 1/4 and 1/2) LC_{50} of ZnOBPs+ vitamins (E + C)	
G8, G9, and G10	Fish groups exposed to (1/8, 1/4 and 1/2) LC_{50} of ZnONPs	
G11, G12, and G13	Fish groups exposed to (1/8, 1/4 and 1/2) LC_{50} of ZnONPs+ vitamins (E + C)	

2.5. Statistical Analysis:

(One-way ANOVA) was applied to analyse data using Turkey's test to compare means at $p<0.05$ at 95%. All data were expressed as mean ± standard error.

3. Results and Discussions

Mortality rate and half lethal concentration (LC_{50}) in *O. niloticus* fish

The results of the 24hr, 48hr, 72hr and 96hr LC_{50} values for zinc oxide bulk and nano particles in *O. niloticus* were presented in Table (2). The results revealed that mortality rate increased with increasing concentration and also with increasing the time of exposure (24, 48, 72 and 96 hr). No mortality was observed during the 96 hr at control group (0.0 mg /L). Also, fish tolerated exposure up to 1.5 mg/L of ZnONPs and 40 mg/L of ZnOBPs. Finally, the test showed that the calculated 96 hr LC_{50} value for *O. niloticus* fish of ZnOBPs was 84 mg/l and ZnONPs was 5.6 mg/l respectively.

Table 2 Half lethal concentration (LC_{50}) of ZnO (BPs & NPs) (mg/l) in *O. niloticus* fish

Concentrations (mg/L)	**No. of exposed fish (N)**	**Mortality at duration hours**				**Overall deaths within 96 hr.**	**Mortality, %**			
		24hr	**48hr**	**72hr**	**96hr**			**A**	**B**	**AxB**
ZnOBPs										
0	**10**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
20	**10**	**0**	**0**	**0**	**0**	**0**	**0**	**20**	**0**	**0**
40	**10**	**0**	**0**	**0**	**0**	**0**	**0**	**20**	**0**	**0**
60	**10**	**0**	**1**	**1**	**0**	**2**	**20**	**20**	**1**	**20**

80	**10**	**1**	**2**	**1**	**0**	**4**	**40**	**20**	**3**	**60**
110	**10**	**5**	**3**	**1**	**0**	**9**	**90**	**30**	**6.5**	**195**
140	**10**	**8**	**2**	**0**	**0**	**10**	**100**	**30**	**9.5**	**285**
ZnONPs										
0	**10**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1	**10**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**
1.5	**10**	**0**	**0**	**0**	**0**	**0**	**0**	**0.5**	**0**	**0**
3	**10**	**0**	**1**	**1**	**0**	**2**	**20**	**1.5**	**1**	**1.5**
5	**10**	**2**	**2**	**1**	**0**	**5**	**50**	**2**	**3.5**	**7**
9	**10**	**7**	**2**	**0**	**0**	**9**	**90**	**4**	**7**	**28**
14	**10**	**9**	**1**	**0**	**0**	**10**	**100**	**5**	**9.5**	**47.5**

LC_{50} *= LC_{100}- ∑ (A x B)/N*
A*: differences between the two consecutive concentrations*
B*: arithmetic mean of the mortality caused by two consecutive concentrations*
∑ ***(A x B)****: sum of AxB*

3.2. Albumin concentration:

Albumin concentration in serum of *O. niloticus* subjected to sublethal concentrations of ZnO bulk and nano particles with and without vitamin (E and C) under different exposure period were represented in Table (3,4).

For ZnO bulk particle Table (3) with increasing the sublethal concentration the concentration of albumin decreased and the same trend were also shown by increasing the exposure time. The results of albumin decreasing were as follows: at 7 days from (2.61-2.2 g/dl), at 14 days (2.36-1.64 g/dl), at 21 days (1.98-1.47 g/dl) and at 28 days (1.94-1.39 g/dl) by comparing with the control group which were 2.79, 2.80, 2.81 and 2.83 g/dl at 7, 14, 21 and 28 days, respectively. The addition of vitamin (E and C) on ZnO bulk particle increased the albumin levels in at all time of exposure and at different concentrations. The value of albumin under the effect of ZnO bulk+ vitamin (E and C) show the same trend as without vitamins this means that, the values deceased by increasing the sub lethal concentration and with increasing the time of exposure. The results of ZnO + vitamins (E and C) were decreased as shown at 7 days (2.77-2.47 g/dl), at 14 days (2.68-2.16 g/dl), at 21 days (2.23- 1.77 g/dl) and at 28 days (2.22- 1.73 g/dl).

For ZnO nanoparticles there were a decrease in albumin levels by increasing sublethal concentrations and with increasing contact time in the two cases, without addition of vitamin (E and C) and with addition of vitamins, Table (4). In case of ZnO nanoparticle only the result were at 1/8 LC_{50} (2.46- 1.75 g/dl), at 1/4 LC_{50} (2.21- 1.23 g/dl) an at 1/2 LC_{50} (2.13- 1.11 g/dl). In case of ZnO nanoparticle + vitamin (E and C) the results were at 1/8 LC_{50} (2.68- 1.86 g/dl), at 1/4 LC_{50} (2.49- 1.50 g/dl) an at 1/2 LC_{50} (2.38- 1.36 g/dl).From the all results in Tables (3 and 4) revealed that , serum albumin levels were significantly decreased (P↑0.05) by subjecting to different concentrations of ZnO nanoparticle than in bulk particle through the different exposure time when compared to the control group. While there were were significantly increased (P↑0.05) in serum albumin levels by addition of vitamin(E and C)than without vitamin at different concentrations ZnO in bulk and nano forms through the different exposure time when compared to the control group.

Table 3: Albumin concentration (g/dl) of *O. niloticus* exposed to sublethal concentrations of ZnOBPs and supplemented with vitamin (E and C).

Treatments / Time (days)	Control	ZnOBPs			ZnOBPs plus vitamin (E and C)		
		1/8 LC_{50}	1/4 LC_{50}	1/2 LC_{50}	1/8 LC_{50}	1/4 LC_{50}	1/2 LC_{50}
7	2.79±0.11^{a}	2.61±0.06^{ab}	2.37±0.04^{bc}	2.2±0.08^{c}	2.77±0.09^{a}	2.53±0.07^{ab}	2.47±0.04^{bc}
14	2.80±0.04^{a}	2.36±0.03^{b}	1.88±0.04^{d}	1.64±0.02^{e}	2.68±0.04^{a}	2.3±0.05^{bc}	2.16±0.09^{c}
21	2.81±0.05^{a}	1.98±0.04^{c}	1.64±0.08^{de}	1.47±0.04^{e}	2.23±0.11^{b}	1.94±0.02^{c}	1.77±0.02^{cd}
28	2.83±0.07^{a}	1.94±0.08^{c}	1.61±0.04^{de}	1.39±0.05^{e}	2.22±0.06^{b}	1.91±0.08^{c}	1.73±0.06^{cd}

-Data are presented as mean± SE of 6 fish. - SE: standard error.
- (a, b, c .) Means within the same row carrying different letters are significant at (P ↟0.05)
- Means having the same letter in the same row are not significantly different.

Table 4: Albumin concentration (g/dl) of *O. niloticus* exposed to sublethal concentrations of ZnONPs and supplemented with vitamin (E and C).

Treatments / Time (days)	Control	ZnONPs			ZnONPs plus vitamin (E and C)		
		1/8 LC_{50}	1/4 LC_{50}	1/2 LC_{50}	1/8 LC_{50}	1/4 LC_{50}	1/2 LC_{50}
7	2.79±0.11^{a}	2.46±0.03^{bc}	2.21±0.03^{de}	2.13±0.07^{e}	2.68±0.10^{ab}	2.49±0.05^{bc}	2.38±0.02^{cd}
14	2.80±0.04^{a}	2.17±0.08^{c}	1.67±0.04^{d}	1.53±0.01^{d}	2.51±0.06^{b}	2.12±0.06^{c}	1.99±0.05^{c}
21	2.81±0.05^{a}	1.78±0.04^{c}	1.23±0.07^{e}	1.13±0.04^{e}	2.07±0.04^{b}	1.52±0.02^{d}	1.40±0.04^{d}
28	2.83±0.07^{a}	1.75±0.02^{b}	1.23±0.02^{ed}	1.11±0.02^{e}	1.86±0.03^{b}	1.5±0.05^{c}	1.36±0.02^{cd}

-Data are presented as mean± SE of 6 fish. - SE: standard error.
- (a, b, c ..) Means within the same row carrying different letters are significant at (P ↟0.05)
- Means having the same letter in the same row are not significantly different.

3.3. Cholesterol concentration:

Table (5,6) showed cholesterol concentration in serum of *O. niloticus* exposed to sublethal concentrations of ZnO bulk and nano particles with and without vitamin (E and C) under different contact times (7, 14, 21 and 28 days).

In ZnO bulk particle Table (5), there were an increase in cholesterol concentration by increasing the contact time at 7 days (130.95-147.61 mg/dl), at 14 days (166.66-195.23 mg/dl), at 21 days (173.80-202.30 mg/dl) and at 28 days (211.90-235.71 mg/dl). Also there were an increase in cholesterol concentration by increasing the sublethal concentrations as shown: at at 1/8 LC_{50} (130.95- 211.90 mg/dl), at 1/4 LC_{50} (135.71- 221.42 mg/dl) and at 1/2 LC_{50} (147.61-

235.71 mg/dl). In case of ZnOBPs plus vitamin (E and C) the results of cholesterol concentration follow the same trend means, the cholesterol concentration increased by increasing contact time and increasing the sublethal concentration. But the whole results in comparing before and after vitamin (E and C) cleared that, there were a decrease in cholesterol concentration when vitamin included.

In Table (6), there was an increase in cholesterol concentration by increasing the time of exposure and with increasing in the sublethal concentration for ZnO nanoparticles with and without vitamins. The minimum value for cholesterol concentration without vitamin was (128.57 mg/dl) at 7days of exposure under sublethal concertation1/8 LC_{50} and the maximum was (311.90 mg/dl) at 28 days of exposure under sublethal concertation1/2 $LC_{50.}$ While with vitamin (E and C) the maximum was (245.23 mg/dl) at 28days of exposure under sublethal concertation1/2 LC_{50} and the minimum was (116.66 mg/dl) at 7 days of exposure under sublethal concertation1/8 LC_{50}. Also Table (6) showed that, the addition of vitamins (E and C) cause a decrease in cholesterol concentration than without vitamins

The results revealed that the concentration of cholesterol was significant increased ($p \leq 0.05$) in all fish groups subjected to sublethal concentrations of ZnO bulk or nano with compared to the control group under exposure time. Also, cholesterol concentration ZnONPs fish groups was higher than groups subjected to ZnOBPs under different studied exposure period. However, ZnOBPs or ZnONPs with vitamin (E and C) fish showed a significant decreasing ($P \uparrow 0.05$) in cholesterol concentration when comparing with groups of no vitamins.

Table 5: Cholesterol concentration (mg/dl) of *O. niloticus* exposed to sublethal concentrations of ZnOBPs and supplemented with vitamin (E and C).

Treatment	**Control**	**1/8 LC_{50}**	**1/4 LC_{50}**	**1/2 LC_{50}**
Days	**ZnOBPs**			
7	**116.66±5.77^{b}**	**130.95±11.54^{ab}**	**135.71±8.66^{ab}**	**147.61±4.04^{a}**
14	**114.28±8.08^{d}**	**166.66±9.23^{bc}**	**185.71±6.92^{ab}**	**195.23±9.23^{a}**
21	**119.04±10.96^{e}**	**173.80±7.50^{bc}**	**197.61±5.77^{ab}**	**202.30±6.92^{a}**
28	**121.42±6.35^{c}**	**211.90±17.89^{a}**	**221.42±4.04^{a}**	**235.71±5.19^{a}**
	ZnOBPs plus vitamin (E and C)			
7	**116.66±5.77^{b}**	**119.04±5.19^{b}**	**123.80±7.50^{ab}**	**126.19±3.46^{ab}**
14	**114.28±8.08^{d}**	**135.71±6.35^{d}**	**140.47±8.08^{cd}**	**164.28±4.61^{bc}**
21	**119.04±10.96^{e}**	**138.09±9.81^{de}**	**150.00±7.50^{cd}**	**169.04±5.19^{c}**
28	**121.42±6.35^{c}**	**171.42±3.46^{b}**	**176.00±4.61^{b}**	**219.04±4.61^{a}**

-Data are presented as mean± SE of 6 fish. - SE: standard error.
- (a, b, c ..) Means within the same row carrying different letters are significant at (P ↑0.05)
- Means having the same letter in the same row are not significantly different.

Table 6: Cholesterol concentration (mg/dl) of *O. niloticus* exposed to sublethal concentrations of ZnONPs and supplemented with vitamin (E and C).

Treatment	Control	1/8 LC_{50}	1/4 LC_{50}	1/2 LC_{50}
Days	ZnOBPs			
7	116.66±5.77^{b}	128.57±4.61^{bc}	138.09±3.46^{ab}	152.38±5.19^{a}
14	114.28±8.08^{d}	171.42±7.50^{bc}	192.85±9.81^{ab}	204.76±6.92^{a}
21	119.04±10.96^{e}	180.95±6.00^{b}	214.28±2.47^{a}	221.42±6.35^{a}
28	121.42±6.35^{c}	250.00±6.92^{b}	295.23±9.23^{a}	311.9±6.35^{a}
	ZnOBPs plus vitamin (E and C)			
7	116.66±5.77^{b}	116.66±4.04^{c}	123.8±7.50^{bc}	130.95±4.04^{bc}
14	114.28±8.08^{d}	138.09±5.77^{de}	152.28±6.92^{cd}	164.28±8.08^{cd}
21	119.04±10.96^{e}	147.61±4.04^{c}	176.19±3.46^{b}	180.95±5.77^{b}
28	121.42±6.35^{c}	216.66±8.08^{b}	240.47±8.66^{b}	245.23±20.20^{b}

-Data are presented as mean± SE of 6 fish. - SE: standard error.
- (a, b, c ..) Means within the same row carrying different letters are significant at (P ↑0.05)
- Means having the same letter in the same row are not significantly different.

The measurement of safe or tolerance levels of a pollutant can be greatly aided by determining LC_{50} values **(Prenter *et al.*, 2004)**. The obtained LC_{50} of ZnOBPs (84 mg/l) was less hazardous to *O. niloticus* fish than the LC_{50} of ZnONPs (5.6 mg/l) in the current investigation. According to **Wigginton *et al.*, (2007)** nanoparticles behave substantially differently when compared to bulk particles of the same chemical composition. Small size, high reactivity, and high surface area per unit volume of nanomaterials, as well as direct nanoparticle toxicity caused by chemical composition and surface reactivity, may all contribute to this **(Navarro *et al.*, 2008)**.

(Ferrari *et al.*, 2007) said that, the use of serum biochemical markers in the detection and diagnosis of metabolic abnormalities and disorders in fishes. Albumin promotes in the transport of lipids as well as fish metabolism in general **(Andreeva, 1999)**. Albumin is only produced in the liver, but Serum proteins come from many different places **(Coz-Rakovac *et al.*, 2005)**.

At the exposure period of the experiment, serum albumin levels in all treatment groups with ZnOBPs or ZnONPs were substantially lower (P↑0.05) than in the control group. Then, compared to groups not supplemented, there was a significant increase (P↑0.05) in groups exposed to the same dose of ZnOBPs or ZnONPs and given with vitamins (E and C).

Reduced feed intake, loss through the kidneys and gut, and changed liver metabolism could all be factors in the lower serum albumin level **(Alkaladi *et al.*, 2015)**. These findings were similar to those of **Abdel-Khalek et al., (2016)**, who found a significant drop in albumin levels in Nile Tilapia; *O. niloticus* when they were exposed to 1/10 and 1/20 LC_{50} 96 hr of both CuO and Cu (BPs & NPs).

Vitamin E and C supplementation, on the other hand, boosted albumin levels in these groups when compared to non-supplemented groups. This could be as a result of increased food consumption and improved liver function. This matches the findings of **Farsani *et al.*, 2017** who

found that dietary vitamin E treatment prevented *Oreochromis niloticus* exposed to ZnONP against the nanoparticles' adverse effects.

After 7, 14, 21, and 28 days, the concentration of cholesterol in fish exposed to sublethal quantities of ZnO bulk and nanoparticles was significantly elevated ($p \uparrow 0.05$) when compared with the control group. Then, compared to the control group, fish supplemented with vitamin and subjected to the same quantity of ZnOBPs or ZnONPs had a significant decrease ($P \uparrow 0.05$) in cholesterol concentration.

The high cholesterol levels observed in this study could be due to the formation of lipid peroxides induced by the effect of pollutants (ZnOBPs or ZnONPs), as previously reported by **(Mousa, 2004)**, as well as the destruction of liver cells and other organs due to the impact of pollutants, which increases total cholesterol levels in the serum **(Shalaby *et al.*, 2007)**.

Toxic chemicals can also elevate total cholesterol levels in fish blood or tissues by a variety of mechanisms, including increased synthesis by the liver and other organs, release of cholesterol and other lipid contents from damaged cell membranes, and impaired hepatic cholesterol excretion **(Metwally, 2009)**. Vitamin E and C supplementation, on the other hand, reduced cholesterol levels in these groups as compared to other groups. As described by **Farsani *et al.*, (2017)** this is likely owing to a protective effect provided by a combination of vitamins E and C against the severe effects of pollutants.

4. Conclusion

To summarize, ZnONPs have a greater harmful effect on *O.niloticus* than ZnOBPs. The findings of albumin and cholesterol tests in supplemented fish groups were better than those in supplemented fish groups, especially at low dosages. Furthermore, the data suggested that vitamin (E and C) addition considered as an effective antioxidant that protects *O.niloticus* from the harmful effects of ZnOBPs and ZnONPs.

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