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Assessment of acute toxicity and behavioural responses of spotted snakehead *Channa punctatus* (Bloch, 1793) exposed to silica nanoparticles

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Abstract- Silica nanoparticles (SiO_2NPs) are primarily made up of silicon dioxide and the size lies in the range of 1-100 nm. These nanoparticles have wide range of applications in nearly every domain because of their unique properties. However, their release in the environment and subsequent implications on aquatic organisms and human beings, is a matter of growing concern. The present study focuses on the assessment of acute toxicity of silica nanoparticles on a most commonly used piscine model, the spotted snakehead *Channa punctatus* (Bloch.). The study also incorporates the behavioural responses of the fish against different concentrations of SiO_2NPs. Medial Lethal Concentration, LC_{50}, was calculated by probit regression method and confirmed by pilot test. Healthy air-breathing fish, *Channa punctatus*, weighing 20-25 grams and length 10-15 cm were procured from Bazar Samiti, Patna, Bihar, and brought to the Aquatic Toxicology Laboratory, Department of Zoology, Patna University, Patna. They were disinfected with 0.01% $KMnO_4$ solution and acclimatized in ideal laboratory conditions for 15 days. They were fed *at libitum*. The physico-chemical analysis of water was done as per the standard methods of APHA (2023). Fishes were exposed to different concentrations of SiO_2NPs under controlled laboratory conditions and mortality rates were recorded after 24 hours, 48 hours, 72 hours, and 96 hours. The behavioural responses including swimming activity, gulping of air, opercula beat, secretion of mucus and equilibrium were also recorded. Abnormal behavioural responses including erratic swimming, air gulping, opercula beat, mucus secretion and loss of equilibrium became more pronounced with the increasing dose and duration of exposure of SiO_2NPs. The results of the present study reflect on the escalating toxicity and cumulative impact of nanoparticles exposure in aquatic organism. It will help in the assessment of environmental risk and the development of regulatory guidelines for nanoparticles exposure. At the same time, it will aid in making strategies for effective management and conservation of the environment.

Key words: Acute toxicity, Behavioural responses, *Channa punctatus*, Conservation, Environment, LC_{50}, Silica nanoparticles.

INTRODUCTION

Nanoparticles (NPs) are tiny particles with dimensions measured in nanometers (one billionth of a meter). Due to their small size, they have unique properties, such as increased surface area to volume ratio and quantum effects. All these unique characteristics enable nanoparticles for a widespread use in industrial, biotechnological, and medicinal applications.[1-3] Silicon dioxide (SiO_2), commonly called as silica, is the most prevalent compound on Earth. Silicate minerals, which are widely dispersed across the Earth's crust, have also been reported in plants and grains.[4] Silica nanoparticles

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(SiO_2NPs) are widely used nanoparticles due to their distinct chemical and physical characteristics.

Silica nanoparticles are obtained from various natural and anthropogenic resources. SiO_2NPs can be synthesized in the laboratory using chemical precursors such as tetraethyl orthosilicate (TEOS), sodium silicate, or silica fume. These precursors undergo controlled reactions to form silica nanoparticles with desired properties. SiO_2NPs can be extracted from naturally occurring sources such as sand, quartz, or diatomaceous earth by the processes like grinding, milling or chemical treatments. These nanoparticles have a wide range of applications across various fields due to their unique chemical and physical characteristics, which include their biocompatibility, huge surface area, small particle size, and controllable diameter.[5] SiO_2NPs are widely used in nano-medicine, diagnostics, gene carriers, drug delivery, bio-sensing, sunscreen lotions, cancer therapy, molecular imaging agents, food additives, stabilizers, cleaning and anticaking agents and in various chemical industries.[6-11] Due to their extra ordinary smaller size and ability to penetrate biological barriers silica nanoparticles have negative impact on the potential health of human beings and other organisms.[12] Environment and open or underground water bodies serve as the final sink for the released silica nanoparticles.[13] Fishes, being at the highest trophic level in the aquatic ecosystem, get easily victimized to the ill effects of these released silica nanoparticles and face the potential risk of their survival.

Fish reacts very fast and sensitively against any changes in the aquatic environment, so considered as bio-indicator species and play an important role in the monitoring of water pollution.[14] They exhibit physiological responses to environmental stressors and pollutants. Teleost fish are considered to be good models to evaluate the toxicity and impacts of toxicant on animals, since their biochemical responses are similar to those of mammals and of other vertebrates.[15]

Channa punctatus, commonly known as the spotted snakehead, is a species of freshwater fish native to South Asia. It has been considered as a model organism in scientific research due to its survivability in wide range of environmental conditions, easily acclimatization in ideal laboratory condition and requiring simple aquatic setups and standard feeding protocols. It shares genetic similarities with other vertebrates, making it relevant for studies in developmental biology, genetics, and evolutionary biology. The present study has been carried out to assess the acute toxicity of SiO_2NPs in the fresh water fish, *Channa punctatus* under ideal laboratory conditions.

MATERIALS & METHODS

Experimental Animal

Channa punctatus of average size 15.2±1.6 cm and average weight 40.4±2.8 g were procured from Fish Market, Bazaar Samiti, Patna, India. In the laboratory, fish were disinfected by giving a bath of 0.1% $KMnO_4$ solution. Fish were acclimated in well-aerated aquaria of 100 L capacity for 15 days in the laboratory. They were fed daily *ad libitum*.

Preliminary Tests

The physico-chemical characteristics of water *i.e.,* temperature, pH, dissolved oxygen were determined as per the standard protocol of Baird & Bridgewater (2017)[16]. These parameters were monitored using the standardized procedures and was maintained throughout the experiment.

Procurement, Characterization and Stock Solution Preparation of SiO_2NPs:

Silica Nanoparticles (SiO_2NPs) of average particle size 20-30 nm and purity 99.9% was purchased from Nano Research Laboratory, Jamshedpur, Jharkhand. The procured nanoparticles were further characterized by x-ray diffraction and scanning electron microscopy. The stock solution was prepared by dispersing SiO_2NPs in double distilled water followed by ultra-sonication at 100 kHz for 45 min prior to the experiment and this was maintained as stock.

Experimental Design:

A range finding test was done. Following that fish were divided into 12 groups (Group I as control and Group II to XII as treated groups) of ten (n=10) fishes each. The test was performed in triplicates. The feeding was stopped a day before or during the test period in order to minimize faeces and surplus food contaminating the test solution. The aquaria were aerated by tube-driven motorized pumps. The control group *i.e.* Group I was kept free of test solution. The test groups were exposed to different concentrations of SiO_2NPs as- Group II- 15000 mg/L, Group III- 16000 mg/L, Group IV- 17000 mg/L, Group V- 18000 mg/L, Group VI- 19000 mg/L, Group VII- 20000 mg/L, Group VIII- 21000 mg/L, Group IX- 22000, Group X- 23000 mg/L, Group XI- 24000 mg/L and Group XII- 25000 mg/

L. Throughout the course of the study, fish behaviour, movement, and death were all closely observed. Fish that remained motionless for a prolonged period of time were regarded as dead and were taken out of the aquarium right away to avoid contamination.

Statistical Analysis:

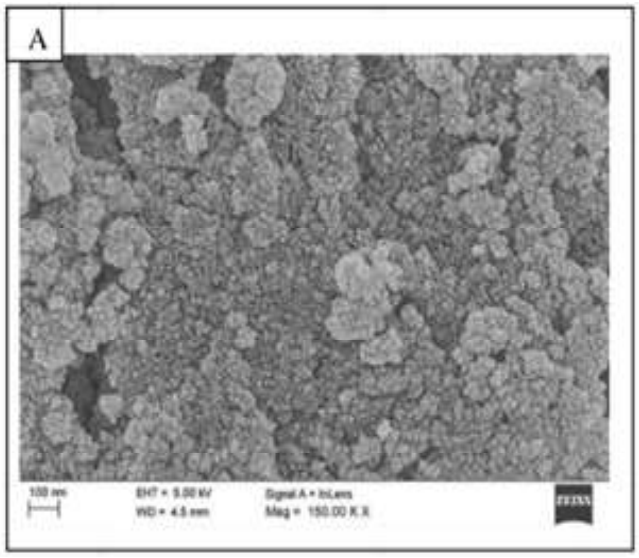
The percentage mortality at 24 hours, 48 hours, 72 hours and 96 hours were recorded. The corresponding LC_{50} values and its confidence limits (95% CLS) were calculated by Finney's probit regression method. The correlation between logarithm of concentration on X-axis and the corresponding probit values of percentage mortalities on Y-axis were taken to obtain the regression equation using MS Excel Office 2021 software. The antilog of concentration gave the value of LC_{50}. All tests were performed in triplicates for the precision of the results.

RESULTS & DISCUSSION

Characterization of SiO_2NPs

Scanning Electron Microscopy (SEM) analysis was done to see the surface morphology and average size of SiO_2NPs. SEM image confirmed the sizes of the particles below 50 nm. The average size was found to be between 20-30 nm. The particles were seen to form agglomerates. This could be overcome by sonicating the dispersion of nanoparticles in a sonicator.[17]

The XRD graph obtained showed a broad peak which confirmed the amorphous nature of this nanoparticle with the value of 2u=22°. The broad peak was seen at 22° which showed the characteristic peak of amorphous SiO_2NPs. No other peaks were seen in the graph obtained.



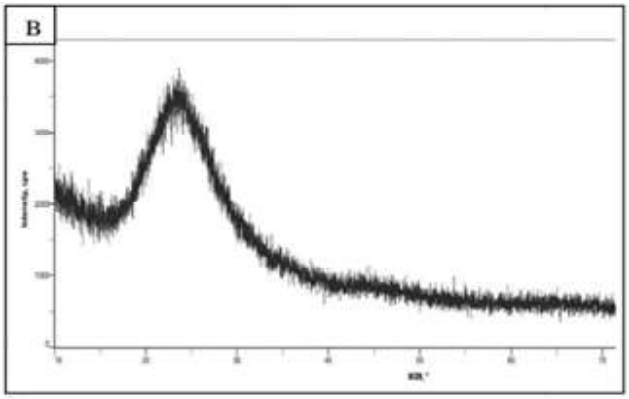


Fig 1: A- SEM image showing size of SiO_2NPs. B- XRD graph showing characteristic peak of SiO_2NPs.

Determination of Medial Lethal Concentration (LC_{50}) for 24, 48, 72 and 96 hours.

There was no mortality seen in the control group during the experimental period. No mortality was recorded when the fish were exposed to 15000 mg/L, 16000 mg/L and 17000mg/L for the entire period of exposure. At 18000 mg/L, 19000 mg/L, 20000 mg/L, 21000 mg/L, 22000 mg/L, 23000 mg/L, 24000 mg/L and 25000 mg/L, percentage mortality were recorded at the end of 24 hours, 48 hours, 72 hours and 96 hours (Table 1).

Median lethal concentration (LC_{50}) for 24 hours, 48 hours, 72 hours and 96 hours were calculated separately by probit regression analysis method. The LC_{50} values of SiO_2NPs for *Channa punctatus* was determined as 25907 mg/L, 24230 mg/L, 23121 mg/L and 22848 mg/L for 24, 48, 72 and 96 hours respectively (Table 2). In this study, it is apparent that the mortality of the fish is positively correlated with concentration of SiO_2NPs. The LC_{50} value were calculated by converting percentage mortality into probit units and plotting against the logarithm of concentration of SiO_2NPs to obtain a regression line and regression equation for each duration of exposure (Figure 2A, 2B, 2C and 2D).

Acute toxicity test plays a vital role in safeguarding animal and human life, protection of environment and guiding human beings towards responsible use and management of substances. Acute toxicity is the apparent undesirable effect induced by a substance to a living being within a short time of exposure.[18] It is the effect that can lead to damage in the organs of a living organism due to exposure of a substance in a short duration of time.[19-20]

Ahmad and Ahmad (2015)[21] have determined 96 hours LC_{50} value for pendimethalin (PND) to the fish

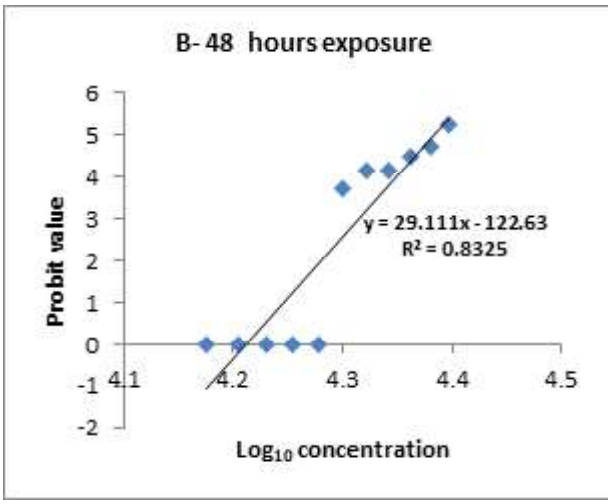
Table 1: Correlation between SiO_2NPs concentrations & mortality rate of *Channa punctatus* at different durations of exposure (24 hours, 48 hours, 72 hours and 96 hours).

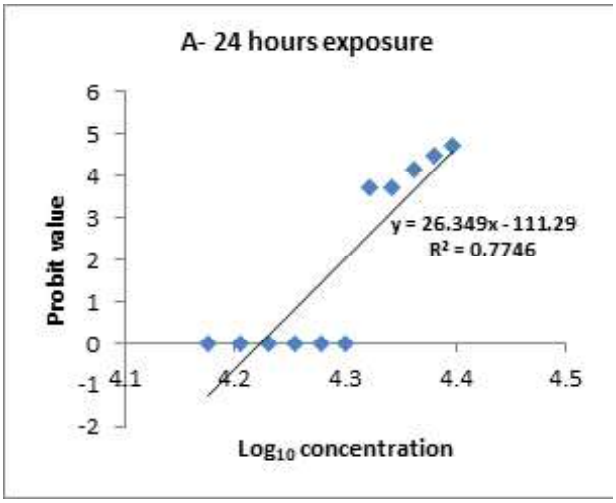
Conc. (mg/L)	Log_{10} values of conc.	24 Hours		48 Hours		72 Hours		96 Hours	
		% mortality	Probit kill	% mortality	Probit kill	% mortality	Probit kill	% mortality	Probit kill
15000	4.176091259	0	-	0	-	0	-	0	-
16000	4.204119983	0	-	0	-	0	-	0	-
17000	4.230448921	0	-	0	-	0	-	0	-
18000	4.255272505	0	-	0	-	10	3.72	10	3.72
19000	4.278753601	0	-	0	-	10	3.72	20	4.16
20000	4.301029996	0	-	10	3.72	20	4.16	30	4.48
21000	4.322219295	10	3.72	20	4.16	20	4.16	30	4.48
22000	4.342422681	10	3.72	20	4.16	30	4.48	40	4.75
23000	4.361727836	20	4.16	30	4.48	40	4.75	40	4.75
24000	4.380211242	30	4.48	40	4.75	50	5.00	50	5.00
25000	4.397940009	40	4.75	60	5.25	60	5.25	60	5.25

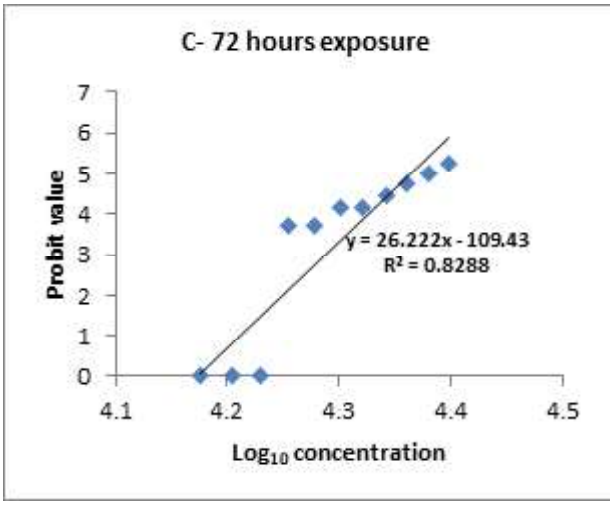
Table 2. The LC_{50} values of SiO_2NPs to *Channa punctatus* at different exposure periods (24 hours, 48 hours, 72 hours and 96 hours).

S. No	Period of exposure	LC_{50} (mg/L)
1.	24 Hours	25907
2.	48 Hours	24230
3.	72 Hours	23121
4.	96 Hours	22848

Channa punctatus as 3.60mg/L. Waghmare and Baile (2017)[22] have reported LC_{50} of applaud (Buprofezin) for 24h, 48h, 72h and 96 h exposure in *Channa punctatus* as 459.29, 326.12, 253.30 and 198.84 ppm respectively. Tiwari and Singh (2004)[23] have reported the 96 h LC_{50} value for oleandrin to *Channa punctatus*. Acute toxicity of cypermethrin and ʎ cyhalothrin have been studied using *Channa punctatus*.[24]







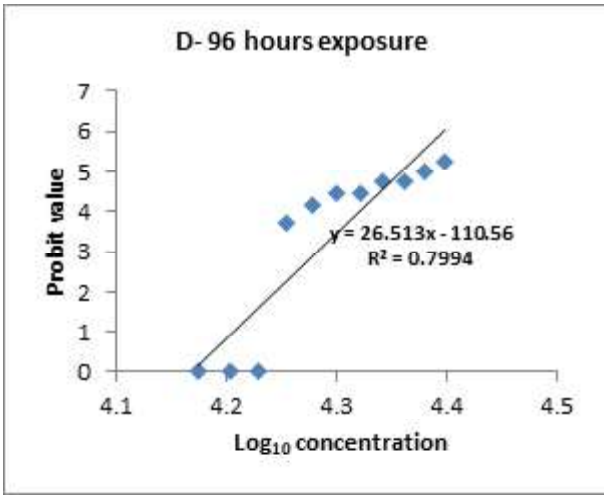


Fig 2: Regression plot between Log_{10} concentration and probit value of SiO_2NPs to *Channa punctatus* at different exposure periods (A- 24 hours, B- 48 hours, C- 72 hours and D- 96 hours).

Behavioural responses in *Channa punctatus* due to acute toxicity of SiO_2NPs

The control group fish exhibited normal behavioural attributes throughout the period of experiment. On the exposure of different concentrations of SiO_2NPs for different durations, the *Channa punctatus* exhibited dose and duration dependent behavioural responses. The behavioural responses observed during the exposure period included erratic swimming, air gulping, opercular beat, mucus secretion and loss of equilibrium. These behavioural responses were seen to be increasing with increase in the concentration of SiO_2NPs and duration of exposure (Table 3).

The behavioural responses of *Channa punctatus* to increasing concentration of SiO_2NPs and period of exposure provide valuable insights into the potential impacts of nanoparticle pollution on aquatic life. The behavioural responses such as erratic swimming, air gulping, opercular beat, mucus secretion and loss of equilibrium become more pronounced as the dose and duration of exposure of SiO_2NPs increases, reflecting on the escalating toxicity and cumulative effect of nanoparticle exposure.

Table 3: Effect of SiO_2NPs on behavioural responses of *Channa punctatus* at different concentrations at different periods of exposure.

Period of exposure	Behavioural parameters	Control group	Dose of SiO_2NPs (mg/L)										
			15000	16000	17000	18000	19000	20000	21000	22000	23000	24000	25000
24 h	ES	-	-	-	-	-	-	+	+	+	++	++	++
	AG	-	-	-	+	+	+	+	++	++	++	+++	+++
	OB	-	-	-	+	+	+	+	++	++	++	+++	+++
	MS	-	-	-	-	-	-	-	+	+	+	+	+
	LE	-	-	-	-	-	-	-	+	+	+	++	++
48 h	ES	-	-	-	-	+	+	++	++	++	++	++	++
	AG	-	+	+	+	+	+	+	++	++	+++	+++	+++
	OB	-	+	+	+	+	+	+	++	++	+++	+++	+++
	MS	-	-	-	+	+	+	+	+	++	++	++	++
	LE	-	-	-	-	-	+	+	+	+	++	++	++
72 h	ES	-	-	-	+	+	+	+	+	+	++	++	++
	AG	-	+	+	++	++	++	++	+++	+++	+++	+++	+++
	OB	-	+	+	++	++	++	++	+++	+++	+++	+++	+++
	MS	-	+	+	+	+	+	++	++	+++	+++	+++	++
	LE	-	-	-	-	+	+	+	+	++	++	++	+++
96 h	ES	-	-	-	+	+	++	++	++	++	++	++	++
	AG	-	+	++	++	++	++	+++	+++	+++	+++	+++	+++
	OB	-	+	++	++	++	++	+++	+++	+++	+++	+++	+++
	MS	-	+	+	+	++	++	++	+++	+++	+++	+++	+++
	LE	-	-	+	+	+	+	++	+++	+++	+++	+++	+++

(Abbreviations: ER = Erratic Swimming, **AG** = Air Gulping, **OB** = Opercular Beat, **MS** = Mucus Secretion and **LE** = Loss of Equilibrium; - = none, + = mild, ++ = moderate, +++ = strong**).**

Narwaria and Saksena (2012)[25] reported similar kind of altered behavioural response in *Puntius sophore* (Bloch) exposed to sodium fluoride. Patel *et al.* (2022)[26] also observed similar kind of changes in locomotor and behavioural attributes in *Channa punctatus* while studying acute toxicity assessment of sodium fluoride to fish. Kumari and Chand (2021)[27] have reported similar irregular, erratic and rapid swimming along with altered physiological responses in air breathing fish *Clarias batrachus,* after exposure to mercury chloride. They revealed that the changes in the behaviour might be due to the hyperactivity of the neural mechanism or secondary stress response in the fish under the influence of stress hormones.

CONCLUSION

The study investigated the acute toxicity of SiO_2NPs on a fresh water fish *Channa punctatus* over a period of 24 hours, 48 hours, 72 hours, and 96 hours. The LC_{50} values decreased with increasing duration of exposure indicating a time-dependent increase in toxicity. The observed behavioural modifications indicate a disruptive impact of SiO_2NPs on the natural behaviour of the fish.

This research contributes to our understanding of the acute toxicity of SiO_2NPs on *Channa punctatus* and provides valuable data for assessing the environmental risks associated with nanoparticle exposure. It also sheds light on the behavioural changes in the fish due to SiO_2NPs exposure. This study highlights the potential ecological implications of SiO_2NPs in aquatic environments and the need for further research to understand their long-term effects on fish populations and aquatic ecosystems. The present study furnishes a launching pad for further study of various toxicological hazards of silica nanoparticles on the aquatic organism at molecular level.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. **Chausali N., Saxena J., and Prasad R. 2022.** Recent trends in nanotechnology applications of bio-based packaging. *Journal of Agriculture and Food Research*, **7:** 100-257.
2. **Fajardo C., Martinez-Rodriguez G., Blasco J., Mancera J. M., Thomas B., and De Donato M. 2022.** Nanotechnology in aquaculture: Applications, perspectives and regulatory challenges. *Aquaculture and Fisheries*, **7(2):** 185-200.
3. **Zhu B., He W., Hu S., Kong R., and Yang L. 2019.** The fate and oxidative stress of different sized SiO_2 nanoparticles in zebrafish (*Danio rerio*) larvae. *Chemosphere*, **225:** 705-712.
4. **Diab R., Canilho N., Pavel I. A., Haffner F. B., Girardon M., and Pasc A. 2017.** Silica-based systems for oral delivery of drugs, macromolecules and cells. *Advances in Colloid and Interface Science*, **249:** 346-362.
5. **Guo C., Liu Y., and Li Y. 2021.** Adverse effects of amorphous silica nanoparticles: Focus on human cardiovascular health. *Journal of Hazardous Materials*. **406:** 124-626.
6. **Slowing I. I., B .G. Trewyn, S. Giri and V. S. Y. Lin. 2007**. Mesoporous silica nanoparticles for drug delivery and biosensing applications. *Advanced Functional Materials* **17:**1225–1236.
7. **Li Z., Barnes J. C., Bosoy A., Stoddart J. F. and Zink J. I. 2012.** Mesoporous silica nanoparticles in biomedical applications. *Chemical Society Reviews.* **1:** 2590–2605.
8. **Tang L., and Cheng J. 2013.** Nonporous silica nanoparticles for nanomedicine application. *Nano today*, **8(3):** 290-312.
9. **Liu Y. Q., Xue S. M., Zhang P., Xu L. N., Wang D. P., Li G., & Cao J. M. 2020.** Silica nanoparticles disturb ion channels and transmembrane potentials of cardiomyocytes and induce lethal arrhythmias in mice. *International Journal of Nanomedicine*, 7397-7413.
10. **Ogawa T., Okumura R., Nagano K., Minemura T., Izumi M., Motooka D., Nakamura S., Iida T., Maeda**

Y., Kumanogoh A. and Tsutsumi Y., 2021. Oral intake of silica nanoparticles exacerbates intestinal inflammation. *Biochemical and Biophysical Research Communications*, **534:** 540-546.

11. **Ahmed H., Gomte S. S., Prathyusha E., Prabakaran A., Agrawal M. and Alexander A. 2022.** Biomedical applications of mesoporous silica nanoparticles as a drug delivery carrier. *Journal of Drug Delivery Science and Technology*, **76:** 103-729.
12. **Napierska D., Thomassen L. C. J., Lison D. & Martens J. A., and Hoet P. H. 2010.** The nanosilica hazard: another variable entity. *Particle and fibre toxicology*, **7(1):** 39.
13. **Rashidian G., Lazado C. C., Mahboub H. H., Mohammadi-Aloucheh R., Prokic M. D., Nada H. S. and Faggio, C. 2021.** Chemically and green synthesized ZnO nanoparticles alter key immunological molecules in common carp (*Cyprinus carpio*) skin mucus. *International Journal of Molecular Sciences*. **22(6):** 32-70.
14. **Srivastava A. K., Mishra D., Shrivastava S., Srivastav S. K., and Srivastav A. K. 2010.** Acute toxicity and behavioural responses of *Heteropneustes fossilis* to an organophosphate insecticide, dimethoate. *International journal of pharma and bio sciences*, **1(4):** 359-363.
15. **Sancho E., Ceron J. J., and Ferrando M. D. 2000.** Cholinesterase activity and hematological parameters as biomarkers of sublethal molinate exposure in *Anguilla anguilla*. *Ecotoxicology and environmental safety*, **46(1):** 81-86.
16. **Baird R., and Bridgewater L. 2017.** *Standard methods for the examination of water and wastewater.* 23rd edition. Washington, D.C., American Public Health Association.
17. **Ghani N. N. A., Saeed M. A., & Hashim I. H. 2017.** Thermoluminescence (TL) response of silica nanoparticles subjected to 50 Gy gamma irradiation. *Malays J Fundam Appl Sci*, **13(3):** 178-180.
18. **Ullah A., Rehman H. U., Saeed W., Faheem M., Raqeebullah W. A., and Saeed K. 2016.** Determination of 96-hr LC_{50} value of cadmium for a fish, *Labeo rohita*. *J. Ent. Zoology Studies*, **4:** 380-382.
19. **Rani K. J., John Milton M. C., Uthiralingam M. and Azhaguraj R. 2011.** Acute Toxicity of mercury and Chromium to *Clarias batrachus* (Linn.). *Bioresearch Bulletin*. **1:**104-108.
20. **Widiastuti I. M., Hertika A. M. S., Musa M., and Arfiati D. 2019.** Acute toxicity test and LC_{50} value of mercury on tubifex tubifex. In *Journal of Physics: Conference Series* (**Vol. 1242, No. 1,** p. 012040). IOP Publishing.
21. **Ahmad I. and Ahmad M. 2015.** Freshwater fish *Channa punctatus*, as a model for Pendimethalin Genotoxicity Testing: A New Approach towards Aquatic Environmental Contaminants. *Environmental toxicology*, Doi: 10.1002/tox.22156:1-10.
22. **Waghmare K. T. and Baile V. V. 2017.** *Channa punctatus* (Bloch, 1793) As a Model for Toxicological Studies. *International Journal of Researches in Biosciences, Agriculture and Technology (IJRBAT)*. **5(2):** 908-911.
23. **Tiwary S. and Singh A. 2004.** Toxic and sublethal effects of oleandrin on biochemical parameter of freshwater air breathing murrel, *Channa punctatus* (Bloch). *Indian journal of Experimental Biology*. **42:** 413-418.
24. **Kumar A. Sharma B. and Pandey R. S. 2007.** Preliminary Evaluation of the Acute Toxicity of Cypermethrin and ʎ Cyhalothrin to *Channa puunctatus*. *Bulletin of Environmental Contamination and Toxicology*. **79:** 613-616.
25. **Narwaria Y. S. and Saksena D. N. 2012.** Acute toxicity bioassay and behavioural responses induced by sodium fluoride in freshwater fish *Puntius sophore* (Bloch). *Fluoride.* **45(1):** 7-12.
26. **Patel K., Kumar M. and Chand G. B. 2022.** Acute toxicity assessment of sodium fluoride in freshwater air breathing fish *Channa punctatus: In vivo* study. *Biospectra.* **17(2):** 89-92.
27. **Kumari K and Chand G. B. 2021**. Acute toxicity assessment of mercuric chloride to freshwater air breathing fish *Clarias batrachus* (Linnaeus, 1758): *In vivo* study. *Agriculture Science Digest*. **41:**242-246.