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Study on the Genotoxic Effect of Copper Sulphate in the Spotted Snakehead Fish *Channa Punctatus* (Bloch, 1793)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The genotoxic effects of a herbicide containing CuSO4 were assessed using the micronucleus assay in Channa punctatus. The study involved intraperitoneal administration of three different doses (1.0, 3.0, and 5.0 mg/kg body weight) and exposure to varying concentrations of copper sulphate (15, 25, and 35 ppm) in laboratory aquaria. Peripheral blood smears stained with 15 to

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20% Giemsa (pH=7.0) were examined. Apart from micronuclei, the herbicide induced other nuclear and cytoplasmic abnormalities. The findings suggest a direct impact of increasing CuSO4 concentration on the biological samples of Channa punctatus. This fish species is commonly found in freshwater habitats like ponds, ditches, wetlands, and rice fields, especially in Odisha, India. India is crucial for maintaining aquatic biodiversity. The study highlights the potential detrimental effects of improper use of CuSO4 containing pesticides in agriculture on Channa punctatus and emphasizes the need for careful management of such chemicals to protect aquatic ecosystems.

Keywords: Channa punctatus; copper sulphate; Micronucleus (MN) Assay; genotoxic potential; peripheral blood smear.

1. INTRODUCTION

Copper sulphate ($CuSO_4$, $5H_2O$) is a significant copper salt with broad-spectrum herbicidal and weedicidal properties, particularly against aquatic weeds Banerjee et al, [1] and as a molluscicide [2]. Metal pollution has escalated due to human technological advancements, including industrial activities, mining, advanced agriculture, household waste, and motor traffic. These activities are considered major sources of metal pollution, leading to metal accumulation in aquatic organisms like fish, persisting in water and sediments [3]. Fish, being vital animal protein resources, serve as suitable bio-indicators of metal contamination due to their position at the top of the aquatic food chain. Metals induce oxidative stress, and assessing oxidative damage and antioxidant defenses in fish reflects metal contamination in aquatic environments [4]. Copper, in small quantities, is an essential trace metal for various fish metabolic functions, incorporated into enzymes such as peroxidase, xanthine oxidase, invertase, glucose oxidase, and protease papain and bromelain [5,6]. Although copper is used as a chemotherapeutic agent in aquaculture, elevated copper levels in aquatic environments stem from sewage, industries (electroplating, mining, metallurgy), and agricultural wastes [7]. The rise in population, industrialization, and agricultural production has led to increased freshwater systems impairment due to contaminants in wastewater releases [8,9]. Several insecticides have exhibited genotoxic potential on various freshwater fish species. For instance, acephate induces DNA damage and nuclear abnormalities in *Clarias batrachus* [Jagyanseni et al, 2022], and in *Heteropneustes fossilis,* it causes DNA damage and changes in hematological parameters [10]. This study aims to evaluate the genotoxic effects of copper sulphate on the freshwater fish *Channa punctatus* using the micronucleus assay.

2. MATERIALS AND METHODS

2.1 Test Chemical

Analytical-grade copper sulphate $CuSO_4$,$5H_2O$ (99% pure) from Fine-chem (India) Ltd was utilized in the experiment. Glass double-distilled (g. d.d) water served as the solvent.

2.2 Dose

Intraperitoneal administration (i.p.) of three different doses (1.0, 3.0, and 5.0 mg/kg body weight) was conducted on *Channa punctatus*. Additionally, fish were exposed to copper sulphate concentrations of 15, 25, and 35 ppm in laboratory aquaria.

2.3 Experimental Animal

Live fish weighing 70 to 100 g were obtained from domestic ponds. Before chemical treatment, the fish were acclimatized in laboratory aquaria for 2 to 3 days and fed commercial fish food twice daily. Proper aeration and daily water changes were ensured in the aquaria, and any feed remains, excretory waste, or dead animals were promptly removed to prevent stress and contamination.

2.4 Time

Blood smear slides were prepared after 24, 48, and 72 hours of exposure.

2.5 Experimental Protocol

There are overall two treatment groups. For **e**ach treatment group healthy, active and strong individuals were selected. In one treatment group; a batch of fish was injected i.p with different doses of copper sulphate dissolved in sterile, distilled water. The injected animals were kept in aquaria containing tap water.

In another set, fish were released into different aquaria containing different concentration of copper sulphate and kept for varying period of time (see Table: 2). Tap water is used for dilution. The animals kept in tap water served as control. Four fish were used for each sampling period and for each concentration level. After treatment, animals were euthanized at 24, 48, and 72 hours. Peripheral blood smear slides were prepared from blood collected via caudal incision, following procedures outlined by A1-Sabti K, [11] and Das et al, [12] with some modification. The full procedure was as followed.

Thin smears of peripheral blood from the caudal region were made on grease-free clean slides and allowed to air-dry. Slides were fixed in absolute methanol for 10-15 minutes and air-dried. Staining was performed in 15-20% Giemsa solution at pH 7.0 for 60 to 90 minutes. Slides were gently washed in tap water and air-dried. All slides were coded before screening. 4000 erythrocytes (1000 per slide) were examined under oil immersion. Non-refractile particles resembling nuclei in all aspects except size were considered micronuclei.

3. RESULTS AND DISCUSSION

3.1 General Toxicity

No external toxicity symptoms were observed following injection (mg/kg) or whole-body exposure to different concentrations (ppm) of the chemical.

3.2 Qualitative

The location and size of micronuclei varied among cells, with generally one micronucleus per cell observed. Micronuclei were predominantly dot-shaped, ranging from 1/5 to 1/25th the size of the principal nucleus (*C. punctatus*). Throughout the study, both small and large micronuclei were observed in treated individuals. In addition to micronuclei induction, various other nuclear anomalies such as sickle-shaped, thinning in mid-region of nuclei, and enucleated cells were recorded.

3.3 Quantitative

In *Channa punctatus* injected intraperitoneally with the chemical, the frequency of micronuclei (MN) significantly increased across all doses and exposure durations compared to respective controls (Table 1) [*P<0.05, **P<0.01 (Student's t-test)]. Dose-response analysis revealed significant variations (F=14.78; degree of freedom (d.f.)=36.3; **P<0.01), with a linear increase in micronuclei frequency observed with increasing doses [Y-intercept (b) =0.64; Coefficient of correlation (r) =0.869; **P<0.01]. However, no significant variation was noted in time-response analysis [F=1.87; d.f. =36.2; P>0.05] (ANOVA).

Table 1. Incidence of micro nucleated peripheral blood cells of fish *Channa punctatus* injected ip with copper sulphate

Dose (mg/kg)	Time (hrs)	No. of micronucleus (MN)	‰ aberration ±S.E.	No. of nuclear abnormalities (NA)	‰ aberration ±S.E
Control	24	2	0.05±0.06	2	0.13±0.07
	48	2	0.11±0.07	2	0.12±0.08
	72	2	0.12±0.08	3	0.17±0.06
	24	5	0.32±0.06*	3	0.18±0.12
	48	5	0.31±0.07*	5	0.32±0.06
1	72	6	0.37±0.07*	5	0.32±0.15
	24	6	0.36±0.07**	4	0.26±0.11
	48	7	0.44±0.08**	5	0.33±0.06
3	72	8	0.51±0.11*	5	0.31±0.07
	24	6	0.38±0.08**	5	0.30±0.06
	48	7	0.44±0.07**	5	0.31±0.08
5	72	9	0.57±0.12**	6	0.35±0.07

Results are mean‰±S.E of four fish; Result is significantly different from the control at P<0.05,**P<0.01(Student's t-test); 16000 cells were scored for each point (4000/fish)*

Table 2. Incidence of micro nucleated peripheral blood cells of fish *Channa punctatus* exposed to copper sulphate contaminated water

Dose(ppm)	Time(hrs)	No. of MN	‰ aberration ± S.E.	No. of NA	‰ aberration ± S.E
	24	1	0.04±0.06	2	0.12±0.08
	48	2	0.11±0.07	2	0.11±0.07
Control	72	2	0.12±0.08	3	0.19±0.06
	24	3	0.19±0.06*	3	0.17±0.05
	48	6	0.36±0.07*	5	0.32±0.06
15	72	6	0.37±0.08*	5	0.30±0.12
	24	4	0.24±0.10**	5	0.32±0.07
	48	6	0.37±0.08**	5	0.38±0.17
25	72	8	0.49±0.11*	6	0.26±0.10
	24	3	0.17±0.07**	4	0.32±0.10
	48	7	0.42±0.08**	5	0.32±0.10
35	72	9	0.55±0.17**	7	0.43±0.07

Results are mean‰±S.E of four fish; Result is significantly different from the control at P<0.05,**P<0.01(Student's t-test); 16000 cells were scored for each point (4000/fish)*

In dermal exposure experiments on *C. punctatus*, the frequency of MN induced by different doses significantly increased compared to respective controls (Table 2). A notable rise in MN incidence was observed in all treated groups after 48 and 72 hours of exposure [*P<0.05; **P<0.01; (Student's t-test)]. Significant variations were also observed in different doses (F=9.81; d.f. = 36.3; **P<0.01) and exposure time (F=8.80; d.f. =36.2; **P<0.01) (ANOVA). Additionally, a linear increase in micronuclei frequency with dose was evident (b=0.008; r=0.947; *P<0.05).

4. DISCUSSION

The safety of our aquatic environment is paramount, as it directly impacts our health and food security. Therefore, evaluating its genotoxicity is crucial for sustainable development. Genotoxic pollutants have the potential to cause gene mutations, posing risks to future generations if left unregulated [13]. While fish may be the first to suffer from these pollutants, humans are next in line. Peripheral blood sampling is an effective method for bio-monitoring projects, enabling multiple samples to be collected from the same individuals without sacrificing those individuals [14]. Unfortunately, the micronucleus (MN) assay has not received sufficient attention in environmental biotechnology and management, leading to a lack of understanding regarding the lethal and sub-lethal effects of certain eco-genotoxicants. These pollutants have been found to be eco-toxic in developed countries [15]. The analysis of DNA changes in aquatic creatures has been a widely accepted method for evaluating the genotoxic potential of contaminants in the environment. This method is appropriate for identifying exposure in a broad variety of species [16-19]. It has been proposed that genotoxicity testing be an essential part of environmental risk assessment programmes due to its significance as a highly important fish biomarker [20]. Additionally, the micronucleus (MN) assay has been extensively employed as a comprehensive technique to assess chromosomal damage, which is specifically scored in once-divided bi-nucleated cells with micronuclei and other abnormalities in the cell. One well-liked early cytotoxicity biomarker that indicates chromosomal breakage and/or complete loss of chromosome is the frequency of micronuclei [21]. Fish are used as indicator organisms in genotoxicity research for a variety of significant reasons [22,23]. These include their place in the food chain, their nutritional worth to people, their capacity to bio-accumulate harmful substances, their susceptibility to minute amounts of mutagenic substances, and even their aesthetic value. The erythrocyte micronucleus test has been applied to a variety of fish species in order to monitor aquatic contaminants that exhibit mutagenic properties [24]. In our study genotoxic potential of cupper sulphate on *Channa punctatus* has been shown using micronucleus assay. The comet assay has been used to investigate the genotoxicity of copper sulphate to planaria, which indicates increased levels of DNA strand breaking and inhibition of DNA repair in planaria preexposed to methylmethan sulphonate [25]. The induction of micronuclei in the blood of *Channa punctatus* exposed to copper has also been reported to exhibit a dose-dependent rise and a time-dependent decline

[26]. It has been reported that CuSO4 was able to induce *Channa punctatus* to form a micronucleus [27]. The gills and erythrocytes of *Labeo bata* fish raised in sewage-fed fish farms that also contain copper and other heavy metals have been discovered to have a genotoxic potential for copper [28-30]. In present study, it has been seen that appearance of micronucleus and nuclear abnormalities increased in *Channa punctatus* with increase in time and concentration of copper sulphate in different treatment groups [31-33].

5. CONCLUSION

Crop production must be intensified to keep up with our fast expanding population. Therefore, it is crucial to manage pests and insects that result in crop output losses. The use of pesticides in the production cycle has become essential in order to eradicate plant diseases and pests that can significantly lower the amount of products that can be harvested. Furthermore, a contaminated environment containing a high concentration of pesticides has negatively impacted a number of aquatic living organisms. Our study unequivocally demonstrates the clastogenic effects of copper sulphate on *Channa punctatus*, raising serious concerns about the potential health implications for humans and other aquatic organisms reliant on aquatic ecosystems and its use in agriculture. Further research employing different test systems is necessary to reconcile the contradictory results observed in various test systems

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Banerjee SM, Mitra E. Preliminary observations on the use of copper sulphate to control submerged aquatic weeds in alkaline waters. Indian journal of fisheries. 1954;204-216.
2. Babu GR, Rao PV. Heavy metal ion toxicity in the freshwater gastropod snail host, *Lymnaea luteola*. Indian Journal of Physiology and Pharmacology. 1982;26 (2):141-146,
3. Luoma SN, Rainbow PS. Sources and cycles of trace metals. In: Metal Contamination in Aquatic Environments: Science and Lateral Management. Cambridge University Press, Cambridge. 2008a;47-66.
4. Livingstone D R. Oxidative stress in aquatic organisms in relation to pollution and agriculture. Revue de Medicine Veterinaire. 2003;154:427– 430.
5. Shukor Y, Baharom NA, Rahman FA, Abdullah MP, Shamaan NA, Syed MA. Development of heavy metals enzymatic-based assay using papain. Analytica Chemica Acta. 2006;566:283-289.
6. Shukor MY, Masdor N, Baharom NA, Jamal JA, Abdullah MPA, Shamaan NA, Syed MA . An inhibitive determination method for heavy metals using bromelain, a cysteine protease. Appl Biochem Biotechnol. 2008;144(3):283-291.
7. Singh D, Nath K, Sharma YK, Trivedi SP. Hepatotoxic effect of Cu (II) in fresh water fish Channa punctatus: A histopathological study. Res. Environ. Life Sci. 2008;1(1):13-16.
8. Di Toro DM, Allen HE, Bergman HL, Meyer JS. Biotic ligand model of the acute toxicity of metals.1.Technical basis. Environ Toxicol Chem. 2001;20(10):2383-2396.
9. Santore RC, Di Toro DM , Paquin PR, Allen HE, Meyer JS. Biotic ligand model of the acute toxicity of metals. II. Application to acute copper toxicity in freshwater fish and daphnia. Environ Toxicol Chem. 2001;20 (10):2397-2402.
10. Jagyanseni S, Mishra S, Sahoo S N. A Study on genotoxic potential of acephate in *Clarias batrachus*. Journal for Research in Applied Sciences and Biotechnology. 2023;2(1):22-25.
11. Al-Sabti K. Clastogenic effects of five carcinogenic mutagenic chemicals on the cells of the common carp *Cyprinus carpio* L. Com Biochem Physiol. 1986;85 (C):5-9.
12. Das R K, Nanda N K. Induction of micronuclei in peripheral erythrocytes of fish *Heteropneustes fossilis* by mitomycin C and paper mill effluent. Mutation research. 1986;175: 67-61.
13. Okpokwasili GC. Contributions of industrial pollution and environmental degradation on the emergence of communicable and non-communicable diseases. A paper presented at the5thAnnualNational Conference of Society for Occupational

Safety and Environmental Health (SOSEH); 2009.

14. Lyne TB, Bickham JW, Lamb T, Gibbons JW. The application of bioassays in risk assessment of environmental pollution. Risk Anal.1992;12(3):361-365.
15. Germain A, Perron F,Van Coillie R. PAHs in the Environment: Fate, Ecotoxicity and Regulations, Environment Canada, Conservation and Protection, Quebec Region, Montreal,Que; 1993.
16. Vuković-Gačić B, Kolarević S, Sunjog K, Tomović J, KneževićVukčević J, Paunović M, Gačić Z. Comparative response of freshwater mussels *Unio tumidus* and *Unio pictorum* to environmental stress. Hydrobiologia. 2013;735:221–231.
17. Rocco L, Peluso C, Stingo V. Micronucleus test and comet assay for the evaluation of zebrafish genomic damage induced by erythromycin and lincomycin. Environ Toxicol. 2012;27:598–604.
18. Sunjog K, Gačič Z, Kolarevič S, Visnjić-Jeftić Ž, Jarić I, Knežević-Vukčević J, Vuković-Gačić B, Lenhardt M. Heavy metal accumulation and the genotoxicity in Barbel (*Barbus barbus*) as the indicators of the Danube River pollution. Sci World J. 2012;1–6.
19. Kolarević S, Knežević-Vukčević J, Paunović M, Tomović J, Gačić Z, Vuković-Gačić B. The anthropogenic impact on water quality in river Danube in Serbia: microbiological analysis and genotoxicity monitoring. Arch Biol Sci. 2011;63:1209–1217.
20. Van der Oost R, Beyer J, Vermeulen N P E. Fish bioaccumulation and biomarkers in environmental risk assessment: A review. Environ Toxicol Pharma. 2003;13: 57–149.
21. Xin L, Wang J, Guo S, Wu Y, Li X, Deng H, Kuang D, Xiao W, Wu T, Guo H. Organic extracts of coke oven emissions can induce genetic damage in metabolically competent HepG2 cells. Environ Toxicol Pharmacol. 2014;37:946–953
22. Szefer P, Szefer K, Skwarzec B. Distribution of trace metals in some representative fauna of the Southern Baltic. Mar Poll Bull. 1990;21:60–62.
23. Visn-Jeftic Z, Jaric I, Jovanovic L, Skoric S, Smederavac-Lalic M, Niksevic M, Lenhardt M. Heavy metal and trace element accumulation in muscle liver and gills of the Pontic shad (*Alosa immaculate* Bennet 1835) from the Danube River (Serbia). Microchem J. 2010;95:341–344.
24. Arslan O Ç, Parlak H. Micronucleus test good biomarker for determination of genetic changes in aquatic organism. J Aquat Pollut Toxicol. 2017;1(3): 18-23.
25. Guecheva T, Henriques JAP , Erdtmann B . Genotoxic Effects of Copper Sulphate in Freshwater Planarian *In vivo*, Studied with Single- Cell Gel Test (Comet Assay). Mutation Research. 2001; 497:19-27.
26. Yadav KK, Trivedi SP. Sublethal exposure of heavy metals induces micronuclei in fish, *Channa punctata*. Chemosphere. 2009;77(11):1495-1500.
27. Kousar S, Javed M. Studies on induction of nuclear abnormalities in peripheral blood erythrocytes of fish exposed to copper. Turk J Fish Aquat Sci. 2015;15(4): 879-886.
28. Talapatra SN, Banerjee SK. Detection of micronucleus and abnormal nucleus in erythrocytes from the gill and kidney of *Labeo bata* cultivated in sewage-fed fish farms. Food Chem Toxicol. 2007;45(2): 210-215.
29. Singh D, Nath K, Trivedi SP, Sharma YK. Impact of copper on haematological profile of fresh water fish *Channa punctatus*. J. Environ. Biol. 2008;29(2): 253-257.
30. Jagyanseni S, Sahoo S, Sahoo SN. Genotoxic and haematological effect of commonly used insecticide on fish *Heteropneustes fossilis.* International Journal of Modern Pharmaceutical Research. 2023;7(7):27-29.
31. Kashyap A, Kumar V. Histopathological Analysis of Hexavalent Chromium Toxicity to Ovary and Testis of Freshwater Food Fish, Channa punctatus (Bloch. 1793). Asian J. Fish. Aqu. Res. 2023;23(4): 18-25.
    Available:https://journalajfar.com/index.php/AJFAR/article/view/607
    [Accessed on 2024 May 22]
32. Akan JC, Salwa M, Yikala BS, Chellube ZM. Study on the Distribution of Heavy Metals in Different Tissues

of Fishes from River Benue in Vinikilang, Adamawa State, Nigeria. Curr. J. Appl. Sci. Technol. 2012;2 (4):311-33.
Available:https://journalcjast.com/index.php/CJAST/article/view/1002
[Accessed on 2024 May 22].

33. Idrees N, Sarah R, Tabassum B, Abd_Allah EF. Evaluation of some heavy metals toxicity in Channa punctatus and riverine water of Kosi in Rampur, Uttar Pradesh, India. Saudi Journal of Biological Sciences. 2020;27(5): 1191-4.

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