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Detection of Heat Shock Protein (*Hsp70)* Gene Expression, Some Physiological and Histological Alterations in the Nile Tilapia (*Orechromis niloticus*) Treated with Boric Acid and Cadmium

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ABSTRACT

The impact of both boron (H_3BO_3) and cadmium ($CdCl_2$) on some physiological and histological parameters was assessed on the Nile tilapia (*Oreochromis niloticus*). Six treatments including a control were used, with concentrations of 29mg/ L and 72.5mg/ L for boric acid, a concentration of 6.25mg/ L for cadmium, and combinations of (B 29mg/ L + Cd 6.25mg/ L) and (B 72.5mg/ L + Cd 6.25mg/ L). The chronic influence of boron and cadmium on *heat shock protein* (*Hsp70*) in musculature, liver function, antioxidant enzymes, and histopathology of liver and gills were studied. The relative expression of HSP70 in B_{29}, $B_{72.5}$, B_{29}+Cd, $B_{72.5}$+Cd, and Cd was P= <0.0001, P= 0.0018, P= 0.0198, P= 0.4573, and P= 0.9814, respectively, in comparison with the control group. ALT, AST, and ALP enzymes activities demonstrated significant differences between treatments. Liver antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities showed significant differences between treatments. Histopathological investigation of fish liver and gills in treated groups revealed severe hemorrhage and congestion of sinusoids for the liver. Moreover, the investigation showed severe lamellar lifting and lymphatic infiltration in the gills. In cadmium-treated groups, the liver showed moderate congestion and lymphatic infiltration, and the gills showed moderate raising of lamellae epithelial and mild lamellar aneurysm.

INTRODUCTION

Industrial and agricultural contaminants that have ruined the aquatic ecosystem may have an effect on fish health, either directly through consumption of the water or indirectly through their diet. Fish populations and health are immensely significant since they are an essential part of human existence and a progressively more important source of protein for humans **(EL-Gazzar *et al.*, 2014)**.

Boron (B) is a trace element naturally occurring in rocks, soils, and natural streams. It is found in many shapes, such as borates, boric acid, boric oxide, and sodium, calcium, and magnesium salts. The most common type of boron available is the boric acid (H_3BO_3) (**Kabu & Akosman, 2013)**. It is a vital mineral for all creatures. Furthermore, it is a necessary micronutrient for many biological processes, such as the activity of enzymes; bone growth; immune response; mineral and endocrine metabolisms; hormone functioning; brain function; improvement of arthritis, and diminishing cancer risk,; however, it becomes toxic in the aquatic environment when its levels are too high **(Kabu & Akosman, 2013; Gülsoy *et al*., 2015; Uluisik *et al*., 2018)**.

Cadmium (Cd) is considered as one of the notorious and detrimental aquatic environmental toxins; it has a variety of human endeavors and products, such as pigments, glasses and plastics. As a result, the environment now contains higher levels of cadmium. It is regarded as a highly toxic heavy metal, and it is currently among the most hazardous metals contaminants; moreover, it is regarded as a non-biodegradable or durable metal. Consequently, it has a substantial risk to both human and animal health **(Al-sawafi *et al*., 2017)**. Cd has many physiological alterations such anorexia and detriment to red blood cells despite its existence with low amounts in fish and causes histopathological changes in various tissues and organs of fish **(Khalesi *et al*., 2017)**.

Heat shock responses were first identified by Ritossa in 1962 after being expressed as *heat shock protein*s (*Hsp*s); they were largely synthesized after being discovered in house fly. It has been observed that *Hsps* could be found in all the creatures from bacteria to humans **(Dubey *et al*., 2015)**. *Hsps* provide a protective role and aid in cell healing after cellular injury; it is considered as a crucial biomarkers for a variety of environmental stresses and pollution, since each time an organism is exposed to contaminants, their expression patterns and physical characteristics change (**Golli-Bennour & Bacha, 2011; Lauritano *et al*., 2012; Li *et al*., 2015)**.

Fish have many biochemical parameters, which are sensitive for the detection potential of various impacts of the metal bioaccumulations. Enzymes, such as aspartate and alanine aminotransferase (AST, ALT), and lactate dehydrogenase (LDH) are considered as beneficial biomarkers to detect pollution levels throughout chronic exposure and also an essential factor for checking water for toxicants existence **(Younis *et al*., 2012; Al-Asgah *et al*., 2015)**.

It is remarkable that oxidative stress of environmental contaminants causes an imbalance between reactive oxygen species (ROS) and the organisms' ability to manipulate them through many mechanisms. The dynamic equilibrium could be disturbed conducive to enhanced ROS' level and detriment to cellular component (DNA, lipids and proteins), which is called "oxidative stress" **(Obaiah *et al*., 2020)**.

Pollutions that are happening by chemical toxicants are the main sources of ROS in all biological systems, which impede the activity of some enzymes of the antioxidative defence system, being the first cause of tissue damage that are decreased by antioxidant enzymes, such as catalase (CAT), superoxide dismutase (SOD), endogen glutathione (GSH), and glutathione S-transferase (GST) **(Alak *et al*., 2013)**.

Histopathological procedures are a sensitive, dependable, low-cost, and easy instrument that is frequently utilized in disease diagnosis and other pathologic problems. It shows the direct influence of toxins on architecture, as well as on cell and tissue morphology. Hence, it is considered a significant tool in toxicity studies of environmental pollutants in aquatic ecosystems (**Vidya & Chitra, 2018**).

The present study was designed to investigate some boric acid and cadmium influences on *hsp70*, liver function, antioxidant enzymes in musculature, and histological changes in both liver and gills.

MATERIALS AND METHODS

Experiment design

The Nile tilapia fish (50± 15g) were purchased from a private farm at El Qantara era and then were delivered to fish physiology laboratory at the National Institute of Oceanography and Fisheries, Suez and Aqqaba Gulfs Branch. Fish were acclimatized in plastic water tanks of 300L for seven days to recover from the stress of capture and transportation. Acute toxicity tests of boric acid (H_3BO_3) and cadmium chloride ($CdCl_2$) were carried out to attain the LC_{100} and LC_{50} concentrations (96hrs) for the calculation of the sublethal concentration, which was used in the current chronic study. Based on our previous study **(Abdel Aliem *et al*., 2022**), sub lethal concentration of boron and cadmium was used. Six different concentrations were employed including a control, using concentrations of 29mg/ L and 72.5mg/ L for boric acid, a concentration of 6.25mg/ L for cadmium, and combinations of (B 29mg/ L + Cd 6.25mg/ L) and (B 72.5mg/ L + Cd 6.25mg/ L). Experiment was performed in 18 glass aquariums of 80L each. Artificial aeration was provided in all aquariums. The fish were stocked at a density of 10 fish/ aquarium, and they were in a static system for two months. Water quality parameters were measured with readings of 0.14ppt for salinity, 7.295 for pH, 5mg/ L for dissolved oxygen and 26°C for temperature.

Expression of *hsp70* gene

Total RNA isolation

Total RNA was isolated from fish tissues by the standard TRIzol® reagent extraction method (cat#15596-026, Invitrogen, Germany), as indicated by **Abdel-Gawad *et al*. (2020)**.

Reverse transcription (RT) reaction

Complete poly (A) $^{+}$ RNA isolation was performed from fish tissue specimens, followed by reverse transcription to cDNA using the RevertAid^{TM} Synthesis Kit in a total volume of 20µl.

Quantitative real time-polymerase chain reaction (qRT-PCR)

StepOne™ real-time PCR purchased from Applied Biosystems (Thermo Fisher Scientific, Waltham, MA USA) was employed to determine the copy number of target genes in the fish tissue samples. The PCR reactions were established in 25µl reaction mixture, which included 12.5µl of 1× SYBR® Premix Ex TaqTM (TaKaRa, Biotech. Co. Ltd.), 0.5µl of 0.2µM sense primer, 0.5µl of 0.2µM antisense primer, 6.5µl distilled water, and 5µl of cDNA template. Each experiment contained a blank distilled water as a control. The specific primer for each utilized genes' sequences are listed in Table (1). By the end of each qPCR, a melting curve analysis was done at 95.0°C to test the quality of the primers used. The scoped relative quantitation to the reference was investigated by utilizing the $2^{-\Delta\Delta CT}$ method.

Table 1. Primer's sequence used for *qRT-PCR*

Gene	Primer sequence	NCBI reference
Hsp70	F: TCA CCA CCT ACT CCG ACA AC	FJ207463.1
	R: CCA CCG CAG ACA CAT TCA AA	
Gapdh	F: GGA TAC ACA GAG CAC CAG GT	XM_005455438.3
	R: GTT CAG CGA CAC CCA AGT TT	

Hsp70: heat shock protein 70; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

Fish liver functions

Aspartate amino transferase (AST), Alanine aminotransferase (ALT), and Alkaline phosphatase (Alp) activities determination

AST and ALT activities were determined according to the method described by **Reitman and Frankel (1957)**. Moreover, the results were expressed as milliunit/ ml. The

ALP activity was defined by using the method of **Garen and Levinthal (1960)**. Furthermore, the results were expressed as milliunit/ ml.

Determination of fish liver antioxidant enzymes

Liver tissue glutathione peroxidase (GPx) activity was determined by the method indicated by **paglia and valentine (1967)**. Moreover, the superoxide dismutase (SOD) activity was estimated following the method of **Beutler (1984**), and catalase (CAT) activity was estimated by method of **Jimenez *et al*. (2009)**. The results were expressed as unit/ ml.

Histological analysis of liver and gills

Specimens of liver and gills were preserved in a suitable fixer (formalin 10%) for histological analysis according to the method of **Drury and Wallington (1980)**. Specimens were then sectioned by microtome at the thickness of 4 microns and stained with hematoxylin and eosin.

The organ index (Iorg), which is relied on the intensity of the lesion was utilized to objectively evaluate the existence of histological changes for each organ following the method of **Bernet *et al*. (1999)**.

Ethical approvement

The experiment was conducted under the ethical approvement of the Institutional Review Board Statement of Suez University No.141123.

RESULTS

1. Expression of *hsp70* gene

The relative expression of *hsp70* in B_{29} (i.e., boron with concentration 29mg/ L) group showed highly significant differences (P= <0.0001) compared to the control group. The relative expression of *hsp70* in $B_{72.5}$ (i.e., boron with concentration 72.5mg/ L) group paled significant differences (P= 0.0018), as did the cadmium group (P= 0.0198) in comparison with the control group. Relative expression of *hsp70* gene in B_{29}+Cd and $B_{72.5}$+Cd treatments showed no significant differences (P= 0.4573 and P= 0.9814, respectively), as compared to control group.

2. Liver functions

For the liver function enzymes, the result of ALT showed high significant differences in B_{29} (P= 0.0003) treatment compared to the control group, and there was also a significant differences in $B_{72.5}$ (P= 0.0494) treatment compared to the control group. Moreover, no significant differences were detected in B_{29}+Cd (P= 0.4381), $B_{72.5}$+Cd (P= 0.1065), and Cd (P= 0.4934) treatments compared to the control group. Regarding the AST result, there was a high significant difference in B_{29} (P= 0.0003) treatment as compared to the control group. Moreover, there was a significant difference

in B_{29}+Cd (*P*= 0.0304) compared to the control group; it was noted, that there were no significant differences in $B_{72.5}$ (*P*= 0.7476), $B_{72.5}$+Cd (*P*= 0.9492), and Cd (*P*= 0.1961) treatments with the control group. In ALP, results showed very highly significant differences in B_{29} (*P*= 0.0001) treatment compared to the control group, and there was also a significant difference in B_{29}+Cd (*P*= 0.0172) compared to the control group. However, there were no significant differences in $B_{72.5}$ (*P*= 0.1388), $B_{72.5}$+Cd (*P*= 0.0647), and Cd (*P*=0.1769) treatments with the control group.

3. Antioxidant enzymes activities

Results showed a significance change between treatments in CAT activity in B_{29}, $B_{72.5}$+Cd, and Cd groups (*P*= 0.0263, *P*= 0.0289, and *P*= 0.0060, respectively) compared to the control group, and there were no significant differences between $B_{72.5}$ and B_{29}+Cd treatments with the control group. GPx activity was observed to show a high significant difference between B_{29} (*P*= 0.0002) and the control group. In addition, significant differences appeared in B_{29}+Cd, $B_{72.5}$, and Cd treatments (*P*= 0.0126, *P*=0.0060, and *P*= 0.0102, respectively) and the control group. The SOD activity revealed that there was a significant difference between B_{29} (*P*= 0.0096) and Cd (*P*= 0.0250) treatments and the control group, while there were no significant differences between B_{29}+Cd, $B_{72.5}$+Cd, and $B_{72.5}$ groups (*P*= 0.3079, *P*= 0.3460, and *P*= 0.1520, respectively) and the control group.

4. Histological studies of chronic experiments

4.1. Gills

Alterations in histomorphology were observed in all boron-treated groups. In contrast, control fish gills showed no discernible alterations. Each gill consists of a primary filament and secondary lamellae (Fig. 8). One or two layers of thick cells make up the main lamellar epithelium. Moreover, the main lamellar epithelium displayed the chloride cells. Fish treated with cadmium (6.25mg/ L) in the gills displayed moderate raising of lamellae epithelial and mild lamellar aneurysm. Furthermore, the gills in fish which were exposed to boron (29mg/vl) showed severe lamellar lifting as well as lymphatic infiltration. Additionally, fish exposed to boron (29mg/ L) + cadmium (6.25mg / L) exhibited severe lamellar fusion and lamellar disorganization besides moderate lamellar hyperplasia. *O. niloticus* gills tissue exposed to boron (72.5mg/ l) displayed sever lamellar hyperplasia and fusion. For those fish that were exposed to boron (72.5mg/ L) + cadmium (6.25mg/ L) severe degeneration of gill lamella and necrosis (The epithelium is completely interrupted as a normal consequences of cell-lysis) were showcased.

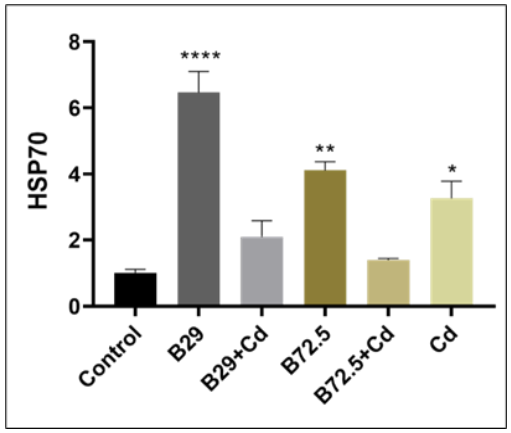

Fig. 1. Sub- lethal levels of boron and cadmium's effect on *hsp*70

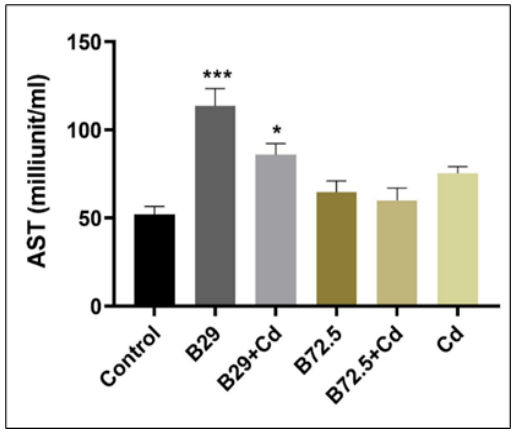

Fig. 2. Sub- lethal levels of boron and cadmium's effect on liver function

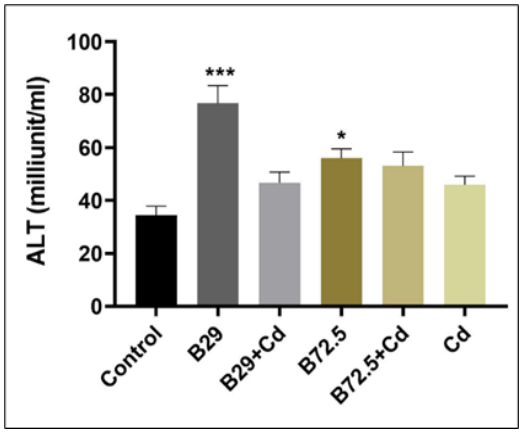

Fig. 3. Sub- lethal levels of boron and cadmium's effect on liver function

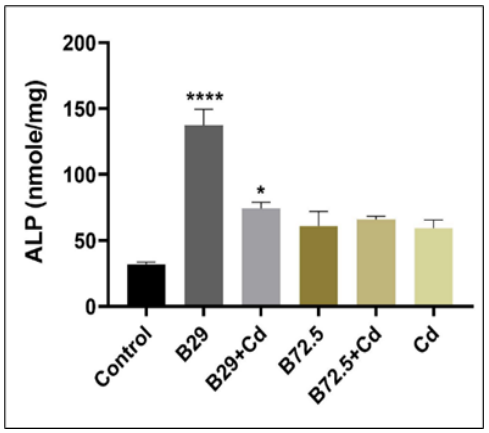

Fig. 4. Sub- lethal levels of boron and cadmium's effect on liver function **enzyme**

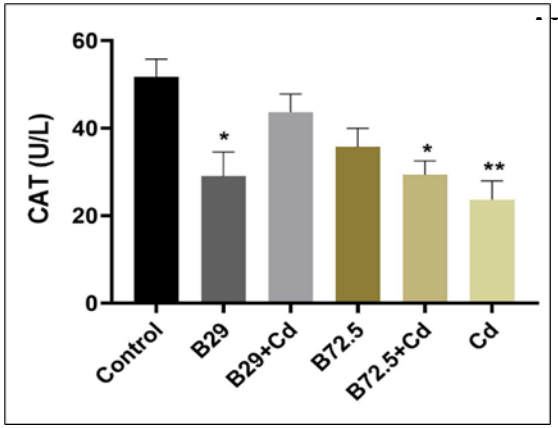

Fig. 5. Sub lethal levels of boron and cadmium's effect on liver antioxidant

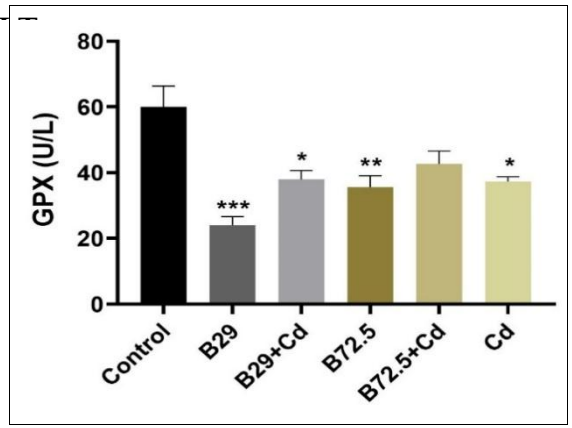

Fig. 6. Sub lethal levels of boron and cadmium's effect on liver antioxidant

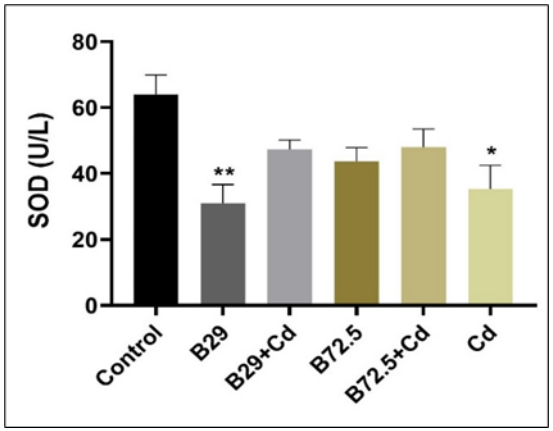

Fig. 7. Sub lethal levels of boron and cadmium's effect on liver antioxidant

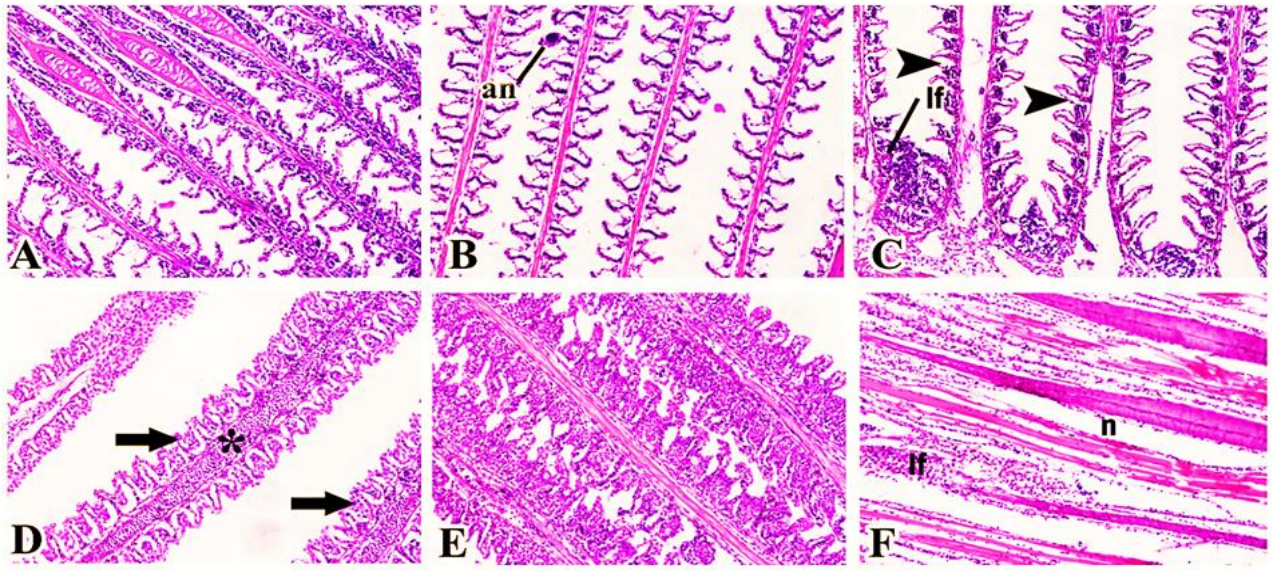

Fig. 8. Histological section of fish gills stained with hematoxylin & eosin showing: **(A)** Control group showing normal architecture of secondary lamellae originate at the superior and inferior surface of primary lamellae, **(B)** Cadmium (6.25mg/ L) treated group showing aneurysm (an) in the secondary lamellae (L2), **(C)** Boron (29mg/ L) treated group showing epithelial lifting (arrowhead) and leukocyte infiltration in the bases of primary lamellae (L1), **(D)** Boron (29mg/ L) + cadmium (6.25mg/ L) treated group displayed epithelial lifting (arrow) associate with intraepithelial edema, **(E)** Boron (72.5mg/ L) treated group showing architectural and structural alteration and epithelial hyperplasia with lamellar fusion, and **(F)** Boron (72.5mg/ L) + cadmium (6.25mg/ L) treated group showing necrosis (n) of lamellae associated lymphatic infiltration (lf)

4.2. Liver

The morphology of the control fish's liver hepatocytes was found to have a parenchymatous look. The hepatopancreas and bile duct separated the liver's irregularly formed lobules at the light microscopic level (Fig. 9). Normal hepatocytes had a polygonal shape, a spherical nucleus in the center, and a nucleolus that was heavily stained. Fish treated with cadmium (6.25mg/ L) in the liver displayed moderate congestion and lymphatic infiltration. Moreoever, fish liver tissues specimens exhibited to boron (29mg/ L) showed a severe haemorrhage as well as congestion of sinusoids. Fish exposed to boron (29mg/ L) + cadmium (6.25mg/ L) displayed severe congestion and hepatocytes hyperplasia besides the deposition of eosinophilic granules in cytoplasm. Furthermore, *O. niloticus* liver tissue of individuals exposed to boron (72.5mg/ L) displayed hyperplasia of pancreatic tissues and necrosis of hepatic tissue. For these *O. niloticus*, individuals exposed to (72.5mg/ L) + cadmium (6.25mg/ L) revealed severe degeneration denoted as cytoplasmic degeneration and cellular rupture along with hyperplasia of the pancreatic tissues and blood vessel congestion. Statistical analysis of organ indices for liver and gills were summarized in Table (2).

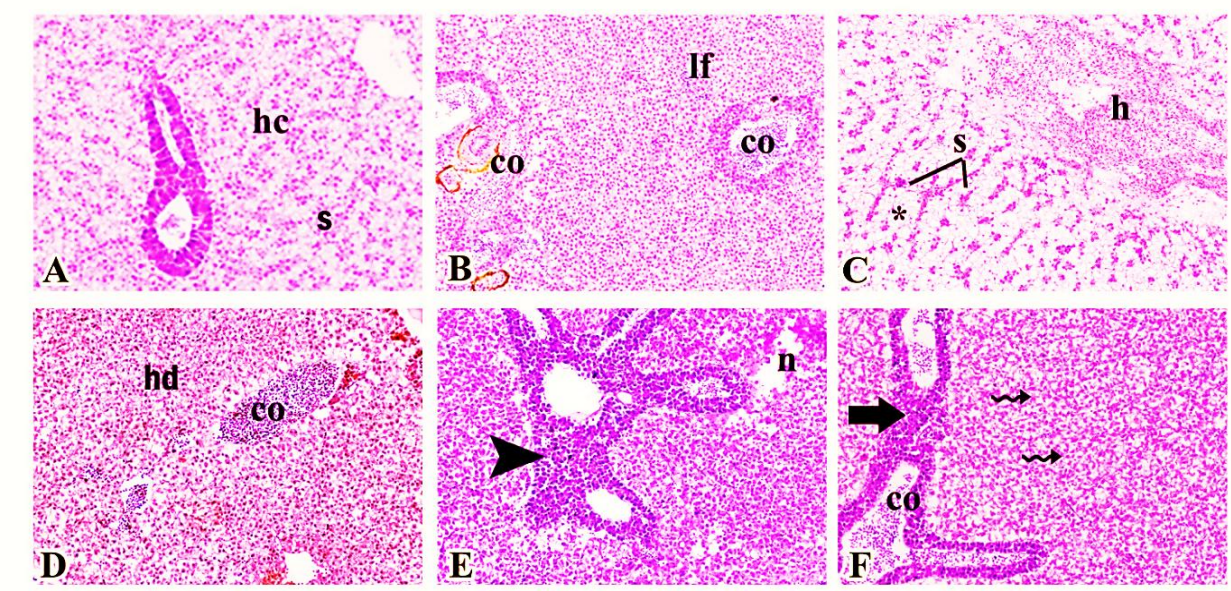

Fig. 9. Histological section of fish liver stained with hematoxylin & eosin showing: **(A)** Control group showing hepatocytes (hc), small darkly stained nuclei, sinusoids (s) and pancreas (p), **(B)** Cadmium (6.25mg/ L) treated group displayed, showing focal necrosis with lymphatic infiltration (lf) and congestion of blood vessels (co), **(C)** Boron (29mg/ L) treated group showed hemorrhagic areas (h), **(D)** Boron (29mg/ L) + cadmium (6.25mg/ L) treated group displayed congestion of blood vessels (co) and hydropic degeneration (hd) of hepatocyte, **(E)** Boron (72.5mg/ L) treated group displayed hyperplasia of pancreatic tissue (head arrow), and **(F)** Boron (72.5mg/ L) + cadmium (6.25mg/ L) treated group showing congestion of blood vessels (co), severe hydropic degeneration of hepatocytes (wavy arrow) and hyperplasia of pancreatic tissue (arrow)

Table 2. Organ indices (mean ± S.E) for gills and liver

Parameter	Control	Cd (6.25mg/l)	B (29mg/l)	B (29 mg/l) + Cd (6.25 mg/l)	B (72.5mg/l)	B (72.5mg/l)+ Cd (6.25 mg/l)
Gills index	ND	9±2.5	12±3.1	16±4.2	20±2.2	35±3.6
Liver index	ND	11.66±1.3	15.0±2.86*	22.2±1.37*	27.36±3.51	33.41±4.11

Data represent as mean ± S. E. (n= 6),
(*) refers to a significant difference between the control and other treated groups,
(ND) not found.

DISCUSSION

The objective of the study was to assess the sensitivity of *O. niloticus* to harmful heavy metals and minerals such as cadmium and boron. *Heat shock protein* 70 (*Hsp70*) levels were examined in individuals exposed to the highest concentration of boron (29mg/ L). As reported in **Capkin *et al.* (2017)**, borax has various effects on the

expression of the *hsp70* gene, and the transcription of the *hsp70* gene was significantly regulated due to borax exposure, leading to increased synthesis of *heat shock protein* 70 (*Hsp70*). *Hsp70* is produced in response to phsiological, environmental, and chemical exposures in both eukaryotes.

Haseeb *et al*. (2018) also revealed the impact of boron, but on the ostrich spleen and level of *hsp70* expression. They found that, *hsp70* expression level was first provoked at low-dose groups, and after that, it dramatically impeded in high-dose groups compared to the control group. At low doses of boron, the synthesis of *hsp70* increasesm affirming that *hsp70* is a beneficial and highly sensitive biomarker for boron exposure in the ostrich spleen. **AlaK *et al*. (2019)** evaluated the efficiency of borax against a different heavy metal exhibition on the *hsp70* gene expression, and they observed that *hsp70* decreased after treatment with borax. They suggested that borax itself is not a factor of antioxidations; however, it can support other antioxidants defence actions of fish are that interrupted by different heavy metals. Moreover, a significant increase in copper treated groups compared to control led to an increase in *hsp70*. In the studies of **Jing *et al*. (2013**), **Savassi *et al*. (2020)** and **Li *et al*. (2021)**, the effect of heavy metals (Cu, Cd) on *hsp70* gene expression in common carp (*Cyprinus carpio L*.) showed increased *hsp70* expression after exposure to cadmium and copper. The study investigated the expression of *heat shock protein* 70 in the Cyprinidae fish *Tanichthys albonubes* in response to copper and cadmium exposure. While, **Jiang *et al*. (2015)** reported that cadmium, copper, and its combination induced *hsp70* overexpression in common carp liver.

In the study of the effect of zinc nano particles on *hsp70* expression in liver and gill tissues of goldfish (*Carassius auratus*), it was noted that, *hsp70* gene expression was significantly increased in treated fish **(Harsij *et al*., 2018)**.

Since all chemical reactions that happened in different body cells are stimulated by enzymatic system and the entry of foreign chemicals in the cell generally disrupted most enzyme -in this system- functions, the enzymes activities alterations in fish have often been utilized as indicators for both intoxication and water pollution **(Mohamed *et al*., 2019)**. The commonly utilized markers of liver injury and stress indicator are ALT, AST, and ALP and are also known as serum aminotransferases **(Atli *et al*., 2015; Oztopuz *et al*., 2019)**. **Al-Asgah *et al*. (2015)** and **Abdelzaher *et al*. (2022)** found that ALT and AST levels raised significantly with the increase of Cd concentration in *Oreochromis niloticus*, which was exposed to environmental pollutants. In African catfish, *Clarias graiepinus* and common carp *Cyprinus carpio* exposed to cadmium where serum ALT and AST, ALP activities were increased **(Elarabany *et al*., 2019; Oz *et al*., 2020a; Aldoghachi *et al*., 2022)**, and this is compatible with our results.

Pawa and Ali (2006) investigated the role of boron in ameliorating fulminant hepatic failure by counteracting the alterations related to the oxidative stress; the results showed no alterations in the activity or levels of AST and ALP when the effect of boron alone was studied relative to the control group; rather a little reduction in ALT was

observed. Similarly, there was a reduction in AST and ALT activity of boric acid treated fish **(Ali *et al*., 2019)**. Furthermore, a substantial decrease in the activity of serum ALT, related to a non-significant change in serum AST activity, was observed in rats exposed to H_3BO_3 **(Ismail, 2022)**.

In our study, antioxidant enzyme activities were significantly decreased in all investigated treatments of boron and cadmium compared to the control group. The findings of **Ince *et al*. (2010)** and **Ince *et al*. (2014)** agree with our result upon investigating the boric acid and borax dietary supplementation influence on antioxidants activity in rats. **Cheng *et al.* (2021)** detected the influence of cadmium on antioxidant enzymes of mud crab (*Scylla paramamosain*). Moreover, studies by **EL-Gazzar *et al*. (2014)** and **Abd-Allah (2019)** focused on oxidative stress in *O. niloticus*.

On the contrary, **AlaK *et al*. (2019)** evaluated the borax efficiency against heavy metal exhibition on the antioxidant enzymes GPX, CAT and SOD; the result showed rises in antioxidant enzymes activity in borax and borax combined groups.

Batool *et al*. (2018) revealed that cadmium caused an increase activity of SOD and reduction in activity of CAT, and GPX in two fish species *Channa marulius* and *Wallago attu.* In the study of the antioxidant system of freshwater fish (*Oreochromis niloticus*) response when exposed to Cd, the results showed an increasing effect of CAT and decreasing activity of SOD and GPX **(Saglam *et al*., 2014)**. Activities of GPX and SOD in freshwater murrel (*Channa punctatus)* were elevated after exposure to cadmium, while the activity of CAT decreased **(Dabas *et al*., 2012)**, and the difference with our study results may be due to using different doses, species and conditions.

Liver has an essential role in various physiological and biochemical processes such as digestion and detoxification of pollutants in water **(Araújo *et al*., 2019**). Gills in fish also have crucial functions in the filtration of the waterborne pollutants due to their large surface area which are exposed to the external environment **(Barbieri *et al*., 2016)**.

Capkin *et al*. (2017) examined the possible effects of borax on the rainbow trout histopathology. They observed histological lesions in the liver and gills, including lamellar fusion, hyperplasia, and epithelial necrosis and pyknotic nucleus, as well as degradation in hepatic cell nuclei, fat vacuoles, and necrotic hepatocytes in liver of fish exposed to low doses of Borax. In this context, **Oz *et al*. (2020b)** studied histopathological changes in the rainbow trout (*Onchorhyncus mykiss*) consuming boric acid and observed lamellar edema, degenerative changes, inflammatory cell infiltrations in and severe lesion in gills. Hydropic and vacuolar degeneration in hepatocytes in the liver were slight to severe; moreover, mononuclear cell infiltrations, biliary hyperplasia in portal areas and necrotic changes in hepatocytes were observed. Furthermore, the histopathology of different (*O. niloticus*) tissues exposed to cadmium showed that there are many histopathological alterations in different tilapia's organs. Gills showed necrosis and atrophy of the gill lamellae, severe edema, fusion, hyperplasia, and focal desquamation of the epithelial lining of the secondary lamellae. The gills arch showed

many mononuclear leucocytic infiltration, edema, congestion, and the apex of gills filaments showed congestion, hyper activation of the mucous and chloride cells. Regarding the liver, histological observations revealed degeneration of hepatocytes with nuclear pyknosis in many cells, as well as the accumulation of the metal binding proteins in their nuclei (**Kaoud *et al*., 2011; Abbas *et al*., 2019)**. In this respect, **Nursanti *et al*. (2017)** and **Otludil *et al*. (2017**) found the same result in the histology of the gills of *O. niloticus* that were exposed to Cd. Alteration in secondary lamellae such as telangiectasis were observed in the gills of fish. Additional changes included lamellar epithelium lifting, epithelial hypertrophy, hyperplasia, fusion of epithelial lifting, as well as hyperplasia of the secondary lamellae.

CONCLUSION

The present study revealed some effects of boron and cadmium alone and their combination on the Nile tilapia (*O. niloticus*) regarding *heat shock protein 70* (*Hsp70*) gene expression, liver functions enzymes, and their histopathological effect in gills and liver. Data revealed that *hsp70* gene participated in response to environmental stressors and proved the importance of *hsp70* as biochemical markers. This study suggested that boron caused the inhibition of liver antioxidant enzymes, and B & Cd caused alterations in histopathology of liver and gills tissues.

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