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The role of *Saccharomyces cerevisiae* to alleviate copper sulfate toxicity in *Cyprinus carpio*

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Abstract

This study aimed to determine the role of *Saccharomyces cerevisiae* in alleviating copper sulfate toxicity in carp fish and by evaluating Acetylcholinesterase (AChE) activity and histopathological alteration in gills and brain with determined the severity of lesions in the gills by semi-quantities analysis. Sixty fish were divided into four groups first was a control group fish in the second group were exposed to $CuSO_4$ 0.53 mg/L, in the third group fish were treated with yeast 5 g/ kg ratio and the fourth group fish treated with yeast and exposed to $CuSO_4$. At the end of the experiment (56 days) it was observed that there was a significant decrease in the AChE activity in the second group, while this activity was improved significantly in the fourth group. The histopathological alteration in the brain was variable in the severity from infiltration of inflammatory, edema, and central chromatolysis of neuron cell bodies. At the same time, the lesions in the gills include edema, congestion, hydropic degeneration, hyperplasia in the mucus cells with shortening in the secondary gill filaments shaped like a drumstick, necrosis, and cartilage disruption. The semi-quantities lesions analysis in the gills classified them as mild, moderate, severe, and irreversible lesions. These lesions in both brain and gills were mild in severity in fish of group fourth. These studies conclude that the activity of AChE is a biological indicator for copper sulfate neurotoxicity and also causes damage to the brain and gills, and yeast is a bio adsorbent agent for copper

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Introduction

Copper (Cu) is an essential trace nutrient for animals and plants, with antioxidant features and regulation of antioxidant enzymes and tryptophan metabolism (1-4). It has other roles as well as protecting the cardiovascular and antidiabetics, reducing inflammatory chemical mediators, and improving activity against zinc oxide nanoparticles (5,6). Cu is one of the pollutants which are introduced into aquatic environments mostly from effluents discharged by industries, sewage treatment plants, and drainage from urban and agricultural regions or through it is application in aquaculture as a chemotherapeutic compound; cu is a time and dose-dependent disinfectant agent (7,8). Cu is one of the most hazardous elements to fish, affecting blood, development, behavior, enzyme function, reproduction, and others (9-11). Cu^{+2} toxicity occurs when metal ions attach to vital membranes, often outcompeting cations causing harm to the physiological process (12). Probiotics are live bacteria that, when given in sufficient proportions, provide a health benefit to the host and improve growth (13-15). *Saccharomyces cerevisiae*, or baker's yeast, includes immunostimulant such nucleic acids, β-glucans, and oligosaccharides, and it can boost the growth of a variety of fish species, increase fish resistance to environmental stress factors (16,17). Acetylcholinesterase (AChE) has a primary biological role in inhibiting acetylcholine, affecting the equilibrium and locomotion of exposed organisms, which is associated with neurotoxicity (18).

Due to copper sulfate's therapeutic trait, which made it one of the primary drugs for use in fish aquaculture despite its toxic effects and shortage of focused studies on nervous and evaluation of AChE, so this study aims to evaluate the histopathological effects of sub-lethal concentration of $CuSO_4$ on *Cyprinus carpio* and estimation AChE.

Materials and methods

Ethical prove

Scientific Ethical Committee on Animal Experimentation at College of Veterinary Medicine, University of Mosul, UM.VET.2022.010.

Trail diet preparation

Stander diet was formulated 34% protein and 10% lipid, and baker's yeast *Saccharomyces cerevisiae* was added to the basil diet as 0 g/kg control diet and 5 g/kg treatment diet (19). The ingredients were combined individually with an additional 100 ml of water/kg diet to form a paste of each diet. The pastes were ground individually and then pelletized at 1 mm diameter in a paste extruder. For later usage, the diets were air-dried and stored in plastic bags in a refrigerator.

Fish rearing and acclimation

Sixty *Cyprinus carpio* (20,21) 75±10 gm was brought from fish hatcheries in Erbil government. Fish were put in aerated -plastic bags and transported to the fish unit College of Veterinary Medicine, University of Mosul -Iraq. Fish were kept in an indoor fiberglass tank system (40*40*60) cm for at least 10 days for acclimation to the laboratory condition. Fish were fed with a commercial diet, with a natural light cycle 12-hour light/12 hour dark and replacement of about 30 cm of fiberglass water with aeration and dechlorinated water /day with kept water quality at pH 7.1-7.5, water temperature 22±2°C and dissolve oxygen concentration 7 mg/L.

Experimental designs

Fish were divided into four groups, with three replications for each group. The first group (G cont) fish were kept in a fiberglass tank with dechlorinated water and fed with a stander commercial pellet at 3%. The second group (GCu) fish have exposed to sub lethal concentration of $CuSO_4$ 0.53 mg/L (22) and fed with a stander commercial pellet. Fish in the third group (GSarcch) were kept in fiberglass tanks with dechlorinated water and fed with a stander commercial pellet with *an S. cerevisiae* 5 g/kg ratio. In the fourth group (GCu+Sarcch), fish were exposed to a sub-lethal concentration of $CuSO_4$ 0.53 mg/L and fed with stander commercial pellet with additive yeast (*S. cerevisiae*) 5 g/kg ratio. After expanding the period of the experiment (56 days), fish were exposed to general anesthesia using MS-222 at a concentration of 150 mg/L (23), and the brain and gills were dissected and divided into two parts, one of them kept in buffer formalin for histopathological technique, and the semi-quantitative analysis for gill lesions was depended according to the (24-26) with slight modification as in table 1.

Table 1: Categories and score of the severity of gill histopathological lesions (25)

Histopathological categories	Score
Normal pathological architecture	-
Mild histopathological lesions	+ /-
Moderate histopathological lesions	+
Severe histopathological lesions	++
Very Severe histopathological lesions	+++

While the other part was kept with muscle for three days in the aluminum foil at freezing point -18°C for estimation of Acetylcholine esterase (AChE) inhibition activity (%), brain, muscles, and gills were homogenized by electric homogenized using natural phosphate buffer at pH 8.1. Then AChE activity was measured in a modified electrometric method (27), and AChE Inhibition activity % was estimated according to the formula: Inhibition % = control group AChE activity - treated group AChE activity/ control group AChE activity x 100.

Statistical analysis

The data of these studies were analyzed using CRD to indicate significant variances in AChE activity in the brain, muscles, and gills tissue of fish using Duncan's multiple range tests at $P \leq 0.05$ (28).

Results

The AChE activity was estimated in the fish exposed to sublethal concentration of $CuSO_4$, which decreased significantly ($P \geq 0.05$) in the brain and muscle at 0.792 and 0.628%, respectively, in comparison to the control group and fish treated with *S. cerevisiae*. In both organs in fish at GCu+Sarcch, there was improve significantly ($P \leq 0.05$) in the AChE activity in the brain and muscles 0.880 and 0.870% in contrast to AChE activity in fish GCu, the statistical analysis suggested there was no significant variation in the AChE activity in the gills of fish in all treated groups (Table 2).

Microscopic examination

The histopathological lesions in the brain of fish treated with a sublethal concentration of copper sulfate ranged from severe infiltration of inflammatory cells with vascular changes represented by edema (Figure 1) hemorrhage and vesicular (watery) astrocyte (Figure 2), with vasogenic edema and infiltration of microglia cells (Figure 3). The microscopic study revealed the alteration in the neuron cell

bodies and axon represented by central chromatolysis of the neuron cell bodies combined with a proliferation of oligodendroglia cells (Figure 4). The irritation of $CuSO_4$ causing demyelination (Figure 5), and the influence of additive *S. cerevisiae* to the diet of fish exposed to $CuSO_4$ was histologically represented by vasogenic edema (Figure 6).

Table 2: The effects of $CuSO_4$ and *S. cerevisiae* on Cholinesterase activity% in brain, muscles and gills of fish

Treatment	Brain		Muscle		Gills	
	ΔpH/30min	Inhibition%	ΔpH/30min	Inhibition%	ΔpH/30min	Inhibition %
G cont	0.995±0.04	0.976±0.089a	1.06±0.03	1.042±0.062a	0.33±0.04	0.367±0.08a
GCu	0.77±0.05	0.792±0.093b	0.64±0.04	0.628±0.071c	0.25±0.02	0.254±0.042a
GSarcch	0.91±0.02	0.928±0.049a	0.95±0.02	0.945±0.049ab	0.3±0.03	0.378±0.216a
GCu+Sarcch	0.88±0.01	0.880±0.029ab	0.83±0.06	0.870±0.106ab	0.28±0.07	0.290±0.078a

Different letters mean there was significant variable (P≤0.05) in same column.

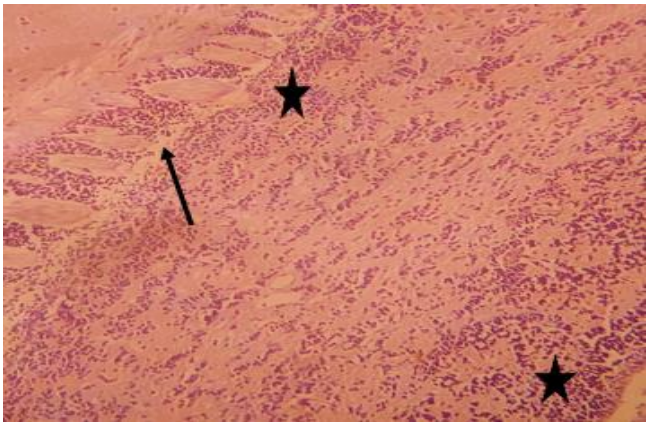

Figure 1: Microscopic examination of the brain in fish treated with $CuSO_4$ 0.53 mg/L for 56 days sever infiltration of inflammatory cells (black star) and edema (black row), H&E, 44x.

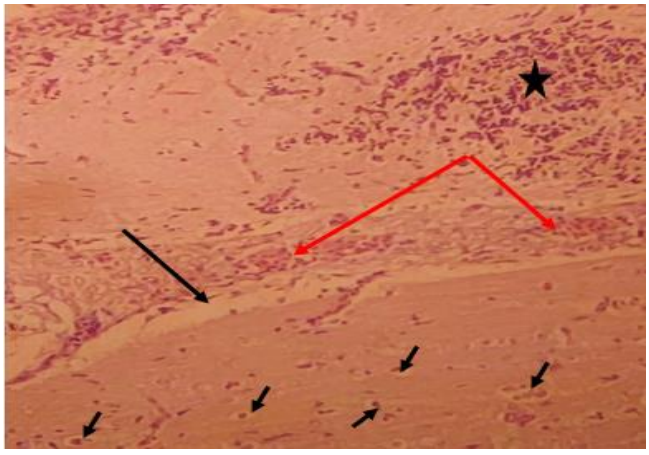

Figure 2: Microscopic investigation of the brain in fish treated with $CuSO_4$ 0.53 mg/L for 56 days revealed hemorrhage (red row), focal infiltration of inflammatory cells (black star), and edema (black row) with vesicular astrocyte (short black row), H&E, 71x.

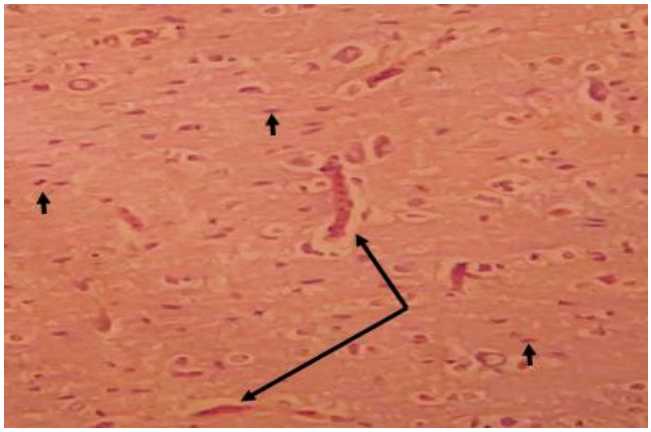

Figure 3: Microscopic investigation of the brain in fish treated with $CuSO_4$ 0.53 mg/L for 56 days revealed vasogenic edema (black row), infiltration of microglia cells (short black star), H&E, 100x.

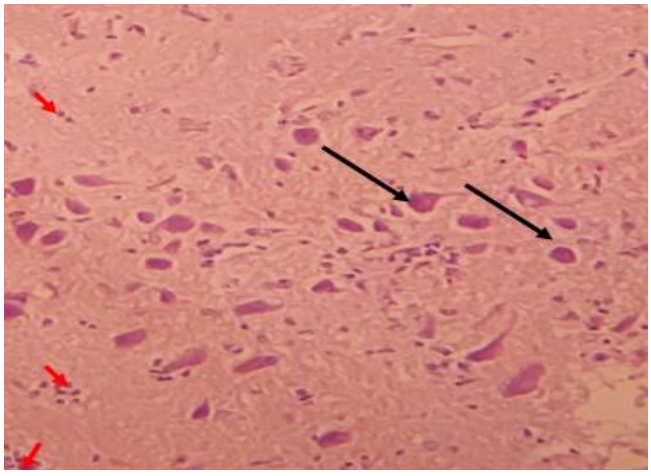

Figure 4: Microscopic investigation of the brain in fish treated with $CuSO_4$ 0.53 mg/L for 56 days infiltration of oligodendroglia cells (red row), and central chromatolysis of neuron cell bodies (black row), H&E, 91x.

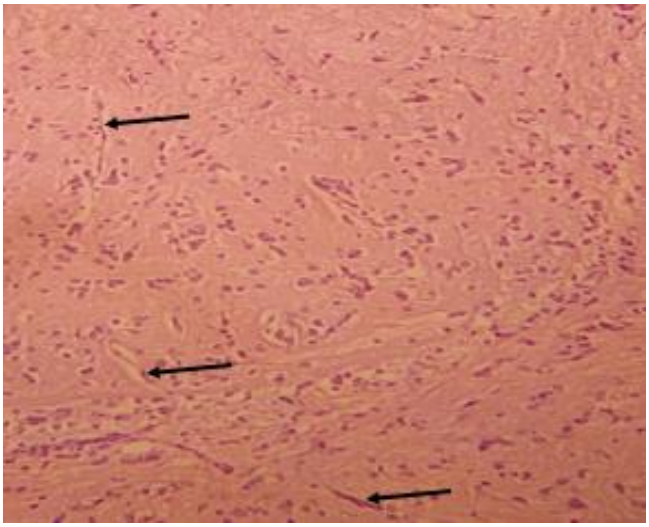

Figure 5: Microscopic investigation of the brain in fish treated with $CuSO_4$ 0.53 mg/L for 56 days show demyelination (black row) H&E, 68x.

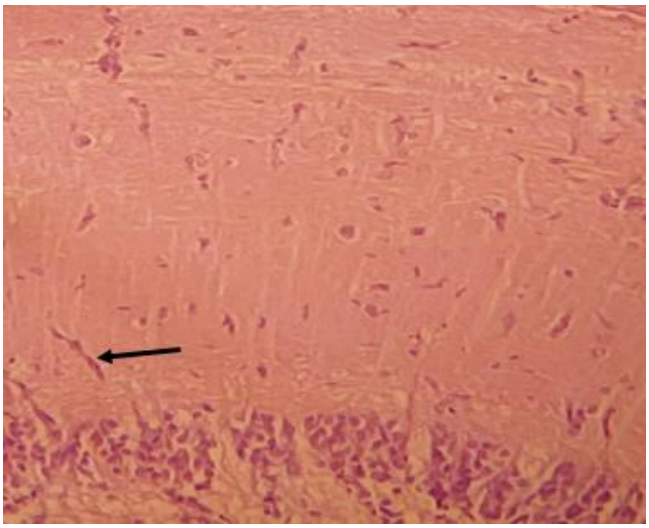

Figure 6: Microscopic investigation of the brain in fish treated with $CuSO_4$ 0.53 mg/L for 56 days show vasogenic edema (black row) H&E, 80x.

The microscopic examination of the gills in the control group revealed normal structure (Figure 7), while fish exposed to $CuSO_4$ 0.53 mg/L for 56 days revealed variable histopathological lesions as lost the straight secondary gill filaments and curling appearance led to adhesion and bulging cyst in the epithelial cells lining primary gill filaments and lifting cells (Figure 8) with cartilage disrupting and damage. Exposed to $CuSO_4$ include variable cell injury from hydropic degeneration in the pillar cell to hyperplasia of mucus cells with circulatory disturbances in the gills as edema with congestion. Also, there was morphological alteration characterized by shortening in the secondary gill filaments (Figure 9). Long exposure of fish to $CuSO_4$ for 56 days induces pathological disturbances in the gill arch, represented by severe infiltration of inflammatory cells, edema, and thrombus in the blood as in vessels (Figure10). The slight adhesion and drumstick appearance in the secondary gill filaments and hydropic degeneration in the pillar cells are the primary histopathological lesions in the gill of fish treated with *S. cerevisiae* (Figure11) with edema and normal cartilage architecture (Figure 12).

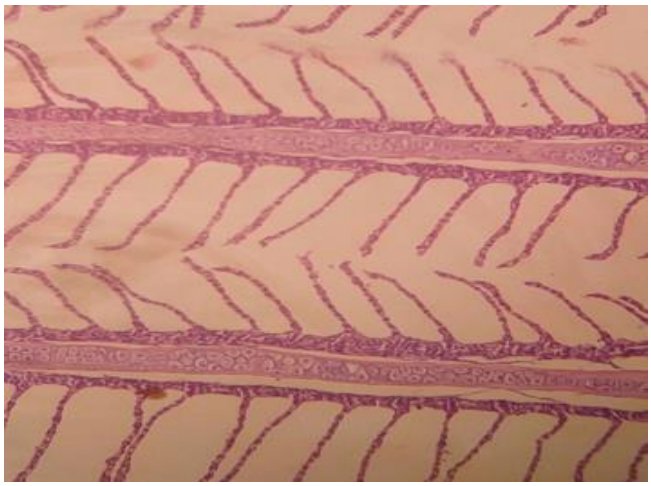

Figure 7: Microscopic investigation of the fish gills in the control group revealed normal structure H&E, 37x.

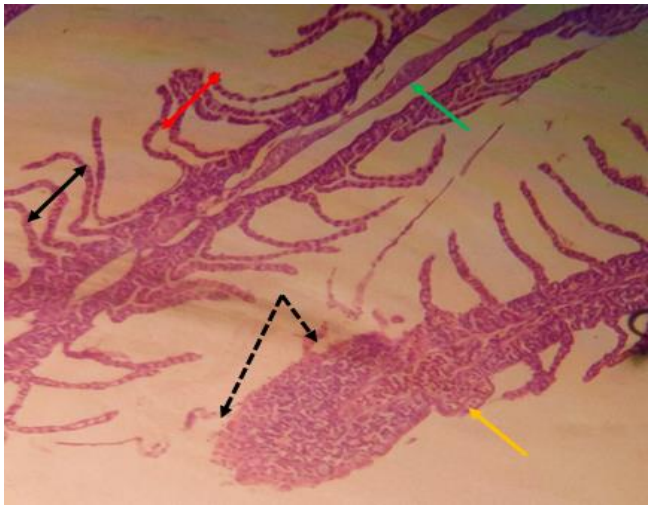

Figure 8: Microscopic investigation of gills in fish treated with ($CuSO_4$ 0.53 mg/L for 56 days) revealed curling of secondary gills filaments (black two-head row), adhesion of secondary gills filaments (red two-head row), cartilage disrupting (green row) and bulging cyst of epithelial lining primary gills filaments (yellow row) with lifting cells (black dot row), H&E, 36x.

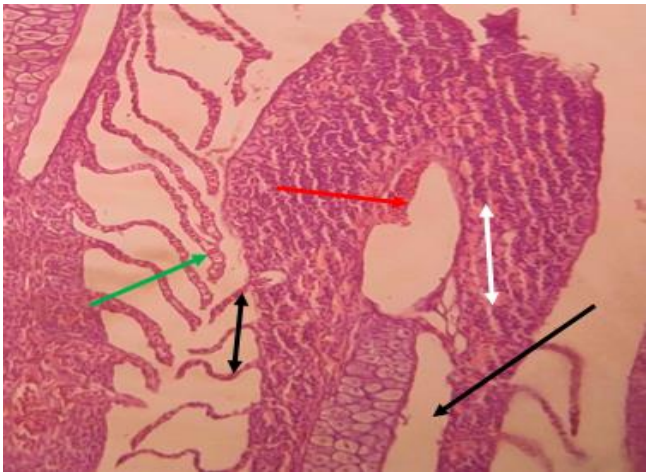

Figure 9: Microscopic investigation of gills in fish treated with ($CuSO_4$ 0.53 mg/L for 56 days) revealed shortening in the secondary gill's filaments (black two-head row), edema (black row), congestion (red row), vacuolar degeneration of pillar cells (green row) and hyperplasia of mucus cells, H&E, 43x.

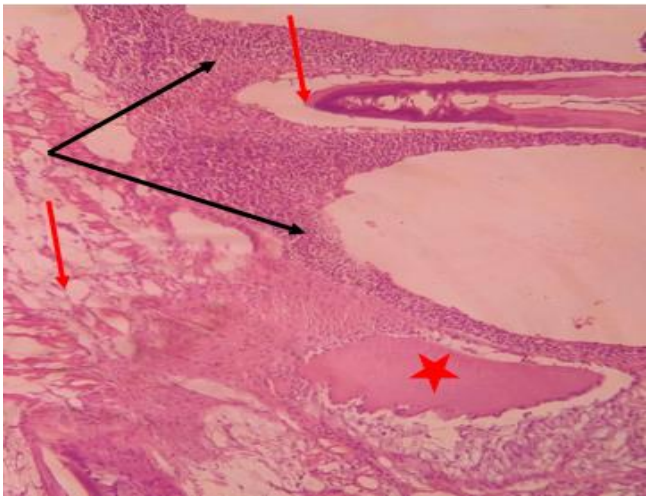

Figure 10: Microscopic investigation of gill arch in fish treated with ($CuSO_4$ 0.53 mg/L for 56 days) revealed severe infiltration of inflammatory cells (black row), edema (red row), and thrombus in gill arch blood vessels (red star), H&E, 30x.

The semi quantities analysis of the gill in the fish that received *S. cerevisiae* and CuSO4 for measuring lesions severity (Table 3). It is clear that treating fish with copper sulfate leads to histopathological changes (circulatory and cell growth disturbances, morphological alteration and cartilage disturbances) that vary in severity from +, ++, and +++, which are more severe than the results of microscopic examination of gills of fish treated with copper sulfate with yeast, as their severity ranged from -, +/-.

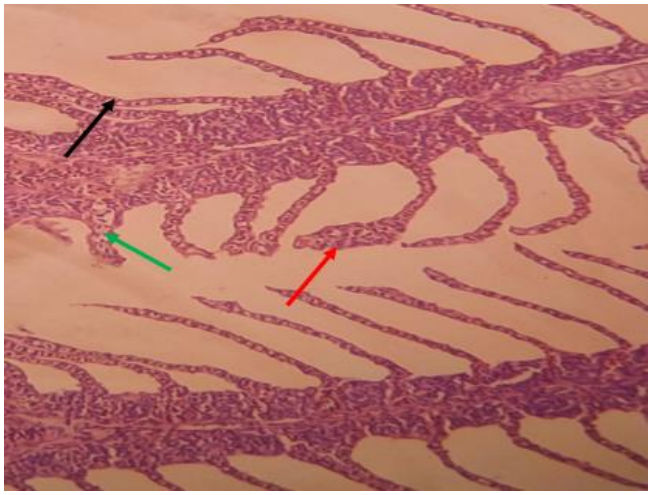

Figure 11: Microscopic investigation of gill arch in fish treated with ($CuSO_4$ 0.53 mg/L and fed with *S. cerevisiae* at 5g/kg diet for 56 days) revealed adhesion of secondary gills filaments (black row) with a drumstick-like lesion (red row) with hydropic degeneration in the pillar cells (green row) H&E, 42x.

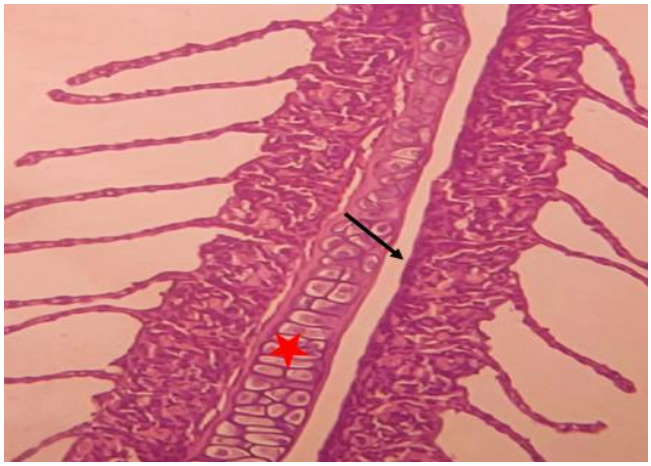

Figure 12: Microscopic investigation of gill arch in fish treated with ($CuSO_4$ 0.53 mg/L and fed with *S. cerevisiae* at 5g/kg diet for 56 days) revealed normal cartilage (red star) and edema (black row) H&E, 81x.

Discussion

Copper is an essential element for aquatic organisms. It is widely used as a chemotherapeutic drug for fish diseases disinfectant or fertilizer agent for plants and phytoplankton, but it is potential toxicity when used at high concentrations and causes adverse pathological and physiological effects (29). Our result revealed the adverse effects of $CuSO_4$ toxicity in the gills and brains of fish. In recent years, there has been an increase in interest in the possible utility of

AChE activity as a biomarker for assessing the quality of the aquatic environment and the health of the aquatic animal. AChE is mainly located at neuromuscular junctions and cholinergic brain synapses, which block synaptic transmission (30). The same results of our study were also obtained by Boareto Acgiareta *et al.* (31) and Al-Zubaidy (32).

Table 3: Semi-quantities of the gills lesions analysis in fish exposed to $CuSO_4$ and treated with *S. cerevisiae* for 56 days

Histopathological categories	G cont	GSarcch	GCu	GCu+Sarcch
Edema	-	-	+++	+/-
Congestion	-	-	+/-	-
Infiltration of inflammatory cells	-	-	+++	-
Proliferation of epithelial cells	-	-	+	-
Hydropic degeneration	-	-	+	+/-
Hyperplasia of mucus cells	-	-	++	-
Lifting cells	-	-	+	-
Shortening secondary gill filaments	-	-	++	-
Morphological alteration	-	-	++	+
Cartilage disrupting	-	-	+++	-

The brain is the regulatory center of all metabolic and physiological processes and fish swimming. The infiltration of inflammatory cells, oligodendroglia, and microglia cells, along with edema and other histopathological alterations, were investigated in the current study (33). These alterations may have been caused by a potential inhibition or reduction in cholinergic activity after exposure to copper sulfate since copper sulfate is a potential neurotoxic mediator which inhibits AChE activity in the brain. Also, membrane-bound Ca^{2+}ATPase and Mg^{+}, K^{+} ATPase activities have been decreased in the brain regions of fish exposed to metal toxicity (34,35), which provides histological alteration in the brain of *C. carpio* in the current study.

Gills are a promising biomarker for aquatic pollution and fish toxicity because it is the more sensitive organ and have direct contact with the external environment. The histopathological investigation of the present study revealed variable microscopic lesions of gills in fish that received copper sulfate 0.53 mg/L for 56 days. This includes circulatory disturbances such as edema, congestion, and thrombus occurrence with cell growth adaption as hydropic degeneration of pillar cells and hyperplasia of mucus cells with a morphological alteration. The same results were also obtained by Atabati *et al.* (29), who found that the 2.5 mg/L copper sulfate led to a degeneration of epithelial cells lining primary and secondary gill filaments and hyperplasia of mucus cells in *Ctenopharynogodon idella* this lesion severity at concentration 5 mg/L. These histological alterations in the gill structure are a fish's response to ingesting toxicants or an adaptive response to restrict the entry of contaminants through the gill surface. As a result, they might act as a defense mechanism. as a greater separation between the blood and the outside world, which also acts as a barrier to the entry of various contaminants (36). The increased distance between the blood artery and the gill epithelium, on the other hand, restricts gas exchange and prolongs the delivery of oxygen to the blood circulatory system. Moreover, the essential toxic mechanisms of Cu^{+2} are disrupting the active Na^{+} and Cl absorption pathways, increasing gill permeability, and oxidative stress mainly through decreased Na^{+}/K^{+} ATPase activity. These mechanisms are the first primary key for cell injury, and the consequent net ion loss may ultimately result in cardiac arrest and death (37-39). The semi-quantitative evaluation of histopathological alteration in the gills is one of the most important scientific methods for distinguishing between the severity of histopathological lesions. The result of this study agrees with the result of Georgieva *et al.* (40).

S. cerevisiae is a more effective biosorbent for metal ions than another biomass (41). This clearly shows in AChE activity and repair of tissue damage in the brain and gills in the treated group fish (GCu+Sarcch), or due to the activity of *S. cerevisiae* in improving the antioxidant activity and detoxification, as well as the *S. cerevisiae* plays a vital role for decline the toxicity of copper sulfate by increasing intestinal absorption and excretion.

Conclusion

It is concluded from this study that exposure of fish to copper sulphate at high concentrations leads to disturbances and histopathological alteration in the gills and brain, and that the evaluation of the activity of AChE in brain tissue is one of the vital indicators for the occurrence of neurotoxicity. Yeast is a more effective biosorbent agent for copper than another biomass.

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Conflict of interests

No conflict of interest.

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تقيم دور الخميرة الجافة في علاج التسمم بكبريتات النحاس في اسماك الكارب الاعتيادي

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الخلاصة

هدفت هذه الدراسة إلى تحديد التأثير التداخلي بين كبريتات النحاس والخميرة الجافة في أسماك الكارب لمدة ٥٦ يوما من خلال تقييم نشاط الأستيل كولين والتغيرات المرضية النسجية في الغلاصم والدماغ مع تحديد شدة الآفات في الغلاصم عن طريق التحليل شبه الكمي. قُسمت ستون سمكة إلى أربع مجموعات، الأولى كانت مجموعة السيطرة، وفي المجموعة الثانية تم تعريض الأسماك لكبريتات النحاس ٠,٥٣ ملغم / لتر، بينما في المجموعة الثالثة تمت معاملة الأسماك بالخميرة ٥ غم/كغم علف، أما المجموعة الرابعة فقد تمت معاملة الأسماك بالخميرة ٥ غم/كغم علف وكبريتات النحاس٠,٥٣ ملغم / لتر، وأظهرت النتائج وجود انخفاض معنوي في نشاط الاستيل كولين في المجموعة الثانية، بينما تحسن هذا النشاط معنويا في المجموعة الرابعة. وكانت التغييرات المرضية النسجية في الدماغ مختلفة في شدتها من ارتشاح الخلايا الالتهابية والوذمة وتحلل الكروماتين المركزي لأجسام الخلايا العصبية، في حين تمثلت الآفات في الغلاصم بالوذمة والاحتقان والتنكس الفجوي مع فرط تنسج الخلايا المخاطية وقصر في طول الخيوط الغلصمية الثانوية مع ظهورها بشكل عصا الطبل ونخر ونحطم في الغضروف ، وتم تصنيف الآفات المرضية النسجية في الغلاصم بالتحليل شبه الكمي الى آفات خفيفة ومتوسطة وشديدة وغير رجعية، وكانت هذه الآفات خفيفة الشدة في كل من دماغ وغلاصم اسماك المجموعة الرابعة. يستنتج من هذه الدراسة إلى أن نشاط الاستيل كولين هو مؤشر بيولوجي للسمية العصبية لكبريتات النحاس والذي يسبب أيضا تلفا للدماغ والغلاصم وأن الخميرة هي عامل ادمصاص حيوي للنحاس.

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