

## Toxicological effect of Boric Acid and Cadmium Chloride on the Nile Tilapia, *Oreochromis niloticus*

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### ABSTRACT

The toxicity of both boron (H<sub>3</sub>BO<sub>3</sub>) and Cadmium (CdCl<sub>2</sub>) was evaluated for Nile tilapia, *Oreochromis niloticus*. Six concentrations including control in Boron (H<sub>3</sub>BO<sub>3</sub>) experiment 0, 1, 20, 40, 80, 500 mg/l and five concentrations including control of Cadmium (CdCl<sub>2</sub>) experiment 0, 15, 30, 60, 90 mg/l were used for evaluation of LC<sub>50</sub>. Duplicate for each concentration was used and ten fish were used with an average weight of 50g ± 15 per aquaria. The 96 hours LC<sub>50</sub> was 290 mg/l for Boron. However, cadmium-treated groups showed no mortality. A histopathological examination of fish liver in Boric acid-treated groups showed moderate to significant changes including hydropic degeneration, congestion, dilated vessel, and focal necrotic changes. While, the control group showed the healthy structure of the liver. In CdCl<sub>2</sub> treated groups, liver examination showed infiltration of inflammatory cells, congestion of blood vessels and cytoplasm vacuolization.

### INTRODUCTION

Trace minerals and enzymes help with a wide range of biochemical processes. They act as cofactors and/or activators of many enzymes, as well as participate in a wide range of biochemical processes (Yatoo *et al.*, 2013). Boron is one of the trace minerals that always bind with oxygen to generate both borate minerals (borax, ulexite, and colemanite) and orthoboric acid. Boron is a trace mineral that is found in small amounts in the environment (Wolska and Bryjak, 2013). Borates, boric acid, boric oxide, and salts are all examples of compounds that may be found in the environment in either solid or liquid form. Studies have shown that Boron has effects on the metabolism of minerals (calcium and phosphorus), vitamin D, enzymes (aldehyde dehydrogenase, xanthine oxidase, cytochrome b5 reductase), hormones {insulin, estradiol (estrogen), testosterone (T3, T4)}, energy substrates (triglycerides, glucose), as well as reactive oxygen species,

and that it may act as a metabolic (**Ince *et al.*, 2010**). Boron is introduced into the aquatic environment through a variety of sources, including boron mining, sewage effluents, coal combustion, agrochemicals, and boron-containing detergents, among others (**Soucek *et al.*, 2011**).

The toxicity of boron in animals has only been studied in a limited number of researches, with the majority of the known studies focusing on rats and humans. The median lethal concentration (LC<sub>50</sub>) of boron in aquatic animals had only been determined in a few species of fish, including salmon (*Oncorhynchus keta*), Japanese flounder (*Paralichthys olivaceus*), and mosquitofish (*Gambusia affinis*), and the values ranged from 5 to 3000 mg/L for freshwater fish and 40 to 74 mg/L for marine fish (**Li *et al.*, 2008**). It is important to know the median lethal concentration (LC<sub>50</sub>) since it is one of the fundamental tests that may help you understand how sensitive animals are to certain heavy metals and minerals.

Contamination of the aquatic ecosystem by industrial and agricultural pollutants may affect the health of fish, either directly by intake from the water or indirectly via their food. Because fish are an integral component of human life and are becoming an increasingly significant source of protein for humans, the population and health of fish are of considerable importance (**EL-Gazzar *et al.*, 2014**).

In surface waters, heavy metals can be found in various concentrations. However, human activity has led to an increase in their concentration in aquatic environments. Occasionally, aquatic animals have been exposed to amounts of these metals that were abnormally high for their environment (**Abdel-Warith *et al.*, 2011**).

Over the last few decades, cadmium (Cd), one of the most well-known and toxic aquatic environmental contaminants, has found its way into a wide range of human activities and products, including pigments, ceramics, plastics, - glasses, vehicle tires, and other synthetics. As a result, the amount of cadmium dispersed in the environment has increased. It is a very poisonous heavy metal that is widely regarded as one of the most dangerous metal pollutants on the planet today. Cd is an unneeded biological metal that is non-biodegradable and permanent; as a result, it poses a considerable risk to human and animal health due to its poisonous potential (**Al-Sawafi *et al.*, 2017**). Because even at low levels, cadmium (Cd) is poisonous to fish and can cause physiological problems including anorexia and damage to red blood cells, it accumulates in fish populations (**Khalesi *et al.*, 2017**). Cadmium builds up quickly in living things. As a result, its buildup is highly beneficial to the environment. Cadmium is toxic to fish, causing pathological alterations in several tissues and organs (**Alak *et al.*, 2013**).

The current study is aimed to investigate the acute toxicological effect of the boron and cadmium on the liver of Nile Tilapia fish, *Oreochromis niloticus*.

## MATERIALS AND METHODS

### Experimental design and toxicity study

Apparent healthy Nile Tilapia fish were purchased from a private farm in El Qantara and transported to the laboratory where they were acclimated in water tanks of 300 L for seven days to allow them to recover from the stress of capture and transportation. The tests of acute toxicity of boric acid ( $H_3BO_3$ ) and cadmium chloride ( $CdCl_2$ ) were performed at the Physiology Laboratory of the National Institute of Oceanography and Fisheries (NIOF) - Suez and Aqaba Gulfs Branch. Following this period, the fishes were employed in the tests as well. The weight of the used fish was  $50 \pm 15$  g. The experiment was carried out in 20 glass aquaria with a total volume of 80 L. Artificial aeration was provided in all aquaria, and the stocking density was maintained at ten fish per aquarium at all times. They were in a static system for a total of 96 hours.

A randomized design was employed with six different concentrations including control of boric acid experiment (0, 1, 20, 40, 80, and 500 mg/l) and five different concentrations including control of Cadmium experiment (0, 15, 30, 60, and 90 mg/l). It was necessary to monitor the animals' behavior and mortality rates every hour for the first 12 hours, then every 6 hours until the experiment was completed (96 hours).

Dead fish were removed as soon as death was confirmed. At the end of the exposure interval, final mortality records were made in all aquariums. In a tabular format, the number of dead fish in each group was recorded against the time of their death, as instructed by **Sprague (1973)**. The 96-hour  $LC_{50}$  values of boric acid and cadmium chloride were computed using the Karber arithmetic approach, as adopted by (**Dede and Kaglo 2001**).

### Histopathological procedures

The treated fish were anesthetized and dissected as soon as possible. Fish liver tissue samples were removed and chopped into small pieces after being excised. Liver-tissue was fixed rapidly in 10% formalin for 24 hours, washed under running tap water, dehydrated in ascending grades of ethyl alcohol (70, 80, 90, 95%) until it reached absolute ethyl alcohol (100%), cleared in methyl benzoate for one day, washed in xylene two times 15 minutes for each, and embedded in four changes of pure paraffin wax. A series of 5 micron-thick transverse slices of all selected organs were cut and placed on clean glass slides stained with hematoxylin (Harris HX) and eosin, cleaned in xylene and mounted in DPX (**Drury and Wallington, 1980** and **Mohamed, 2009**).

Damage **stage I**: which affects normal function, **stage II**: which affects normal function more severely and **stage III**: is the most severe stage and causes the most damage to the tissue. Degree of Tissue Change (DTC) was assessed according to the following formula:

$$\text{DTC} = (\text{SI}) + (\text{SII}) + (\text{SIII})$$

Where: **I**, **II** and **III** represent the number of damage stage; and **S** represents the total of alterations in each stage. Histopathological alterations are categorized into three severity levels. For **stage I**: look for signs of vacuolization and inflammation, as well as the appearance of fat cells (steatosis); for **stage II**: which represents degeneration of the cytoplasm Degeneration of the nucleus Pyknotic nuclei and dilation of vessels for. DTC was determined for each individual.

The Degree of Tissue Change (**DTC**), which is based on the severity of the lesions, was used to assess the presence of histological alterations in hepatic tissue. Changes to the tissue were divided into three stages, each with increasing severity and potential for irreversible up of fat cells (steatosis),

To determine the average index, we used the DTC value that we acquired. While numbers between 0 and 10 show healthy functions, those between 11 and 20 suggest minor modifications, those between 21 and 50 indicate significant changes, those between 51 and 100 denote serious lesions, and those above 100 denote irreparable damage (**Poleksic and Mitrovic-Tutundzic, 1994**).

## RESULTS

It was observed that the acute toxicity of Boron and Cadmium is exactly proportional to the concentrations of the investigated materials, but the percentage of death is essentially non-existent in the control group (**Tables, 1 & 2**).

**Table 1. Boric acid concentrations and mortality rate of *Oreochromis niloticus* on time (24-96 hrs)**

CONC. (mg/l)	24 hours		48 hours		72 hours		96 hours	
	% Alive	% Dead	% Alive	% Dead	% Alive	% Dead	% Alive	% Dead
<b>Control</b>	100	0	100	0	100	0	100	0
<b>1</b>	100	0	100	0	100	0	100	0
<b>20</b>	100	0	100	0	100	0	100	0
<b>40</b>	100	0	100	0	100	0	100	0
<b>80</b>	100	0	100	0	100	0	100	0
<b>500</b>	100	0	60	40	20	80	0	100

LC<sub>50</sub> value was determined as follows:  $LC_{50} = LC_{100} - \frac{\sum [(Mean\ death\ x\ Conc.\ Diff.) / No.\ of\ organisms\ per\ group]}{}$

Results revealed that the susceptibility of *O. niloticus* to the influence of Boron toxicity increased in mortality with an increase in concentration, whereas mortality was nearly nonexistent in the control group of animals. Following the Arithmetic approach of Karber adopted by (**Dede and Igbigbi, 1997**), the median lethal concentration (LC<sub>50</sub>) of

Boron to *O. niloticus* was determined to be 290 mg/l. However, cadmium exhibited no mortality for *O. niloticus* at the same time intervals (**Table, 2**).

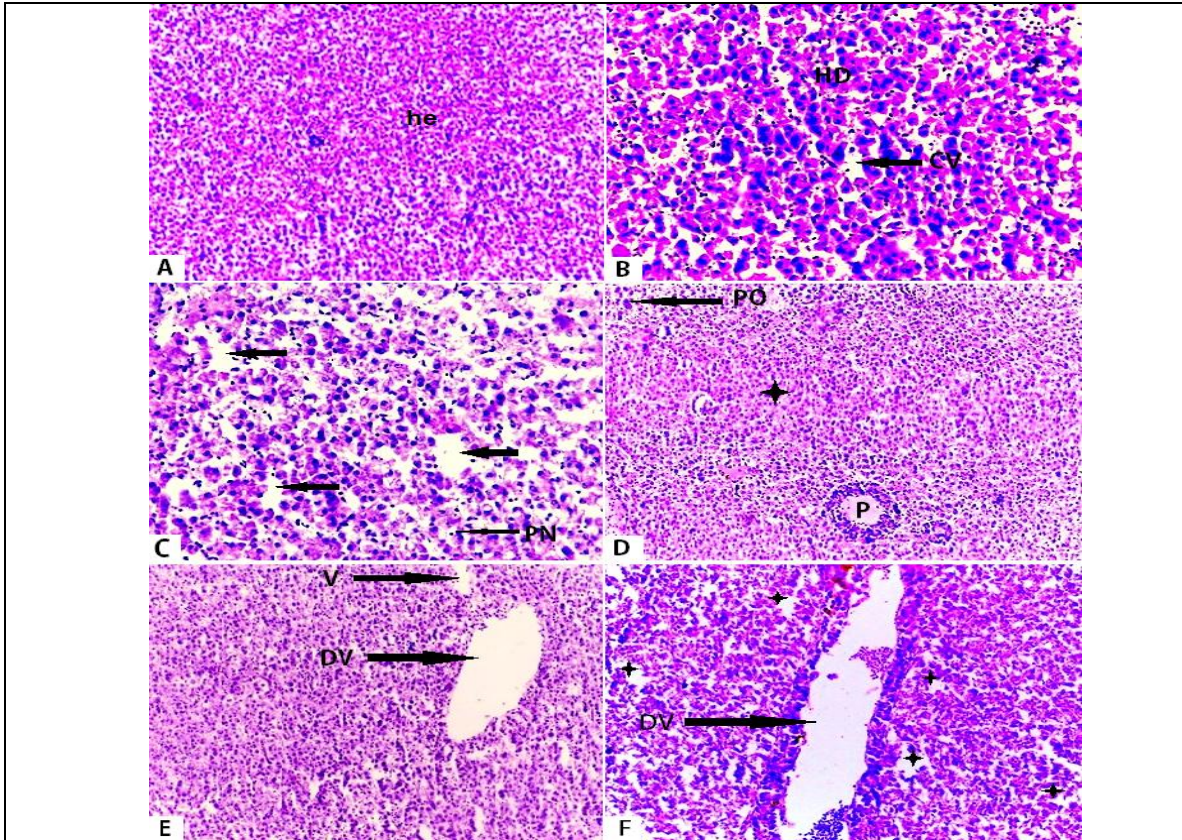
**Table 2. Cadmium concentration and mortality rate of *Oreochromis niloticus* on time (24-96 hrs).**

CONC. (mg/l)	24 hours		48 hours		72 hours		96 hours	
	% Alive	% Dead	% Alive	% Dead	% Alive	% Dead	% Alive	% Dead
<b>control</b>	100	0	100	0	100	0	100	0
<b>15</b>	100	0	100	0	100	0	100	0
<b>30</b>	100	0	100	0	100	0	100	0
<b>60</b>	100	0	100	0	100	0	100	0
<b>90</b>	100	0	100	0	100	0	100	0

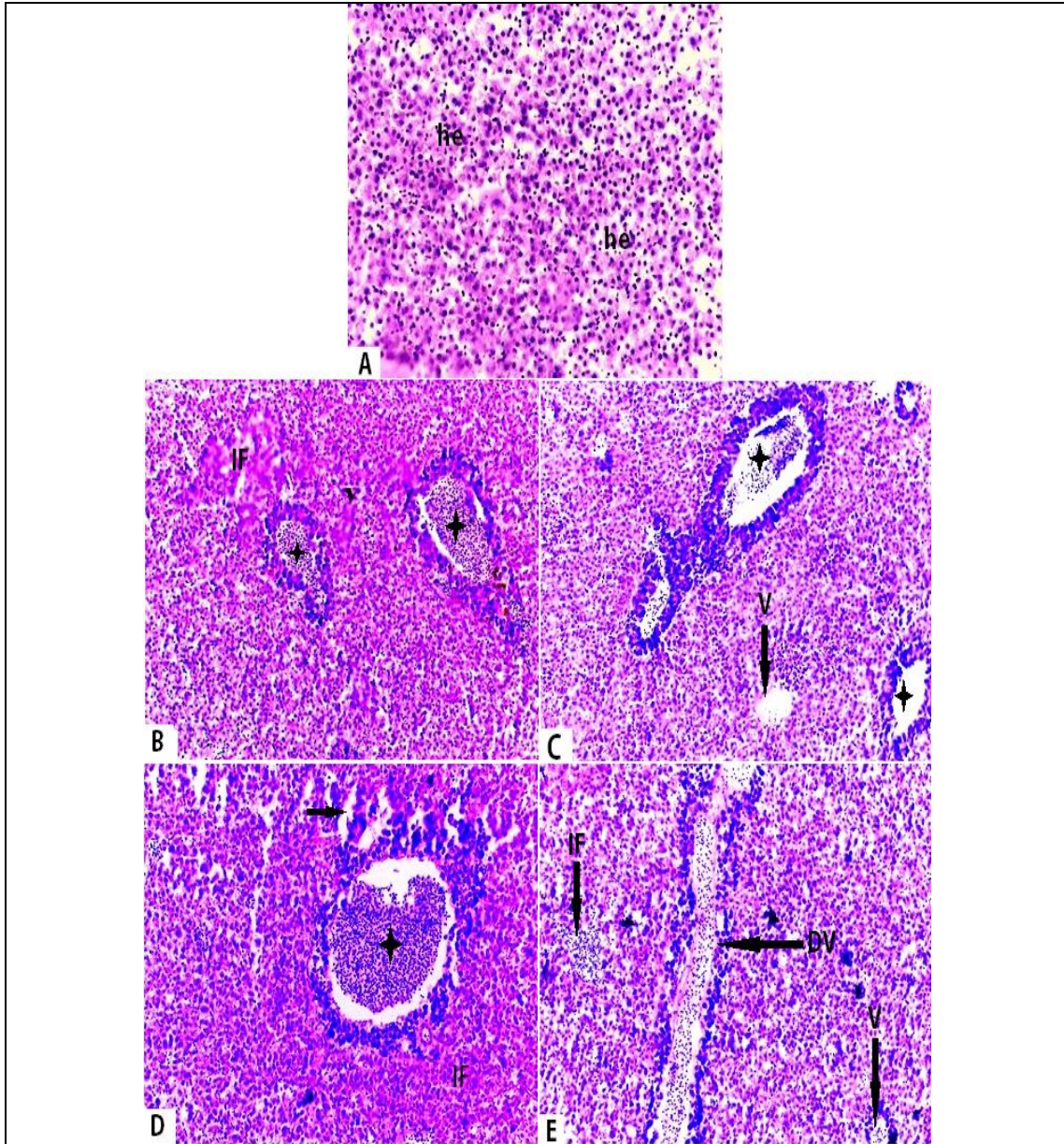
LC<sub>50</sub> value was determined as follows:  $LC_{50} = LC_{100} - \frac{\sum [(Mean\ death\ x\ Conc.\ Diff.) / No.\ of\ organisms\ per\ group]}{}$

Liver tissue of *O. niloticus* control group showed normal hepatic structure. Liver tissue of *O. niloticus* exposed to 1 mg/l Boric acid showed focal necrotic changes, cytoplasm vacuolation and hydropic degeneration. Liver tissues in fish specimens exposed to 2 mg/l Boric acid showed cytoplasm vacuolation and hepatocytes contain pyknotic nuclei. Liver tissue of fishes exposed to 40 mg/l Boric acid showed congestion of blood vessels and polymorphonuclear degeneration. Liver tissue of *O. niloticus* exposed to 80 mg/l Boric acid displayed dilation of blood vessel and hepatocyte vacuolation. While, liver tissues of *O. niloticus* exposed to 500 mg/l Boric acid revealed severe degeneration on hepatic structure with highly dilated vessel, necrotic tissue and lymphatic infiltration (**Fig. 1**).

Liver tissue of *O. niloticus* exposed to 15 mg/l Cadmium chloride showed infiltration of inflammatory cells and mild congestion of blood vessels. Liver tissue of fish specimens exposed to 30 mg/l Cadmium chloride showed severe congestion of blood vessels and vacuolization. Liver tissue of *O. niloticus* exposed to 60 mg/l Cadmium chloride showed degeneration in hepatic structure and cytoplasm vacuolization, infiltration of inflammatory cells and congestion of blood vessels. Liver tissue of fishes exposed to 90 mg/l Cadmium chloride displayed inflammation, vacuolization, and severely dilated vessels (**Fig. 2**).



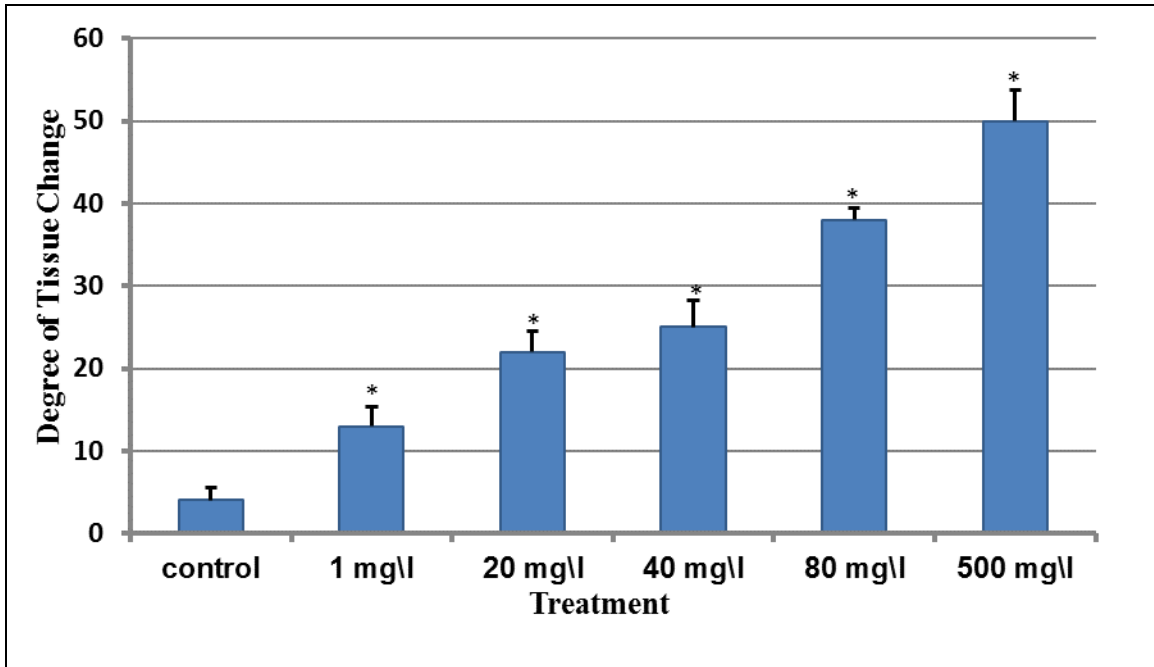
**Fig. 1. Histopathological photographs in liver tissues of *O. niloticus*.** (A) Control group. (B) Group exposed to 1mg/l Boric acid. (C) Group exposed to 2 mg/l Boric acid. (D) Specimens exposed to 40 mg/l Boric acid. (E) Specimens exposed to 80 mg/l Boric acid. (F) Group exposed to 500 mg/l Boric acid. (H&E; A, D, E 200X, B, C, F 400X). **CV:** cytoplasm vacuolation; **DV:** dilated vessel; **HD:** hydropic degeneration; **he:** hepatocytes; **P:** Pancreas; **PN:** pyknotic nuclei; **PO:** polymorphonuclear degeneration; **V:** vacuolation; \*: blood vessel congestion; focal necrotic changes (arrows).



**Fig. 2. Histopathological photographs in liver tissues of *O. niloticus*.** (A) Control group. (B) Group exposed to 15 mg/l Cadmium chloride. (C) Group exposed to 30 mg/l Cadmium chloride. (D) Specimens exposed to 60 mg/l Cadmium chloride. (E) Specimens exposed to 90 mg/l Cadmium chloride (H&E; 400X). **CV:** cytoplasm vacuolation; **DV:** dilated vessel; **HD:** hydropic degeneration; **he:** hepatocytes; **IF:** inflammatory cells; **P:** Pancreas; **PN:** pyknotic nuclei; **PO:** polymorphonuclear degeneration; **V:** vacuolation; **\***: blood vessel congestion; focal necrotic changes (arrows).

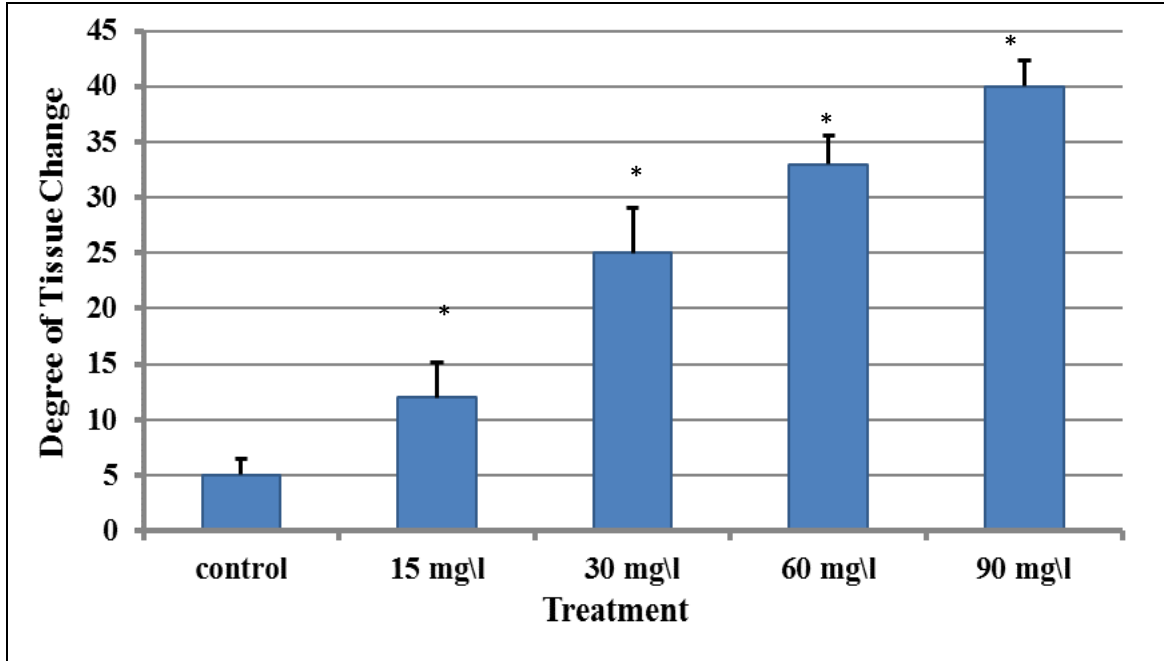
According to the histological index (DTC), the liver histology of the ten control specimens looked to be rather normal and was evaluated following the criteria listed in the index. The typical liver anatomy was readily evident, with just minimal congestion of the blood arteries in the vicinity. Under the index criteria, the histological features of the control specimen were regarded as normal.

The mean DTC for liver treated with boron (values ranging from 13 to 50), indicated that the hepatic lesions at low doses cause moderate damage to the tissue. However, the high dose of 500 mg/l causes significant lesion changes (**Fig. 3**). The DTC values for cadmium-treated groups were significantly higher as compared with control fish (**Fig. 4**). DTC values range from 12 to 40. The current results showed that boron had a higher toxic effect on hepatic tissue than cadmium.



**Fig. 3.** Degree of hepatic Tissue changes of *Oreochromis niloticus* treated with Boron (mean  $\pm$  SE). \*indicates statistical difference as compared with control; ( $P < 0.05$ )





**Fig. 4.** Degree of hepatic Tissue changes of *Oreochromis niloticus* treated with cadmium (mean  $\pm$  SE). \*indicates statistical difference as compared with control; ( $P < 0.05$ )

## DISCUSSION

The goal of this study was to see if the *O. niloticus* was susceptible to potentially harmful heavy metals and minerals like boron and cadmium. Boron's median lethal concentration ( $LC_{50}$ ) to *O. niloticus* was determined and found to be 290 mg/l after 96 hours of exposure, whereas Cadmium's median lethal value ( $LC_{50}$ ) was zero. Increases in Boron concentration and exposure time resulted in a higher percentage of death.

**Taylor et al. (1985)** investigated the effects of a range of metals on *Limanda limanda* and discovered an 88.3 mg B/l 24h-  $LC_{50}$  concentration. **Thompson et al. (1976)** conducted static renewal tests on sub yearling and alevin Coho salmon (*Oncorhynchus kisutch*) using saltwater and sodium metaborate. The 96h-  $LC_{50}$  was determined to be 40.0 mg B/l. **Hamilton and Buhl (1990)** used boric acid to conduct static acute toxicity studies on Coho salmon in brackish water to determine the 96h-  $LC_{50}$  at 600 mg B/l. When they tested Chinook salmon (*O. tshawytscha*), they got similar findings.

The acute toxicity of boron compounds to fish, as measured by median lethal concentrations ( $LC_{50}$ ) at 96 hours, ranges from 14.2 mg/l in zebra fish to 978 mg/l in mosquito fish, according to published data (**Loewengart, 2001**). Furthermore, **Topal et al. (2016)** calculated the median lethal concentration ( $LC_{50}$ ) of boric acid for juvenile rainbow trout (*Oncorhynchus mykiss*) after 96 hours, and the result was 138 mg/l. The researchers also discovered that borax has acute toxicity in rainbow trout, with the lethal

concentration (LC<sub>50</sub>) being 1 g/l after 96 hours. As previously stated, **Acar *et al.* (2018)** observed that the median lethal concentration (LC<sub>50</sub>) for Nile tilapia after 96-hour exposure to boric acid was 141.42 mg/l (*O. niloticus*). **Suresh (2009)** observed that the median lethal concentration (LC<sub>50</sub>) for Nile Tilapia after 120 hours of exposure to cadmium chloride was (20.93mg/l) after 120 hours of exposure to cadmium chloride. In contrast, the results of (**Kaoud *et al.*, 2011**) on fish Nile tilapia (*O. niloticus*) revealed that the 96-hour LC<sub>50</sub> values of cadmium for fish were 40.533 mg/l. **Nursanti *et al.* (2017)** determined the 96-hour LC<sub>50</sub> of cadmium in Nile tilapia (*O. niloticus*) to be 7.53 mg/l, which was higher than the previous estimate. **Abd-Allah *et al.* (2019)** also observed that the fatal concentration of CdCl<sub>2</sub> for fish Nile tilapia was (132 mg/l) at the 96-hour lethal concentration (96- LC<sub>50</sub>) (*O. niloticus*). On the other hand, the results of (**Paulo *et al.*, 2020**) on juveniles of the fish *O. niloticus* indicated that the fish had a 96-hour LC<sub>50</sub> value of 1.8 mg/l for cadmium. Other research' toxicity reports varied from this one, most likely because of the diverse species employed, age, organism size, test methodologies, and water quality, such as water hardness, which might impact toxicity (**McCahon and Pascoe, 1988**). **Garcia-Santos *et al.* (2006)** revealed that Nile tilapia (*O. niloticus*) 96 h LC<sub>50</sub> of cadmium chloride was 24.66 mg/L, their results support the hypothesis that Nile tilapia (*O. niloticus*) can tolerate high levels of waterborne cadmium because they found no significant changes in some parameters between control and Cd treated groups.

Many hazardous compounds are metabolized, digested, and stored in the liver; this includes those that are harmful to fish. Studies on the toxicological consequences of aquatic animal exposure to environmental toxins can be conducted using fish liver histopathology as a kit indicator (**Nelson and Sheridan, 2006**).

It is uncommon for a single toxicant to be linked to only one kind of histopathological pathology. Some various environmental conditions and variables can affect the histological state of aquatic species. Most of these variables may be controlled or even eliminated in the laboratory, allowing the researcher to look for species- and metal-specific lesion-specific information (**Overstreet, 1988**). This can only be achieved if all metals' toxicological effects have been studied. The results of this investigation showed that simultaneous exposure to different concentrations of boron and cadmium *O. niloticus* showed a clear toxic reaction in the histological structure of the exposed liver when compared to control specimens.

As a result of adding boric acid (BA) to their food, (**Oz *et al.*, 2020**) discovered the presence of hydropic and vacuolar degenerations in the liver of rainbow trout (*Oncorhynchus mykiss*), as well as an increase in the number of bile ducts and mononuclear cell infiltrations in portal areas, while necrosis was rarely seen. As a result, it was hypothesized that vacuolar degenerations would result in liver macroscopic growth.

Exposure to cadmium may produce histopathological alterations in numerous tissues and organs, making histopathological investigation of certain target structural organs an efficient approach to researching and diagnosing acute and chronic illnesses (Paulo *et al.*, 2020). The Cadmium chloride-treated group revealed infiltration of inflammatory cells, slight congestion of blood vessels, vacuolization, and hepatic structure degeneration in the current study, whereas the control group had normal hepatic cord structure and hepatocytes. Cadmium can cause vacuolization of hepatocytes and vascular congestion in the liver (Van Dyk *et al.*, 2007 and Samanta *et al.*, 2018). Furthermore, vacuolated hepatocytes, irregular shape of hepatocytes, blood vessel congestion, and blood cell infiltration were all seen in the liver of cadmium-treated fish (Paulo *et al.*, 2020). Kaoud *et al.* (2011) observed congestion as well as significantly damaged and necrosis of hepatocytes in the livers of cadmium-treated tilapia. Furthermore, after cutting through the gill, (Kaoud *et al.*, 2011) discovered extensive fatty vacuolations, severe necrosis of hepatocytes and congestion of liver sinusoids, lymphocytic filtration, and congestion of the central vein in cadmium-exposed fish. Gaber (2007) found considerable rupture of the hepatic cords, cell membrane breakdown, and hyperplasia of nuclei with eosinophilic granular cytoplasm, as well as fibrosis in the cadmium-treated group's liver (Abbas *et al.*, 2019). Suresh (2009) discovered extensive hazy edema, necrotic changes, vacuolar degeneration, and infiltration of mononuclear cells in-between hepatocytes with activation of melanoma-macrophage centers. In addition, Mahrous *et al.* (2015) discovered cytoplasmic vacuolization of hepatocytes and hepatopancreas necrosis in cadmium-treated fish liver sections. According to Younis *et al.* (2013) cadmium-treated fish's liver revealed increased vacuolation in hepatocytes, damaged nuclei, and dilatation of sinusoids.

## CONCLUSION

Results from the present study indicated that median lethal concentration (LC<sub>50</sub>) of boron to *O. niloticus* was 290 mg/l after 96 hours of exposure and the toxicity value of cadmium cannot be estimated under our conditions and that may be due to the variation of many factors like age, species, and environmental condition. The results also showed that both Cadmium Chloride and boric acid have a histopathological effect in fish liver such as infiltration of inflammation cells, congestion of blood vessels Degeneration in hepatic structure and Cytoplasm Vacuolization.

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