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Exposure of Nile Tilapia *Oreochromis niloticus* to Chlorantraniliprole Inducing Biochemical Alterations and Histopathological Perturbations Aggravated in The Presence of Nano Zinc Oxide

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Abstract

Nile tilapia *Oreochromis niloticus* fish exposed to different concentrations of chlorantraniliprole (CAP) as well as its mixture with nano zinc oxide (ZnO-NP) as a heavy metal pollutant at 10 µg/ml for four days. Additionally, the histological and biochemical alterations which can be employed as biomarkers in monitoring aquatic toxicity were investigated. The 96 h LC_{50} value was 10.607 µg/ml for CAP alone, whereas the toxicity of CAP-ZnO-NP mixture (0.386 µg/ml) was significantly (P<0.05) 27- fold more. *In vivo* study, Nile tilapia fish were exposed to CAP (1/10 LC_{50} of 96 h) to determine the sublethal effects on acetylcholinesterase (AChE), alanine transaminase (ALT), and aspartate aminotransferase (AST) enzymes. The obtained results indicated that the brain AChE activity of fish exposed to CAP was increased significantly (P<0.05) at the early period, 24h and 48h posttreatment (155 and 193 % activity of control, respectively). Liver enzymes were reduced significantly (P<0.05) where AST and ALT activity at 96 h posttreatment were 67.5 and 54 % activity of control, respectively. After 14 days of recovery, all examined enzymes resumed their basal level. The histopathological effect of insecticide and its mixture with zinc on the gill and liver of freshwater fish *O. niloticus* were also observed by light microscope. Gills and liver of tilapia exposed to CAP or ZnO-NP were found to be seriously affected in comparison with that of the control. However, more vigorous histopathological perturbations induced in fish exposed to CAP-ZnO-NP. The gills showed oedema, epithelial lifting, necrosis, hyperplasia, lamellar disorganisation, and lamellar fusion. On the other hand, the liver showed fatty degeneration, increased sinusoidal space, cytoplasmic degeneration, necrosis, and focal fibrosis. These findings may be used as biomarkers for water pollution and early warning ecotoxicological parameters for CAP if it interacts with another contaminant such as ZnO-NP in the environment.

Keywords: Biomarker; Chlorantraniliprole; Enzyme; Fish; Histopathology; Pollution; ZnO-NP

1. Introduction

Due to urban, industrial, and agricultural activities, freshwater sources are dumped with different kinds of chemicals that affect the inhabiting biota. These chemicals are continuously released into the environment, impairing water quality and causing pollution. These pesticides have an adverse effect on aquatic organisms due to their movement from diffuse and point sources. The pesticides are found to damage vital organs of the fish, and skeletal system and cause biochemical alterations in the exposed fishes [1].

Chlorantraniliprole (CAP) has been applied in rice,

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sugarcane, and tomato pest control. It is particularly efficient against early shoot borer and offers more durable protection from yellow stem borer. Its application specifically affects the ryanodine receptors (RyRs) which leads to uncontrolled calcium in the cell and ultimately results in insect death [2]. Additionally, metals are distinct from other contaminants in that they are often pervasive in the environment and occur naturally, yet they can also have a negative impact on health [3]. According to reports, zinc (Zn) metal can be found in freshwater because Zn compounds are comparatively highly soluble. Zn is a crucial component of aquatic life. For instance, the enzyme carbonic anhydrate, which catalyses the creation of carbonic acid from carbon dioxide in the blood, contains Zn. Therefore, it is crucial to consume a tiny amount of Zn in your diet or in your water [4]. When the amount of Zn in the water reaches a certain level and enters the organism through the gills in excess of what is needed, a film of mucus forms on the surface of the gill, which results in death [5]. Galvanized ironwork, zinc chloride used in plumbing, and zinc-based paints are major sources of Zn. Fisheries may be impacted by zinc alone or more frequently in combination with copper and other metals, and zinc wastes may have direct toxicity to fish at elevated waterborne levels. The gills are the primary site of waterborne Zn poisoning, where the disruption of Ca^{2+} uptake results in hypocalcaemia and final mortality [6].

Nano zinc oxide (ZnO-NP) is necessary to produce dyes, glass, cement, cosmetics, sunblock, sun protection ointments, and optical filters [7]. As a result, it enters the aquatic environment directly or indirectly, negatively affecting the biology of aquatic species and ultimately humans [8].

Through the gills, digestive system, and skin, nano zinc oxide enters the body of the fish. [9]. Due to its small particle size, it can enter cells and induce an imbalance in the permeability of the cell membrane, which contributes to the release of free oxygen radicals and the development of oxidative stress [10]. One of the most significant mechanisms of toxicity of nanomaterials within the cell is oxidative stress (OS), which is caused by the overlap of the electron donor or essential receptor sites and acceptor active site with an oxygen molecule, which results in the development of Superoxide radical.

Since fish can metabolise, absorb, and retain toxins detected in water samples, they are a perfect organism to investigate the effects of numerous pollutants. The Freshwater fish tilapia *Oreochromis niloticus* serves as both the average person's protein source and an efficient experimental aliquot [11].

A significant biomarker for pesticides made of organophosphates and carbamates is acetylcholinesterase (AChE) [12]. AChE is also susceptible to metals, detergents, and complex combinations of contaminants, according to numerous studies [13]. By hydrolysing the neurotransmitter acetylcholine in cholinergic synapses, AChE is recognized to play a crucial role in cholinergic neurotransmission [14].

A biomarker of toxicity in living creatures is the alteration of the enzymes Aspartate aminotransaminase (AST) and Alanine aminotransaminase (ALT). These enzymes are found in various tissues besides the liver, including red blood cells, heart cells, muscle tissue, and organs including the pancreas and kidneys. The behaviour of AST and ALT would demonstrate the effects of contaminants on fish [15]. These enzymes are naturally present in the cells of various organs, including the liver, heart, gills, and kidneys [16] and the rise in these enzymes' activity within the liver is a sign of tissue damage or malfunction [17].

The current study therefore sought to determine the effects of CAP alone or mixed with nano zinc oxide as a heavy metal pollutant on the histology and biochemical levels in *O. niloticus*, which can be employed as biomarkers in monitoring aquatic toxicity.

2. Materials and methods

2.1. Chemicals

The insecticide CAP, coragen 20% SC was provided by DuPont Crop Protection. Zinc oxide nanoparticles (ZNO-NP, Particle size < 100 nm NPs, 50% WT in water) were purchased from Sigma Chemical Co., St. Louis, Mo, USA.

All biochemicals used in the experiments were of the highest analytical grade. Acetylthiocholine iodide (ATChI), 5,5'dithio-bis 2 nitrobenzoic acid (DTNB), and bovine serum albumin (BSA) and other chemicals were purchased from Sigma Chemical Co., St. Louis, Mo, USA. Aspartate aminotransaminase (AST) and

Alanine aminotransaminase (ALT) Kits was obtained from Diamond Co., Egypt.

2.2. Water analyses

The Water sample was collected from the tank where the experimented fish were treated. The water was analysed for pH, dissolved anions, dissolved cations, electrical conductivity according to the standard method described by the American Public Health Association [18]. The results presented in Table (1) show the Water quality analysis.

Table 1

Water quality analysis

Parameter	Results	Units
Electrical conductivity (EC)	0.53	dSm^{-1}
pH	7.00	meq L^{-1}
Ca^{++}	2.7	meq L^{-1}
Mg^{++}	2.3	meq L^{-1}
Na^{+}	2.2	meq L^{-1}
K^{+}	0.5	meq L^{-1}
$CO3^{--}$	0.0	meq L^{-1}
$HCO3^{-}$	2.0	meq L^{-1}
Cl^{-}	2.8	meq L^{-1}

2.3. Fish collection and maintenance

Nile tilapia *O. niloticus* (Linn.), weighing 32±1.81 g on average procured from El max fish farm, National Instituteof Oceanography and Fisheries (NI OF), Alexandria, Egypt, and adapted to laboratory circumstances for 15 days in 200 L tank with sufficient aeration. The aerator was adjusted to prevent oxygen depletion and to resist any changes brought on by carbon dioxide tension. Fish were fed natural nutrients (fish meal+ Soybean + corn flour + wheat bran + wheat + oil + vitamin) at a rate of 4% of body weight once daily during assimilation and testing. The water in glass aquariums was changed and cleaned every day.

2.4. Acute toxicity test

Nile tilapia fish were exposed to different concentrations of CAP (2, 4, 6, and 10 µg/ml). all concentrations were also mixed with ZnO-NP as a heavy metal pollutant at 10 mg/l. Fish were subjected to 10 µg/ml of ZnO-NP during the test as a positive control and water as a negative control. ZnO-NP concentration in the assay was decided by considering relevant literature. All treatment were replicated thrice, 10 fish/ replicate. Mortality was recorded daily for four days.

2.5. In-vivo histopathological effects

Tilapia fish were subjected to 1/10 LC_{50} of CAP at 96 h, 10 µg/ml of ZnO-NPs and their mixture. Fish were sampled and tissues (gills and liver) were dissected daily for four days. The tissues were also isolated from control.

Physiological saline solution (0.85% NaCl) was used to rinse and clean the tissues. They were fixed were fixed in 10% neutral buffered formalin, and then the samples were processed for routine wax histological evaluation (dehydrated and embedded in paraffin). Sections of 5µm were done and stained with haematoxylin and eosin stains for light microscopic analysis.

2.6. In-vivo biochemical studies

In order to determine the biochemical effects of CAP on tilapia fish, it was treated with 1/10 of 96 h-LC_{50}. Samples were taken daily for four days where brain and liver were dissected as a source of AChE, AST, and ALT. After 4 days, survival individuals were transferred to fresh water without insecticides for recovery study. Samples were also taken after 1 week and 2 weeks of recovery.

Tilapia brain and liver were homogenized in 50 mM of ice-cold phosphate buffer (100 mM, pH 7.4). The homogenate was centrifuged using an IEC-CRU-5000 cooling system at 5,000 rpm for 30 min at -5 ^{0}C. The resulting supernatant was used as an enzyme source.

Ten µl of brain supernatant were used for AChE which measured using ATChI as a substrate and the yellow colour was developed using DTNB as described by Ellman et al. [19]. Absorbance was determined at 412 nm using a Sequoia-Turner Model 340 spectrophotometer. A free enzyme test was used as a blank. Enzyme activity was expressed as % activity of control.

Additionally, liver supernatant was used for the measurement of AST and ALT activities. Activity was determined by adding 0.5 ml enzyme source to 0.25 ml of 100 mM phosphate buffer (pH = 7.4) containing 0.1 M L-asparagine acid + 2 mM alpha-ketoglutaric acid for AST and 0.2 M DL-alanine + 2 mM alpha-ketoglutaric acid for ALT. These mixtures were incubated for 30 min at 37˚C. After that, 500 µl of

developing colour reagent (4 mM 2, 4-dinitrophenylhydrazine) was added and the solution was incubated for 20 min at room temperature. Lastly, 5 ml of 0.4 N NaOH was added then mixed and left at room temperature for five min. An assay mixture without enzyme source was used as blank and the absorption was measured at 550 nm using a Sequoia-Turner Model 340 spectrophotometer. Activities were calculated as % activity of control.

Protein estimation has been done using bovine serum albumin (BSA) according to Lowry *et al.* [20].

2.7. Statistical Analysis

All data of enzyme activities were statistically evaluated using one way analysis of variance (ANOVA) by SAS software version 9.4 [21]. Means were compared for significance by Tukey HSD test at $P \leq 0.05$.

The mortality data were subjected to Probit analysis according to Finney [22], values of LC values, 95% confidence limits, slopes, and Chi^2 were established by Polo plus program [23]. Relative potency ratio, which was estimated as REP=LC of CAP/LC of CAP-ZnO-NP (Mix) and their 95% confidence limits were evaluated, as well. The ratio test of LDs at $P=0.05$ was evaluated according to Robertson et al. [24].

3. Results

3.1. *Acute toxicity of CAP and its mixture with ZnO-NP*

The lethal effect of CAP and its mixture with ZnO-NP at 10 µg/ml on Tilapia fish was investigated. The percent kill after transforming to Probit, mortality was plotted against log concentration of CAP and its mixture with ZnO-NP using Probit method. Thus, the 96 hrs LC_{50} value determined for CAP and its mixture by the above Probit methods were 0.386 and 10.607 µg/ml, respectively. According to the LC_{50} values, the toxicity of the mixture is about 27-fold significantly ($P<0.05$) more than CAP alone (Table 2).

Table 2

Log conc. Probit-mortality data for Tilapia fish in response of CAP and its mixture with ZnO-NP

Treatment	N[a]	LC_{50} (µg/ml)[b] (95% FL)[c]	Slope±SE	χ^2 (df)[d]	REP[d] (95% FL)[f]
CAP	180	10.607 (8.79-13.55	5.14±1.55	0.18 (3)	27.498 (16.08-47.04)[*]
Mix	180	0.386 (0.21-0.80)	1.95±0.67	0.22 (2)	

[a]N = number of fish tested

[b]LCs were calculated after 96 hr for CAP and its Mixture with 10 mg/L ZnONPs.

[c]95% fiducial limits estimated using Polo-Plus program (LeOra 2003) [23].

[d]Chi-square testing linearity of conc–mortality response ($\alpha = 0.05$).

[e]Relative potency (REP) = LC value of CAP/LC value of Mix.

[f]95% confidence limits were estimated using lethal concentration ratio significance test according to the method described by Robertson et al. [24].

[*]Indicates that Relative potency was significant ($\alpha = 0.05$) according to the method described by Robertson et al. [24].

3.2. Biochemical studies

The present results showed that the brain AChE activity increased significantly ($P<0.05$) at the early period 24 and 48h posttreatment with CAP with 155 and 193% activity of control, respectively (Fig. 1A). AChE activity recovered after 7 days recovery period (Fig. 1B), after a 14-day recovery time in water devoid of pesticides, AChE activity resumed its basal level. The exposure of fish to tested concentration of CAP resulted in significant ($P<0.05$) decreasing activity of the liver AST and ALT where their activities at 96 h posttreatment were 67.5 and 54 % activity of control, respectively (Fig. 2A and 3A, respectively). Thus, Inactive transamination and oxidative deamination occurred in the tissues affected the metabolic process. This could have an impact on the fish's physiological functions since hepatoxicity causes the release of these enzymes from the liver cytosol through the membrane into the bloodstream. Decreases in the AST and ALT enzymes typically signify tissue injury. However, AST and ALT activity elevated significantly ($P<0.05$) after 7 days recovery period (190.5 and 184.1 % activity of control, respectively) in contrast to the treated condition and then started to resemble the control value after 14 days (Fig. 2B, 3B).

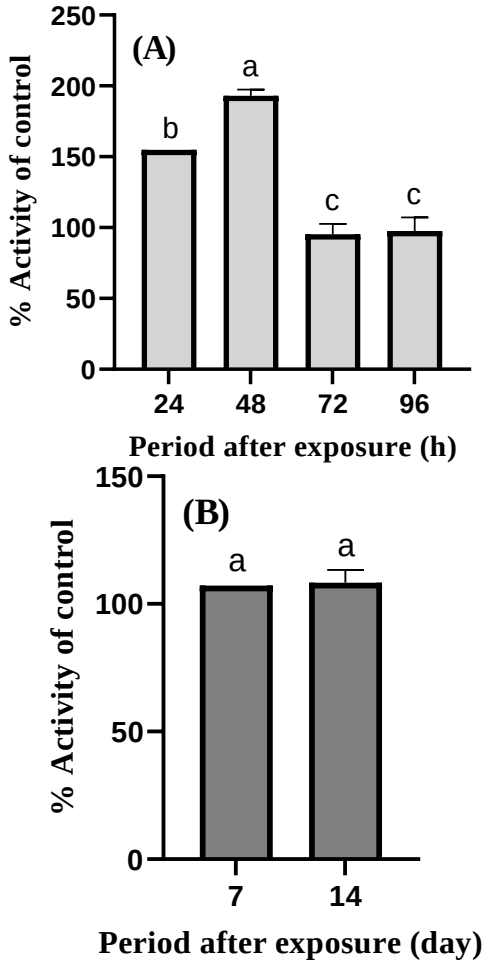

Fig. 1. *In vivo* effect of CAP at 1/10 LC_{50} on AChE enzyme in Nile tilapia. (A) Brain AChE Activity of Tilapia exposed to CAP. (B) Brain AChE Activity of Tilapia exposed to CAP after recovery period. Means bars followed by the same letter at the top of the error bars are not significantly different (P < 0.05) from each other according to Tukey's test.

3.3. Histological studies

The control fish's gill anatomy matched those of other species that have been documented [25]. In a frontal section, two specialized epithelia with different blood compartments could be seen in Fig. 4A. The primary lamellae are mostly used for ionic regulation, while the secondary lamellae are in charge of gas transmission [25]. One cell thick squamous epithelial layer line the respiratory lamellae. The lamellar blood sinus, which is internal to the epithelium, is lined and bridged by pillar cells with contractile activity. Abasement membrane lies between the pillar cells and the epithelial cells. At the tip of the lamellae, expanded lacuna (blood channel) is observed in which there were many blood cells. In the Basi lamellar region situated on the primary filament, were groups of chloride cells which are oval and with acidophilic cytoplasm. Their nuclei are lightly basophilic and oval. In addition, at internals along the superficial layer of epithelial cells there were mucous cells. It is significant to note that the primary lamellae's epithelial cells in most of the control gills tested showed signs of hyperplasia.

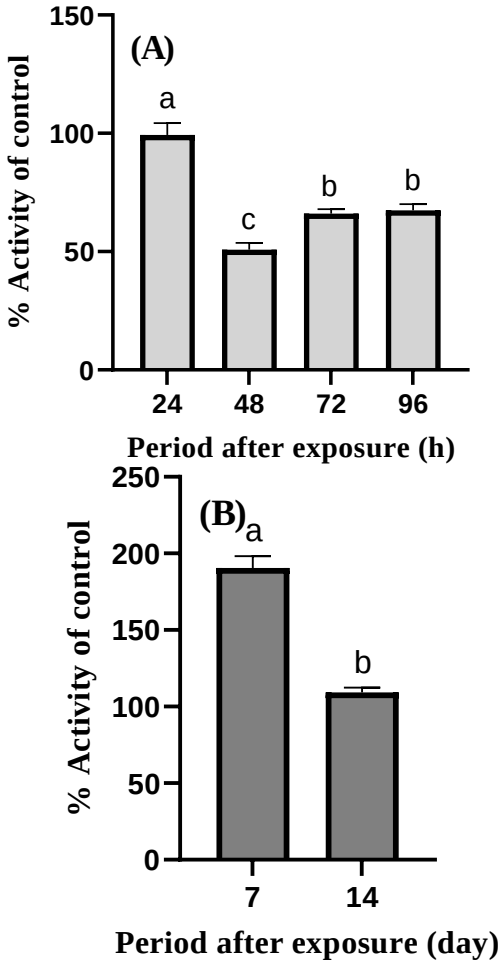

Fig. 2. *In vivo* effects of CAP at 1/10 LC_{50} on AST enzyme in Nile tilapia. (A) Liver AST Activity of Tilapia exposed to CAP (B) Liver AST Activity of Tilapia exposed to CAP after recovery period. Means bars followed by the same letter at the top of error bars are not significantly different (P < 0.05) from each other according to Tukey's test.

Exposure of *O. niloticus* to CAP produced histological changes in gills where the hypercellularity and sloughing of the respiratory epithelia and lifting of epithelial cells from their basement membranes were pronounced (Fig. 4C). In the zinc treated fish, Obvious gill damage was clear (Fig. 4B).

In most gill filaments, the respiratory epithelium showed lysis leaving the blood sinus and supportive pillar cells naked. In the case of CAP-ZnO-NP-treated fish vascular damage of the branchial blood vessels,

blood capillaries, and supportive pillar cells was clear. Lifting of the primary epithelium from the branchial blood vessels was also indicated (Fig.4D).

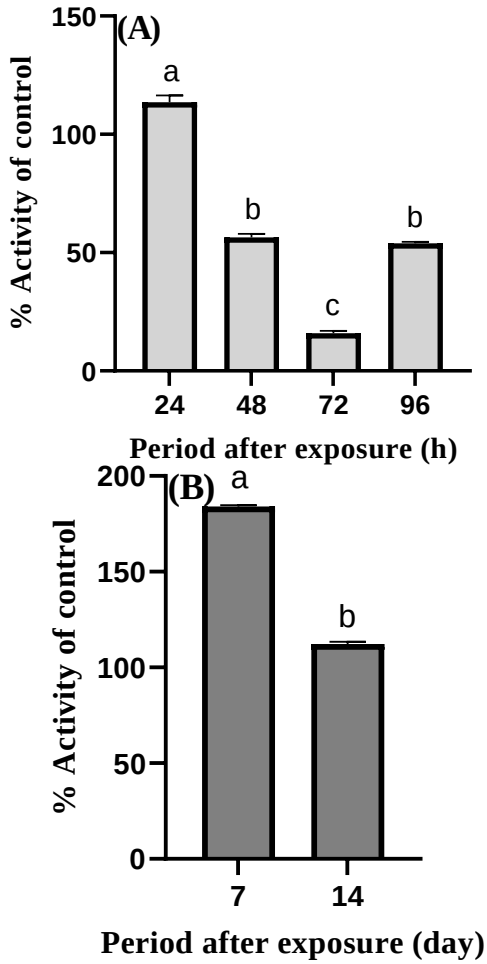

Fig. 3. *In vivo* effects of CAP at 1/10 LC_{50} on ALT enzyme in Nile tilapia. (A) Liver ALT Activity of Tilapia exposed to CAP (B) Liver ALT Activity of Tilapia exposed to CAP after recovery period. According to Duncan's test, the mean bars followed by the same letter at the top of the error bars are not significantly different ($P < 0.05$) from each other according to Tukey's test.

The histological structure of the liver in a healthy fish control shows the histological section of *O. niloticus* liver which appears that there are cords of polyhedral parenchymal cells (hepatocytes) that are two cells thick and lead to the conspicuous central vein. Sinusoids with nucleated red blood cells separate the columns of hepatic cells. The oval Kupffer cells with densely stained oval nuclei occur at various points along the sinusoidal lumen.

Between the hepatic cells, blood vessels can be seen, and each of them is encircled by a fine layer of smooth muscle tissue. Generally, hepatocytes possess large vesicular round pale-stained basophilic typically, nuclei have one prominent central nucleolus that is positioned in the middle. The cytoplasm of a hepatic cell is moderately acidophilic and contains faintly stained fine granules. It should be noted that, in some of the control livers, some hepatocytes' cytoplasm is dotted with tiny vacuoles of various sizes. Some hepatocytes nuclei also show signs of pyknosis (where the nucleus is shrunken and stained more deeply with basophilic dyes) and karyolysis (i.e., the nucleus or nuclear fragments lost the ability to stain with basic dyes) (Fig.5A).

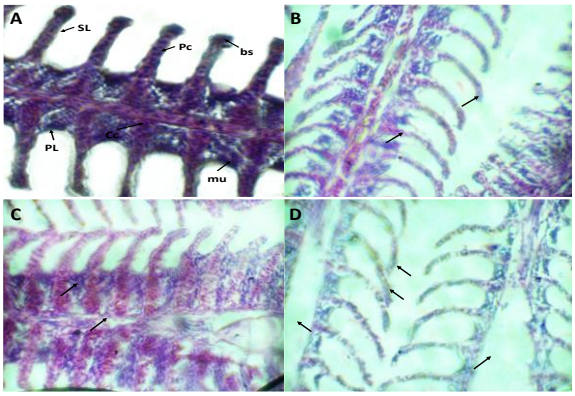

Fig. 4. Histopathological alterations observed in Nile tilapia gills due to exposure to ZnO-NPs, CAP, and their mixture for four days in comparison with control stained by haematoxylin-eosin x 40. (A) Normal structure of two gill epithelial layers primary lamellae (PL), secondary lamellae (SL), blood channel, blood sinus (bs), pillar cells (pc), mucous cell (mu) and chloride cells (cc) in gill of untreated fish. (B) Gill epithelial damage; naked blood sinus and supported pillar cells, (C) Hypercellularity and lifting of gill epithelia from their basement membrane and (D) Twisting of secondary lamellae, vascular damage of the branchial blood vessels in gills of fish exposed to ZnO-NPs, CAP, and their mixture, respectively.

The liver of *O. niloticus* subjected to CAP had histological alterations that revealed evident vacuolar degeneration of the hepatocytes as well as clear nuclear degeneration. Prevalence of sinusoidal collapse, hepatocellular vacuolization, and necrosis are apparent. In addition, there was necrosis of the endothelial lining of the blood vessels (Fig. 5C). In fish liver exposed to ZnO-NP, large spaces between degenerated hepatocytes were observed where necrotic changes caused a vacuolized appearance which in turn replaced the liver parenchymatous structure (Fig. 5B). In fish liver exposed to CAP-ZnO-NP seen in Fig. 5D showed remarkable vacuolar degeneration of the hepatocytes with clear nuclear degeneration. The parenchymal cells were isolated, and shrunken, and a great reduction in their number and size was clear. In addition, congestion of the blood vessels was clear.

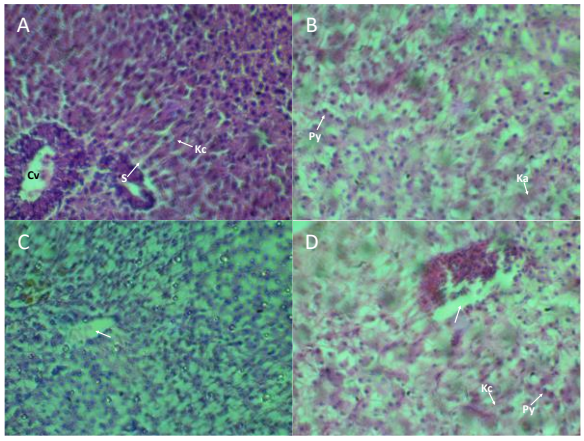

Fig. 5. Histopathological alterations observed in Nile tilapia liver due to exposure to ZnO-NPs, CAP and their mixture for four days in comparison with control stained by haematoxylin-eosin x 40. (A) Normal structure of liver having Central vein (Cv) with hepatocytes (two cell thick) leading to it, Sinusoids (S) and Kupffer cell (Kc) in liver of untreated fish.(B) karyolysed (Ka) and pyknotic nuclei (Py) (C) Sinusoidal collapse (S) haemolysis and necrosis of blood vessel with vacuolated hepatocytes (D) Vacuolated hepatocytes with karyolysed (Ka) and pyknotic nuclei (Py), Sinusoidal collapse (S) and congestion of the blood vessel in liver of fish exposed to ZnO-NPs, CAP and their mixture, respectively.

4. Discussion

Risk assessment of environmental pollutants has drawn the interest of a diverse cross-section of people worldwide and has since emerged as a major topic of discussion among academic researchers who work in the field of environmental protection. Pollution poses a major threat to the life of all organisms and the well-being of millions of people worldwide [26]. The most important toxic pollutants of aquatic ecosystems are pesticides and heavy metals that can form mixtures in the environment [26, 27].

The current study aims to evaluate the toxicity of the formulated CAP, coragen 20% SC, on the freshwater fish, Nile tilapia (*O. niloticus*). Experimentally, the 96h median lethal toxicity (LC_{50}) is used to classify the toxicity of any chemical as nontoxic (LC_{50}>100 mg/l), highly toxic (0.1>1 mg/l), moderately toxic (1>10 mg/l), and slightly toxic (10>100 mg/l) according to US EPA category [28]. The aquatic 96-hour LC_{50} data reveals that CAP poses slightly toxicity. Although CAP toxicity may differ with the species, Coragen 18.5% SC was also slightly toxic to the freshwater fish *Channa punctatus*, *Ctenopharingodon Idella*, *Labeo rohita* and *Cirrhinus mrigala* with acute 96h LC_{50} of 14.424, 11.008, 12.7 , and 16.465 mg/l, respectively [29-31]. CAP is, however, less toxic compared to the organophosphate insecticide, fenitrothion, and the pyrethroid insecticide, deltamethrin with acute 96h LC_{50} of 0.84 and 0.015 mg/l, respectively [32, 33].

It is crucial to define and understand pollutant impacts in biochemical terms to outline pathways of pollutant action and lessen its negative consequences as biochemical processes indicate the most delicate and comparatively early stages of pollutant harm. Enzymes are among the most often employed biomarkers to evaluate how organisms react to toxins, offering crucial hints to anticipate the effects of the chemicals on both target and non-target organisms [34]. *In vivo* effects of CAP at 1/10 LC_{50} on AChE, ALT, and AST enzymes were investigated.

AChE activity increased during the early period, 24h and 48h following treatment with CAP. This result is not strange as CAP acts by disrupting calcium homeostasis in central neurons [35]. Additionally, it is consistent with prior research on various organisms where exposure to CAP only temporarily increased AChE and esterase (EST) activity in Locusta migratoria (Meyen) grasshoppers after treatment [36]. AChE activity was also increased in *Eisenia fetida* earthworm due to exposure to soil treated by CAP [37]. AChE activity elevated actually by 7 days recovery period, while it later restored to its original level at 14- days recovery period in pesticide-free water. Since CAP disrupts calcium levels in the central nervous system, its ability to inhibit AChE as an anticholinergic substance is not anticipated [36]. The rise in AChE activity, however frequent, is little understood, but it appears to be related to oxidative stress and changes in intracellular ion balance [37]. Increased AChE activity was characterized as a sign of exposure to apoptosis-inducing chemicals, i.e., a reaction of cells going through apoptosis, which may be caused by an overcompensation phenomenon or may be associated to tissue inflammation [37]. After a recovery period of two weeks, AChE activity resumed its basal level.

Among other useful enzymatic biomarkers, the hepatic aminotransferases (ALT and AST), are one of the main pathways for the formation and deamination of amino acids, enabling the interconversion of protein and carbohydrate metabolism under stress-induced circumstances to provide the high energy requirements of all species [38]. The results clearly showed that the activity of the liver ALT and AST was significantly reduced.

Several freshwater fish had decreased aminotransferases activity in their liver tissues such as

Anabas testudineus exposed to deltamethrin [39], *Heterobranchus bidorsalis* [40], and *Clarias gariepinus* [41] subjected to cypermethrin. Furthermore, the present result agreed with observations of [42] who noted that the activity of the enzymes ALT and AST was lowered in different organs of mercury-exposed Pontius conchonius fish. Adams observed that fish subjected to paper mill runoff at the outflow also had lower ALT activity than fish downstream [43].

There may be a repressive mode for preventing the pesticide's impact on the fish, according to decreased hepatic aminotransferases activity in the liver [40] additionally, oxidative deamination and inactive transamination in the tissues took place, thereby altering the metabolic process could have an impact on the fish's physiological systems [41] due to hepatoxicity, which causes these enzymes to leak through the membrane out from liver cytosol into the bloodstream. assessed within a shorter time [44].

ALT and AST activity was higher throughout the recovery period than it was during the treatment condition, and after 14 days, it started to resemble the control value. Bálint et al. [45] noted that when exposed to a xenobiotic, the liver-specific enzymes ALT and AST are sensitive to assessing histopathologic alterations and can be measured quickly after exposure. In addition, histopathological evaluation is a sensitive bio-monitoring approach that can be used in toxicant impact studies to show how toxicants affect fish in pesticide-polluted aquatic habitats. Numerous researchers have also looked into such structural variations in fish as indicators in different tissues from distinct species [46, 47]. Accordingly, the histopathological changes in the liver, as well as gills of fish exposed to CAP, were also investigated in comparison with unexposed fish.

Fish gills regulate osmoregulation, respiration, and stress in a manner that is closely related to their morphology [48]. The appropriate respiratory and osmoregulatory activities become unstable when the gill morphology is altered or damaged.

Histopathological changes in the gill of *O. niloticus* including Hypercellularity and lifting of gill epithelia from their basement membrane were also observed unequivocally in gills of *O. niloticus* exposed to cypermethrin [49] and other freshwater fish such as common carp, Cyprinus carpio treated with chlorpyrifos [50], *Capoeta capoeta* exposed to different kinds of heavy metals [51]. Because of the Gills of *C. mrigala* direct contact with the toxin, Rathnamma and Nagaraju [31] noted that it was the organ that was most severely affected by Coragen 18.5% SC compared to the liver. They also noted the significant histopathological changes, that included epithelial lifting, aneurysm, necrosis, and secondary lamellae degeneration.

The primary metabolic organ that can detoxify toxicants and extremely hazardous secondary metabolites is the liver. As a result, the liver is exposed to and accumulates these harmful substances [52]. The current study demonstrates the important consequences of CAP, which resulted in sinusoidal collapse, hemolysis, and necrosis of blood vessels with vacuolated hepatocytes. Rathnamma and Nagaraju [31] also observed the histopathological changes in the liver of *C. mrigala* fish subjected to Coragen 18.5% SC which demonstrates significant necrosis, oedemas, and hepatocyte degeneration. These results revealed that CAP induces histopathological changes in gill and liver as well as biochemical perturbations in *O. niloticus* fish that can work as good biomarkers in monitoring CAP intoxication in the aquatic environment. In the current investigation, fish were subjected to ZnONPs, which are often employed in a variety of commercial and industrial applications [53]. There is no doubt that ZnONPs have the capacity to cause histopathological changes, gill epithelial damage; naked blood sinus, and supported pillar cells in gills as well as karyolysis and pyknotic nuclei in the liver.

Kaya et al. [54] conducted sub-chronic exposure in tilapia *O. niloticus* with concentrations of 1 and 10 mg/L and found that ZnO-NPs cause various histopathological changes including oedema, mononuclear cell infiltrations, fatty swaps, pyknotic nuclei, and hepatic vacuolations in the liver, as well as hyperplasia, aneurysms, and epithelial liftings in the gills. Shahzad et al. [48] found histological changes in the liver of the tilapia *Oreochromis Mossambicus* exposed subchronically to ZnO-NPs, including necrosis and apoptosis with condensed nuclear bodies and pyknotic nuclei. Gill oedema and hyperplasia, a fusion of gill lamellae, and thickening of primary and secondary gill lamellae.

In the environment, organisms are exposed to many different chemicals simultaneously. To clarify the interaction between CAP and ZnO-NPs, the toxicity of their combination was studied. Regarding 96h LC_{50} of the mixture, a synergistic effect has occurred and CAP

became highly toxic in the presence of ZnO-NP according to the US EPA category [28]. Moreover, the CAP-ZnO-NP combination resulted in histopathological changes in gills, Twisting of secondary lamellae and vascular damage of the branchial blood vessels, and Vacuolated hepatocytes with karyolysis and pyknotic nuclei, Sinusoidal collapse, and congestion of the blood vessel in the liver. It can be concluded that the histopathological changes caused by the CAP-ZnO-NP mixture are more vigorous as compared to that of fish individually exposed to its components. Heys et al clarified that carbon disulfide which is a hepatotoxic pollutant exhibits synergistic toxicity in certain mixtures [26]. Heys et al also mentioned that some chemical pollutants interact altering the toxicity to more or less than expected compared to their individual components toxicity summation [26].

5. Conclusions

The present study reveals that sublethal exposure of tilapia fish *O. niloticus* to CAP results in biochemical perturbations on brain AChE and liver ALT and AST for 96 h. All enzymes were recovered after 14 days. Besides, CAP has the potential to induce histopathological alterations in gills and the liver. It can be stated that *O. niloticus* could be a suitable biomarker and AChE, ALT, and AST as well as induced histopathological alterations are very important early warning ecotoxicological parameters for CAP. The toxicity synergized and histopathological perturbations exaggerated by the CAP-ZnO-NP mixture confirm that some environmental chemical pollutants can be interacted altering their effects to be more than predicted as compared to their individual components.

6. Conflicts of interest

The authors confirm that this article content has no conflict of interest.

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8. References

[1] Sabullah, M. K., S. A. Ahmad, M. Y. Shukor, A. J. Gansau, M. A. Syed, M. R. Sulaiman and N. A. Shamaan (2015). Heavy metal biomarker: Fish behavior, cellular alteration, enzymatic reaction and proteomics approaches. International Food Research Journal, 22(2): 435-454.

[2] Satpathy, S., B. S. Gotyal and V. R. Babu (2020). Role of novel insecticides in crop protection and their selectivity to natural enemies: A review. Journal of Environmental Biology, 41(2): 149-160.

[3] Ritter, K. S., P. Sibley, K. Hall, P. Keen, G. Mattu and B. Linton (2002). Sources, pathways, and relative risks of contaminants in surface water and groundwater: a perspective prepared for the Walkerton inquiry. Journal of Toxicology and Environmental Health Part A, 65(1): 1-142.

[4] Morel, F.M., A. J. Milligan and M.A. Saito (2003). Marine Bioinorganic Chemistry: The Role of Trace Metals in the Oceanic Cycles of Major Nutrients. In Treatise on Geochemistry, 6:113-143.

[5] Babv, J., J.S. Rai, E.T. Bibv, P. Sankarganesh, M.V. Jeevitha, S.U. Ajisha and S.S. Rajan, (2010). Toxic effect of heavy metals on aquatic environment. International Journal of Biological and Chemical Sciences, 4(4): 939-952.

[6] Niyogi, S. and M.W. Chris (2003). Assessment, Effects of chronic waterborne and dietary metal exposures on gill metal-binding: implications for the biotic ligand model. Human and Ecological Risk Assessment, 9(4): 813-846.

[7] Ali A., A.R. Phull and M. Zia (2018). Elemental zinc to zinc nanoparticles: Is ZnO NPs crucial for life? Synthesis, toxicological, and environmental concerns. Nanotechnology Reviews, 7:413–441.

[8] Lu, L., T. Luo, Y. Zhao, C. Cai, Z. Fu and Y. Jin (2019). Interaction between microplastics and microorganism as well as gut microbiota: A consideration on environmental animal and human health. Science of the Total Environment, 667: 94-100.

[9] Al-Taee, S. K. E. and H. A. A. Alaa (2015). Effect of $CuSO_4$ on toxicity of nano zinc oxide (nZnO) in carp fish (*Cyprinus carpio* L.). Journal of Limnology and Freshwater Fisheries Research, 1(3): 99-10.

[10] Wang, Z. and T. Meng (2021). Research progress on toxicity, function, and mechanism of metal oxide nanoparticles on vascular endothelial cells. Journal of Applied Toxicology, 41(5): 683-700.

[11] Ali, F.k., A. M. El-Shehawi and A. M. Seehy (2008). Micronucleus test in fish genome: A sensitive monitor for aquatic pollution. African Journal of Biotechnology, 7(5): 606-612.

[12] Fulton, M. H. and P. B. Key (2001). Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticide exposure and effects. Environmental Toxicology and Chemistry, 20(1): 37-45.

[13] Monteiro, M., C. Quintaneiro, F. Morgado, A. M. V. M. Soares and L. Guilhermino (2005). Characterization of the cholinesterases present in

head tissues of theestuarine fish Pomatoschistus microps: application to biomonitoring. Ecotoxicology and Environmental Safety, 62(3): 341-347.
[14] Jebali, J., S. B. Khedher, M. Sabbagh, N. Kamel, M. Banni and H. Boussetta (2013). Cholinesterase activity as biomarker of neurotoxicity: utility in the assessment of aquatic environment contamination. Journal of Integrated Coastal Zone Management, 13(4):525-537.
[15] Alkaladi, A., Y. Y. I. Mosleh and M. Afifi (2013). Biochemical and histological biomarkers of Zn pollution in Nile Tilapia, (*Oreochromis niloticus*). Archives Des Sciences, 66: p. 295-311.
[16] Ahmad, Z. (2012). Toxicity bioassay and effects of sub-lethal exposure of malathion on biochemical composition and haematological parameters of Clarias gariepinus. African Journal of Biotechnology, 11(34): 8578-8585.
[17] Kavitha, C., A. Malarvizhi, S. S. Kumaran and M. Ramesh (2010). Toxicological effects of arsenate exposure on hematological, biochemical and liver transaminases activity in an Indian major carp, *Catla catla*. Food and Chemical Toxicology, 48(10): 2848-2854.
[18] Bhatnagar, A. and P. Devi (2013). Water quality guidelines for the management of pond fish culture. International Journal of Environmental Sciences, 3(6): 1980-2009.
[19] Ellman, G. L., K. D. Courtney, V. Andres Jr and R. M. Featherstone (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology, 7(2): 88-95.
[20] Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall (1951). Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry, 193(1): 265-275.
[21] SAS I. (2017) Base SAS 9.4 procedures guide: Statistical procedures. SAS Institute, Inc: Cary, North Carolina, USA.
[22] Finney, D. J. (1971). Probit analysis, 3rd ed. Cambridge University, London, UK.
[23] LeOra, S. (2003) Poloplus, a user's guide to probit or logit analysis. LeOra Software, Berkeley, CA
[24] Robertson, J. L, M. M. Jones, E. Olguin and B. Alberts (2017). Bioassays with arthropods. CRC press.
[25] Newstead, J. D. (1967). "Fine structure of the respiratory lamellae of teleostean gills. Zeitschrift für Zellforschung und Mikroskopische Anatomie, 79(3): 396-428.
[26] Heys, K. A., F. S. M. Richard, P. Glória, C. J. Kevin and L. M. Francis (2016). Risk assessment of environmental mixture effects. RSC Advances, 6(53): 47844-47857.
[27] Khoshnood, Z. (2017). Effects of environmental pollution on fish: a short review. Transylvanian Review of Systematical and Ecological Research 19(1): 49-60.
[28] Kovács, R., K. Bakos, B. Urbányi, J. Kövesi, G. Gazsi, A. Csepeli, Á. J. Appl, D. Bencsik, Z. Csenki and Á. Horváth (2016). Acute and sub-chronic toxicity of four cytostatic drugs in zebrafish. Environmental Science and Pollution Research, 23: 14718-14729.
[29] Bantu, N. and R. V. Venkata (2013). Acute toxicity of chlorantraniliprole to freshwater fish *Channa punctatus* (Bloch). Advances in Zoology and Botany, 1(4): 78-82.
[30] Rathnamma, V., and B. Nagaraju (2013). Acute toxicity of chlorantraniliprole to fresh water fish *Ctenopharingodon idella* (Valenciennes, 1844). Innovare Journal of Life Science, 1(2): 17-20.
[31] Rathnamma, V. and B. Nagaraju (2014). Acute toxicity and histopathological changes in freshwater fish *Cirrhinus mrigala* exposed to chlorantraniliprole. The Journal of Zoology Studies, 1(4): 23-30.
[32] Benli, A. Ç. K. and A. Özkul (2010). physiology, Acute toxicity and histopathological effects of sublethal fenitrothion on Nile tilapia, *Oreochromis niloticus*. Pesticide Biochemistry and Physiology, 97 (1): 32-35.
[33] Boateng, J. O., F. K. E. Nunoo, H. R. Dankwa and M. H. Ocran (2006). Acute toxic effects of deltamethrin on tilapia, *Oreochromis niloticus* (Linnaeus, 1758). West African Journal of Applied Ecology, 9(1):1-18.
[34] Gonçalves, A. M., P. R. Carolina, C. M. Joao and J. M. G. Fernando (2021). Enzymes as useful biomarkers to assess the response of freshwater communities to pesticide exposure–A review. Ecological Indicator, 122: 107303.
[35] Li, Y., M. Mao, Y. Li, L. Xiong, Z. Li and J. Xu (2011). Modulations of high-voltage activated Ca^{2+} channels in the central neurones of *Spodoptera exigua* by chlorantraniliprole. Physiological Entomology, 36(3): 230-234.
[36] Jia, M., G. Cao, Y. Li, X. Tu, G. Wang, X. Nong, D. W. Whitman and Z. Zhang (2016). Biochemical basis of synergism between pathogenic fungus Metarhizium anisopliae and insecticide chlorantraniliprole in *Locusta migratoria* (Meyen). Scientific Report, 6(1): 1-15.
[37] Hackenberger, D. K., G. Palijan, Ž. Lončarić, O. J. Glavaš and B. K. Hackenberger (2018). Influence of soil temperature and moisture on biochemical biomarkers in earthworm and microbial activity after exposure to propiconazole and chlorantraniliprole. Ecotoxicology and Environmental Safety, 148: 480-489.
[38] Van Waarde, A. and M. D. W. V. B. Henegouwen (1982). Nitrogen metabolism in goldfish, *Carassius auratus* (L.): Pathway of aerobic and anaerobic glutamate oxidation in goldfish liver and muscle mitochondria. Comparative Biochemistry and Physiology b-Biochemistry and Molecular Biology, 72(1): 133-136.
[39] Devi, M. S. and G. Abhik (2014). Physiology, Sublethal toxicity of commercial formulations of deltamethrin and permethrin on selected biochemical constituents and enzyme activities in liver and muscle tissues of *Anabas testudineus*. Pesticide Biochemistry and Physiology, 115: 48-52.
[40] Gabriel, U. G., I. R. Jack and E. Egobueze and O. S. Edori (2011). Impact of cypermethrin on selected enzymes in tissues of *Heterobranchus bidorsalis*. West African Journal of Applied Ecology, 18(1):121-127.

[41] Gabriel, U. U., A. Ojo, and V. S. Ariweriokuma (2012). Changes in metabolic enzymes activities in selected organs and tissue of Clarias gariepinus exposed to cypermethrin. Journal of Chemical Engineering, 1(1): 25-30.
[42] Goel, K., S. K. Tyagi and A. K. Awasthi (1982). Effect of malathion on some haematological values in Heteropneustes fossilis. Comparative Physiology and Ecology, 7:259-261.
[43] Adams, S. M., E. B. James and B. M. Richard (1985). Cumulative stress-induced mortality of gizzard shad in a southeastern US reservoir. Environmental Biology of Fishes, 13(2): 103-112.
[44] Oluah, N.S. (1999). Toxicology, Plasma aspartate aminotransferase activity in the catfish Clarias albopunctatus exposed to sublethal zinc and mercury. Bulletin of Environmental Contamination and Toxicology, 63(3): 343-349.
[45] Bálint, T., J. Ferenczy, F. Kátai, I. Kiss, L. Kráczer, O. Kufcsák, G. Láng, C. Polyhos, I. Szabó, T. Szegletes and J. Nemcsók (1997). Similarities and differences between the massive eel (*Anguilla anguilla* L.) devastations that occurred in Lake Balaton in 1991 and 1995. Ecotoxicology and Environmental Safety, 37(1): 17-23.
[46] Boamponsem, L. K., J. I. Adam, S. B. Dampare, E. Owusu-Ansah and G. Addae (2013). Studies on the histopatholoigcal changes in selected tissues of fish *Labeo rohita* to phenol. Journal of Chemical and Pharmaceutical Research, 34(2): 247-251.
[47] Marchand, M. J., J. C. Van Dyk, G. M. Pieterse, I. E. J. Barnhoorn and M. S. Bornman (2008). Histopathological alterations in the liver of the sharptooth catfish *Clarias gariepinus* from polluted aquatic systems in South Africa. Environmental Toxicology, 24(2): 133-147.
[48] Shahzad, K., M. N. Khan, F. Jabeen, N. Kosour, A. S. Chaudhry, M. Sohail and N. Ahmad (2019). Toxicity of zinc oxide nanoparticles (ZnO-NPs) in tilapia (*Oreochromis mossambicus*): tissue accumulation, oxidative stress, histopathology and genotoxicity. International journal of environmental science and technology, 16(4): 1973-1984.
[49] Korkmaz, N., E. I. Cengiz, E. Unlu, E. Uysal and M. Yanar (2009). Cypermethrin-induced histopathological and biochemical changes in Nile tilapia (*Oreochromis niloticus*), and the protective and recuperative effect of ascorbic acid. Environmental Toxicology and Pharmacology, 28(2): 198-205.
[50] Ghaffar, A., R. Hussain, G. Abbas, M. Kalim, A. Khan, S. Ferrando, L. Gallus and Z. Ahmed (2018). Fipronil (Phenylpyrazole) induces hemato-biochemical, histological and genetic damage at low doses in common carp, *Cyprinus carpio* (Linnaeus, 1758). Ecotoxicology, 27(9): 1261-1271.
[51] Dane, H. and T. Şişman (2015). Histopathological changes in gill and liver of *Capoeta capoeta* living in the Karasu River, Erzurum. Environmental toxicology, 30(8): 904-917.
[52] Dowling, A. J. U. (2004). Nanoscience and Nanotechnology: Opportunities and uncertainties. A Report by The Royal Society and The Royal Academy of Engineering.
[53] Asghar, M. S., N. A. Qureshi, F. Jabeen, M. S. Khan, M. Shakeel and A. Noureen (2015). Toxicity of zinc nanoparticles in fish: a critical review. Journal of biodiversity and environmental sciences, 7(1): 431-439.
[54] Kaya, H., F. Aydın, M. Gürkan, S. Yılmaz, M. Ates, V. Demir and Z. Arslan (2016). A comparative toxicity study between small and large size zinc oxide nanoparticles in tilapia (*Oreochromis niloticus*): Organ pathologies, osmoregulatory responses and immunological parameters. Chemosphere, 144: 571-582.