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Article

Acute toxicity test of monosodium glutamate in *Channa punctatus* and its ecotoxicological implications

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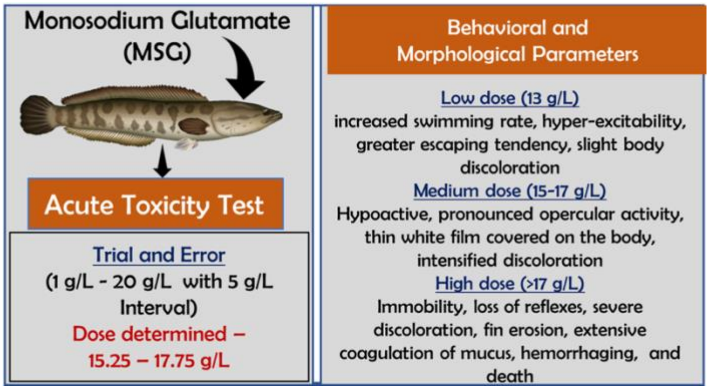

Highlights

- *The increased use of MSG, a food additive, raises ecotoxicological concerns.*
- *This study presents toxicity assessment of MSG and its 96 h LC_{50} value in Channa punctatus.*
- *MSG exposure caused distinct behavioral and morphological alterations as early biomarkers of stress.*
- *Fish health was compromised at low MSG concentrations, highlighting the need for its ecotoxicological monitoring in aquatic environments.*

Abstract

Monosodium glutamate (MSG), the umami substance commonly used in the food industry, is known for its ability to enhance savory flavors. Although it is generally recognized as safe by Food Safety Regulatory Agencies, several studies have questioned its long-term safety. Despite its widespread use, the potential toxicity of MSG therefore remains under-researched. This study aimed to evaluate the acute toxicity of MSG in terms of its median lethal concentrations (LC_{50}) as well as the potential to induce morphological and behavioral changes in the freshwater fish *Channa punctatus* upon short-term exposure. Static bioassay tests were conducted by exposing the fish to different concentrations of MSG and the mortality of the fish was recorded at 24, 48, 72, and 96 hours of exposure. The physicochemical parameters of the aquarium water, such as pH, temperature, dissolved oxygen, total hardness, and total alkalinity, were maintained within their optimal ranges. The LC_{50} value was determined using probit analysis following standard guidelines to ensure accuracy and reproducibility. The results indicate that MSG exhibited acute toxicity to *C. punctatus* within the tested range of concentrations. Furthermore, a dose-dependent increase in mortality as well as marked behavioral and morphological changes were observed. The severity of symptoms increases at higher MSG concentrations, indicating induction of physiological stress in exposed fish. This is the first study to assess the acute toxicity of MSG on the fish *C. punctatus*. These findings provide baseline information that will aid further toxicological research and monitoring of the long-term effects of MSG on aquatic organisms.

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1 Introduction

People are increasingly exposed to a myriad of chemical agents due to their changing lifestyles including food habits (FAO and WHO, 2008). Several of these chemical substances are likely to be toxic even at the level of DNA and chromosomes (Sharma and Chadha, 2021). Toxicity assessment of these substances is, therefore, very crucial for detecting and predicting their potential hazards for humans.

Monosodium glutamate (MSG), the sodium salt of glutamic acid, is one of the widely used food additives popularly referred to as "China Salt" (Niaz et al., 2018). It enhances the natural flavors of poultry, meat, snacks, seafood and stews (Adeyemo and Farinmade, 2013), and is also added to soups, sauces and meal preparation products (Jinap and Hajeb, 2010). It was traditionally extracted from protein-rich foods like fish, algae and certain vegetables (Yamaguchi and Ninomiya, 2000; Stańska and Krzeski, 2016). As a prototypical umami ligand, MSG is frequently used in Asian cuisine (Li et al., 2013) as a fifth distinct taste (Ninomiya, 2002).

Although regulatory authorities generally regard MSG as safe at normal dietary intakes, growing preclinical and mechanistic evidence indicates that excessive or chronic exposures may trigger organ dysfunction, neurotoxic changes, oxidative stress and metabolic disturbances in mammalian models. Recently, the use of MSG has been linked to the appearance of various adverse health effects in human including obesity, neurological disorders like Alzheimer's disease, arthritis, fibromyalgia and other neurodegenerative diseases including those of Parkinson's, Huntington's, and amyotrophic lateral sclerosis (Lau and Tymianski, 2010). It affects gastrointestinal physiology, and contributes to oxidative stress, endocrine disruption and metabolic changes (Chaohua, 1994; Moreno et al., 2005; Chakraborty, 2019). MSG has further been associated with nephrotoxicity, hepatotoxicity, asthma, urticaria and abnormal cell growth (Nnadozie et al., 2019). Additionally, studies report its impact on the pancreas, liver, kidney, spleen, brain and reproductive system, causing functional impairments and inflammatory changes (Al-Mosaibih, 2013; Alalwani, 2014; Ajibade et al., 2015). Recent studies continue to document hepatic, neurological and metabolic perturbations after prolonged MSG exposure (Essawy et al., 2025; Otomewo et al., 2025; Vaithilingam et al., 2025). Moreover, concerns about MSG's safety arise from reports of adverse reactions in individuals consuming MSG-containing foods, emphasizing the need for its toxicological evaluation (Farombi and Onyema, 2006).

Ecotoxicological data on MSG are relatively scarce. In recent decades, there has been a substantial increase in the discharge of MSG into aquatic environment which stems from sources such as effluents from ready-to-eat food processing industries, household cooking and MSG production plants. Consequently, assessing the ecotoxicological risks of MSG in aquatic ecosystems has become highly pertinent. Fish, a constituent of aquatic food chain, have been considered as an efficient and effective model to explore the toxic potential of chemical substances (Monteiro et al., 2010; Sharma and Chadha, 2021). They occupy various zones of aquatic habitat, exhibit sensitivity to toxicants even at low concentrations, tend to bioaccumulate them, and provide early warning signals for environmental changes (Sharma et al., 2018). The first response to environmental change in organisms is the frequent alterations in their behavior (Nagelkerken and Munday, 2016; Sharma and Chadha, 2021) which provides a comprehensive measure of exposure to multiple stressors. Fish are sensitive bioindicators that integrate environmental stressors and frequently exhibit early behavioral and morphological responses before overt mortality occurs. The behavior of an organism results from several complex processes of development and physiology (Wong and Candolin, 2015; Sharma and Chadha, 2021). Behavioral endpoints often manifest earlier and even at lower doses than classical lethal endpoints, making them a suitable model in aquatic toxicology (Scott and Sloman, 2004; Gabriel et al., 2011; Porras-Rivera et al., 2024).

Standardized testing protocols are a critical component of risk assessment and regulatory processes (Burden et al., 2020; Pulido-Reyes et al., 2024). The fish acute toxicity test, a frequently used aquatic ecotoxicity test for environmental risk assessment, is a crucial method used to evaluate the effects of a test substance at its various concentrations on a group of test organisms during short-term exposure based on OECD test Guideline 203 (OECD, 2019), or similar internationally recognized guidelines (US EPA, 2016). Among the various methods available for short-term toxicity evaluation, the determination of the median tolerance limit (TLM) or median lethal concentration (LC_{50}) through probit analysis (a statistical bioassay) is a widely recognized and accepted approach (Dean et al., 1972; Lewis and Finney, 1972; Chaohua, 1994; APHA, 2023).

Studies on the toxicity of MSG in aquatic organisms, particularly fish, are limited. Some aquatic studies provide preliminary insights. In *Labeo rohita*, Perumalsamy et al. (2024) reported a 96 h LC_{50} (1.5 g/L) for MSG exposure accompanied by mucus secretion, erratic swimming and marked histopathological changes in gills, liver and kidney. Similarly, Zebrafish embryo studies demonstrated significant developmental malformations, cardiotoxicity, and reduced survival at high MSG concentrations (Suthamnatpong and Ponpornpisit, 2017; Malathi et al., 2023). More recently, Zhao et al. (2025) highlighted how dietary glutamate alters metabolism and growth in cultured fish species, highlighting the ecological relevance of such exposures.

Despite these studies, major gaps in our understanding remain. Data for *Channa punctatus*, an ecologically and commercially important freshwater fish, are lacking. This fish, used in various toxicological experiments, has several ecotoxicological characteristics (Pandey et al. 2005; Jha et al. 2019). Moreover, very few investigations have simultaneously examined acute lethality, external morphological alterations and behavioral responses at sublethal, environmentally relevant concentrations of MSG. Therefore, the primary aim of this investigation is to assess the toxicity of MSG through behavioral and morphological parameters in the freshwater fish, *Channa punctatus*, using acute toxicity test to determine LC_{50} over a 96-hour period. This attempt represents the first comprehensive study to integrate LC_{50} estimation with sublethal behavioral and morphological endpoints in *Channa punctatus,* which potentially links early warning signs with mortality. Additionally, it intends to provide a more ecologically relevant assessment of MSG toxicity in freshwater systems.

2 Materials and methods

The methodological flowcharts used in the study are summarized in **Figure 1**.

2.1 Ethical approval

The Institutional guidelines for the care and use of animals in the experiment were followed by the authors.

2.2 Procurement of fish

Adult and healthy specimens of *Channa punctatus* (20–25 g in weight, and 10–15 cm in length) were procured from local market and were subsequently transported to the Ecotoxicology Laboratory, Patna University, Patna, India.

2.3 Acclimatization in the laboratory

To prevent injuries and bacterial infection, the fish were treated with 0.01% potassium permanganate solution and then housed in aerated 90-liter aquaria containing dechlorinated water. They were acclimatized for 15 days, and the aquaria water was changed daily. They were fed on pieces of boiled egg and fish food during the period of acclimation. Feeding was discontinued 24 hours prior to the onset of experiment to avoid metabolic interference.

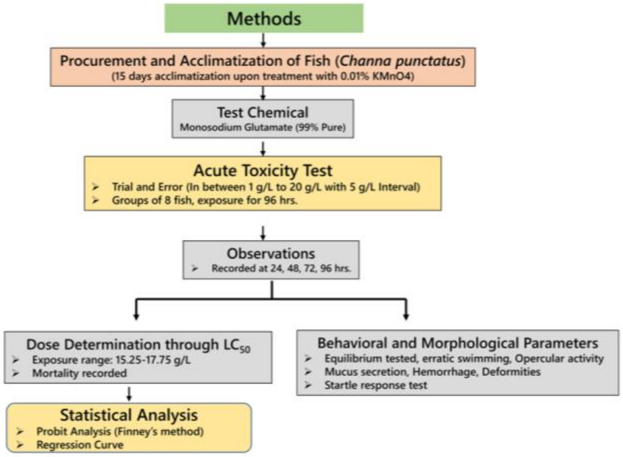

Figure 1. *Flow diagram of the methods followed in the study.*

The fish were properly acclimatized in the laboratory at the optimum physicochemical conditions, and such conditions were maintained throughout the experiment to ensure the optimum growth and survival of fish, as well as to prevent any stress arising due to physiological disruption. It will further ensure the reproducibility, accuracy, experimental integrity and analytical precision.

2.4 Test chemical

Monosodium glutamate as extra-pure (99%) white crystals was obtained from Sigma Aldrich (USA). A stock solution (1% v/v) of MSG was prepared in double-distilled water and stored in a clean standard flask at room temperature. The physicochemical parameters of the tap water were noted periodically before the determination of 96 h LC_{50}.

2.5 Behavioral and morphological indicators

Upon exposure of the fish to various concentrations of MSG, observations were made on their behavioral and morphological responses at 24, 48, 72 and 96 hours. Control fish specimens were monitored simultaneously to provide a reference for assessing the occurrence of any behavioral or morphological change. The observed behavioral and morphological responses include loss of equilibrium, mucus secretion, opercular activity, erratic movement, startle response, hemorrhage and deformity. Startle responses were monitored by the following procedure in sequence: passing a hand over the test tank (overhead moving visual stimulus), rapping on the tank (vibration stimulus) and lightly touching the fish with a stick (tactile stimulus). Therefore, along with mortality, the behavioral responses and morphological alterations in the fish specimens exposed to different concentrations of MSG in separate 90L tanks were observed.

2.6 Acute toxicity test

An acute toxicity test was conducted as per APHA (2023) guidelines based on OECD or USEPA protocols (US EPA, 2016; OECD, 2019). It was used to determine the lethal concentration of MSG that causes death in 50% of test population. Separate tanks were used for each exposure level, ranging from 1 g/L to 20 g/L with an interval of 5 g/L (as there was no previous data). It was tested in triplicate to identify the appropriate exposure levels in order to verify the results. Accordingly, the test fish were exposed to all these concentrations of MSG till the occurrence of 100% mortality. Water was changed daily, and the concentration of MSG was regularly monitored in aquaria water. Dead fish specimens were removed promptly.

2.7 Median lethal concentration (LC_{50}) test

The mortality percentage of fish exposed to different concentration of MSG were recorded at an interval of 24 hours for LC_{50} determination as per the experiment performed by Perumalsamy et al. (2024) with certain modifications. As mortality was reported above the MSG concentration of 15 g/L, tanks were further subdivided into narrow range of concentrations, starting from 15.25 g/L with an interval of 0.5 g/L. Accordingly, experimental fish specimens were divided into separate groups, each with 8 specimens which were exposed to a specific MSG concentration. To maintain optimal dissolved oxygen levels, the test solution was renewed daily throughout the experiment. Each test was conducted in duplicates, each repeated three times, to ensure reliability of results.

2.8 Probit analysis

The mortality data from the definitive test were statistically analyzed using Microsoft Excel 2019 by converting the concentrations into their logarithmic values and corresponding mortality percentages into probit values using Finney's probit analysis method (Lewis and Finney, 1972).

2.9 Regression analysis

A regression curve was plotted with log concentrations on the X-axis and probit values on the Y-axis to evaluate the relationship between MSG dose and mortality rates. Mortality data, recorded at 24, 48, 72 and 96 hours, were used to determine the LC_{50} value through dose-response analysis.

3 Results

3.1 Physicochemical parameters

The optimum physicochemical parameters of water, recorded and maintained from the period of acclimatization to toxicity test, were pH 7.0 ± 0.6, temperature 26°C ± 2°C, dissolved oxygen 6.6 ± 0.3 mg/L, total hardness 176 ± 2 mg/L, total alkalinity 74 ± 3 mg/L and electrical conductivity 255-282 µS/cm.

3.2 LC_{50} determination

The mortality rate in the control group was within 10% and the remaining specimens of fish looked healthy throughout the experiment upon exposure to MSG. However, differential mortality of fish was observed at its various levels during the estimation of LC_{50} value (**Table 1, Fig. 2**). The study revealed a direct relationship between increasing MSG concentration and the degree of fish mortality. The LC_{50} value for MSG at 96 hours of exposure was determined following the method of Miller and Tainter (1944) as mentioned by Randhawa (2009), and it was derived as 16.45 g/L. The regression equation highlighted that the longer the exposure duration, the higher the mortality rate.

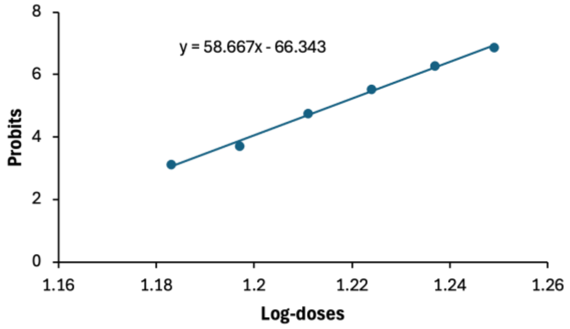

Figure 2. *Correlation between log-concentration of MSG and fish mortality (probit).*

3.3 Behavioral responses

Behavioral responses of *Channa punctatus* following exposure to MSG showed a clear dose-dependent pattern (**Table 2**). At lower concentrations (up to 10 g/L), no noticeable behavioral alterations were observed. However, exposure to 13 g/L induced increased swimming rate, startle response signs of hyperexcitability and greater escaping tendency. At 15 g/L, fish became hypoactive with darting movements and frequent surfacing. A further increase to 17 g/L resulted in marked behavioral impairments, including pronounced opercular activity, loss of buoyancy and significant reduction in movement. At the highest concentration level (19 g/L), severe behavioral deterioration was evident, characterized by the absence of reflexes, complete loss of schooling behavior, frequent gasping at the water surface and immobility.

Table 1. *Dose-response during the determination of LC_{50} of MSG in Channa punctatus at a 96-hour exposure period.*

Experimental Groups	Dose (g/L)	Log value	Mortality (%)	Corrected mortality (%)	Probit value
1	15.25	1.183	0	3.1	3.13
2	15.75	1.197	10	10	3.72
3	16.25	1.211	40	40	4.75
4	16.75	1.224	70	70	5.52
5	17.25	1.237	90	90	6.28
6	17.75	1.249	100	96.9	6.87

Table 2. *Behavioral and morphological changes in Channa punctatus after sub-acute exposure to MSG.*

Dose (g/L)	**Behavioral changes**	**Morphological changes**
<10	No visible changes.	No visible changes.
10 – 13	Startle response, increased swimming rate, increased escaping tendency, and hyperexcitability.	Slight discoloration.
13 – 15	Hypoactive, darting movements, and frequent surfacing.	Mild discoloration, and the body appeared covered with a thin white film.
15 – 17	Significant reduction in movement, loss of buoyancy, and high opercular activity.	Severe discoloration, and destruction of scales.
17 – 19	Loss of schooling behavior, immobility, loss of reflexes, gasping at the surface.	Change in color of the gill lamellae, coagulation of mucus, extensive hemorrhaging, and fin erosion.

3.4 Morphological alterations

Morphological alterations also followed a concentration-dependent trend as these changes became more prominent with increasing concentration (**Table 2**). No visible external changes were noted up to 10 g/L MSG exposure. Exposure to 13 g/L induced slight body discoloration, which became more pronounced at 15 g/L along with the appearance of a thin white film covering the body. At 17 g/L, scales showed signs of destruction with intensified discoloration, so the external damages became apparent. The highest concentration (19 g/L) caused extensive morphological abnormalities, including severe discoloration, fin erosion, changes in the color of gill lamellae, extensive hemorrhaging and coagulation of mucus.

4 Discussion

The present study deals with the assessment of the acute toxicity of MSG in *Channa punctatus*, an unexplored area of aquatic toxicology, coupling LC_{50} with behavioral and morphological endpoints. A linear correlation was observed between the logarithm of MSG concentration and fish mortality (probit), based on values mentioned in **Table 1**, as shown in **Figure 2**. Hence, the result clearly demonstrated that exposure to MSG from its lower to higher levels in *C. punctatus* produced a concentration-dependent increase in mortality. This agrees with earlier reports on other teleost species such as *Labeo rohita* (Perumalsamy et al., 2024) and zebrafish embryos (Suthamnatpong and Ponpornpisit, 2017), as previously noted. As also highlighted in studies by Hrovat et al. (2009) and Spurgeon et al. (2020), such variability in LC_{50} values across species and life stages reflects differences in their physiology, size, metabolic activity, and sensitivity to glutamate.

In the present study, *C. punctatus* exposed to MSG exhibited marked behavioral ailments with increasing concentrations (such as no response at lower concentration, hyper excitability at middle concentration followed by hypoactive responses at higher concentration). These observations are consistent with previous findings by Perumalsamy et al. (2024) showing distinguished behavioral abnormalities in *Labeo rohita* at a controlled 96 h exposure to a sub-lethal dose of MSG. Comparable findings (discoloration of body, gulping of air, reduced opercular movement and loss of swimming activity with increasing exposure) were also reported in Zebrafish under MSG stress (Malathi et al., 2023). The abnormal behaviors observed in the fish might be caused by the disturbances in neural transmission, aggravations in metabolic pathways and by the irritation to the perceptive system of the body (Sharma and Chadha, 2021). Behavioral changes are often the earliest and most sensitive indicators of sublethal toxicity, integrating multiple physiological pathways including neurotoxicity, oxidative stress, and respiratory impairment (Scott and Sloman, 2004; Hellou, 2011; Witeska, 2024; Formicki et al., 2025). The similarity of the observed behavioral changes in *C. punctatus* to those previously reported in other fish models suggests the occurrence of a common stress response pattern to MSG exposure.

In addition to behavioral changes, morphological alterations (including excessive secretion of mucus) were also observed in *C. punctatus* following exposure to MSG. The secretion of excessive mucus is probably caused by the irritation of the skin due to direct contact with the toxicant

(Sharma and Chadha, 2021). These changes are consistent with those observed in previous studies in other fish species exposed to MSG (Flores-Lopes and Thomaz, 2011; Perumalsamy et al., 2024). Similar morphological manifestations have also been documented in fish exposed to other food additives and agrochemicals (Hoyle et al., 2007; Loganathan et al., 2024). Such alterations often reflect impaired osmoregulation, disruption of tissue homeostasis and increased oxidative stress, thereby providing visible endpoints that can be directly linked to physiological dysfunction.

Malathi et al. (2023) observed clear morphological and metabolic changes in adult zebrafish exposed to MSG (96 h LC_{50} ≈23.98 ppm). The morphological and behavioral alterations observed in fish specimens exposed to MSG might be resulting from significant decreases in whole-body protein and carbohydrate content with higher MSG doses (Malathi et al., 2023). Loss of skin pigmentation may result from the modification in the capacity of melanocyte stimulating hormone and melanin concentrating hormone (Madelaine et al., 2020; Vissio et al., 2021). Prolonged increase in the swimming activity leads to weakening of muscles in the fish and significant energy depletion, making them more vulnerable to other environmental stressors (Magnoni et al., 2013; Ibrahim and Mahmoud, 2024). Kurnianingsih et al. (2016) observed raised activity of neural apoptosis markers (bax/bcl-2 ratio, caspase-3, Ca^{2+}, and apoptotic cells in brain) at early embryonic stage of zebrafish, raised in MSG containing water, expressing reduced overall locomotor and increased stereotypic (circular) swimming. In a similar embryonic study, zebrafish eggs showed dose-dependent deformities where high-MSG groups developed growth retardation, yolk-sac edema, chorion shrinkage, tail curvatures, loss of pigmentation and scoliosis (Mahaliyana et al., 2016).

Undoubtedly, the exposure of *C. punctatus* to MSG has resulted in significant behavioral and morphological changes, reflecting the toxic impact of MSG on physiological functions of the fish, particularly on its neurophysiology. Contamination of water bodies with MSG, therefore, appears to be a potential threat to the survival and health of aquatic organisms. While laboratory concentrations used in acute toxicity assays are generally higher than its environmentally relevant levels, the observed morphological disruptions and behavioral responses at sublethal levels highlight the ecological relevance of chronic, low-level MSG exposure in aquatic systems. Studies in *Oreochromis niloticus* and *Clarias batrachus* have already shown that even non-lethal chemical exposures can compromise reproductive output, predator avoidance and feeding behavior (Abu Zeid et al., 2021; Mukherjee et al., 2022). Therefore, the present study contributes to growing evidence that MSG, though widely regarded as a safe food additive, poses measurable ecotoxicological risks in aquatic environments. The calculated LC_{50} value for the same toxicant, however, may differ substantially among fish species under similar exposure conditions due to their differential sensitivity and response (Shuhaimi-Othman et al., 2013; Islam et al., 2021). While using MSG containing wastewater as fertilizer, it has been reported that MSG concentration below 1% produces a positive effect on the germination indices of seeds (Singh et al. 2009). However, MSG concentration above 2% inhibits germination in seeds (Liu et al. 2007). Though the levels of MSG exposure used in the present study (1 g/L or 0.1% to 20 g/L or 2%) are within the non-toxic limit of MSG concentration, but still capable of producing toxic consequences in the fish, reflecting its environmental relevance for aquatic animals. Nevertheless, it highlights the need for species-specific data on MSG toxicity to better understand the broader ecological implications.

5 Conclusion

The study demonstrates that MSG exhibits dose-dependent toxicity in fish, with mortality increasing as exposure levels rise. The current study offers novel insights into the acute toxicity of MSG, including its LC_{50} value for *Channa punctatus,* where limited or no prior information was available. Moreover, it shows that MSG poses a potential risk to aquatic life, capable of causing notable behavioral and physiological changes in the exposed individuals. This may further lead to broader ecological consequences including disturbances in population dynamics and trophic levels within the ecosystem. These findings emphasize the importance of further research into the environmental impacts of MSG on diverse aquatic species, extending beyond the widely studied zebrafish.

6 Data availability statement

The data that supports this research will be shared upon reasonable request to the corresponding authors.

7 Ethical statements

The entire experimental design, including the use of test organisms, was approved by the Animal Ethical Committee (IEC) of the Department of Zoology, Patna University, Patna.

8 Conflict of interest

The authors declare that they have no actual or potential competing financial interests.

9 Acknowledgements

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10 Author contributions

Farha Ashique: Conceptualization, writing original draft, methodology, data collection, data analysis, software, and visualization. Pankaj Kumar: Review of draft, data analysis, and visualization. Kainat Masih: Data analysis and review.

Amod Kumar: Data analysis and review. Parimal Kumar Khan: Conceptualization, draft editing, and supervision.

11 Copyright statement

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