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Developmental Toxicity of Monosodium Glutamate on Zebrafish Embryos

Megha Madhusudhanan[1], M R Srinivasan[2], P Jalantha [3] and Azhahianambi P[4]

[1]Laboratory animal veterinarian, University of Calicut, Kerala.
[2]Assistant Professor, Veterinary Clinical Complex, Veterinary College and Research Institute, Tirunelveli, Tamil Nadu Veterinary and Animal Sciences University.
[3]Assistant Professor, Laboratory Animal Medicine unit, Tamil Nadu Veterinary and Animal Sciences University, Chennai.
[4]Assistant Professor and Scientist, Translational Research Platform for Veterinary Biologicals, Tamil Nadu Veterinary and Animal Sciences University, Chennai.

ABSTRACT

Monosodium glutamate (MSG) is widely used as a food additive worldwide and the safety of MSG has always been a matter of concern. This study was conducted to assess the effect of MSG on foetal development using zebrafish embryos. Zebrafish embryos were collected from the spawning group of a male: female ratio of 1:2. Fertilized embryos in the blastula stage were transferred into 24-well plates and kept at 26.5 ±1°C. MSG in the concentration of 300 mg/L, which was then serially two-fold diluted to 150 mg/L, 75 mg/L, 37.5 mg/L, 18.75 mg/L, 9.38 mg/L, 4.687 mg/L, 2.34 mg/L, was used for treatment, and the control wells contained 2mL of RO water. Observations were recorded 24, 48, 72, and 96 h post-fertilization. MSG induced toxicity, mainly delayed development, reduced melanin pigmentation, and pericardial edema in zebrafish embryos at concentrations of 100 mg/L and above. MSG caused a retarded growth from 24 h to 72 h, but growth by 96 h could recover the developmental delay. Hatching was delayed in the treatment group, and by 96h, all surviving embryos had hatched. Overall, it can be concluded that these developmental defects may be due to oxidative stress and neurotoxic effects of glutamate at concentrations above 100 mg/L. Thus, MSG is toxic to developing embryos and can delay growth and metabolism in a concentration-dependent manner.

Keywords: Developmental delay, Monosodium Glutamate (MSG), Melanin deposition, zebrafish embryo.

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INTRODUCTION

Food additives and taste enhancers have been used for many decades to preserve and enhance the palatability of foods. Nowadays, many people prefer consuming "ready-to-eat" foods, which are easy and fast to prepare, but may contain added taste enhancers and preservatives to preserve and enrich the quality of the food. Monosodium glutamate (MSG) is one such taste enhancer that mainly contributes to the "umami" taste, meaning a delicious taste. It was first introduced in Japan by Professor Ikeda in 1909 and was isolated from a specific seaweed, which

*Corresponding author.
Megha Madhusudhanan.
Laboratory animal veterinarian, University of Calicut, Kerala
Email- meghamadhu2019@gmail.com

has since been used worldwide as a taste enhancer. It is later produced by the fermentation of different carbohydrate sources. Monosodium glutamate is a sodium salt of glutamic acid, which is normally present in all protein foods, and irrespective of ethnicity, adult humans consume approximately 10–20 g of MSG daily (Rhodes *et al*.,1991). On average, in European countries, glutamate salts were consumed at the rate of 0.3-0.5g/day, and in Asian countries, it is 1.2-1.7g/day (Beyreuther *et al*., 2007). The first report of the adverse effect of MSG as a food additive was published in the year 1968, describing it as "monosodium glutamate symptom complex" (Williams & Woessner, 2009). Since then, the safety of MSG as a food additive has been the subject of research and analysis. According to the Food Safety and Standards (Food Products Standards and Food Additives) regulation 2011, the use of monosodium glutamate is restricted to selected food items and should be indicated in the list of contents. According to the FSSAI, the maximum uptake of monosodium glutamate in food products (canned crab meat) is to 500 mg/kg of meat. Chemicals, especially those used as food additives, can have harmful effects on humans, particularly during fertilization, pregnancy, and infancy. Various preclinical studies have revealed that the administration of MSG can lead to cardiotoxicity, hepatotoxicity, low-grade inflammation, metabolic disarray, premalignant alterations, and behavioral changes (Zanfirescu *et al*.,2020). Chronic consumption of MSG in humans can lead to obesity, oxidative stress, neurotoxicity, genotoxicity, diabetes, and kidney failure (Kazmi *et al*.,2017). The use of MSG in food is correlated with the development of metabolic disorders in Thailand (Insawang *et al*., 2012). When used as a food additive in a high-lipid diet (HLD), it can act as a silent killer of immune cells and lead to immunomodulation and splenomegaly (Das *et al*., 2022). The concentration of glutamate and aspartate in plasma was found to be high upon chronic consumption of MSG (Graham *et al*., 2000). Excessive daily intake of MSG causes elevated plasma levels of glutamate. The results of the effect of MSG on developing fetus were equivocal. Exposure of the mother to MSG could lead to the induction of skeletal abnormalities in newborns, as MSG could cross the placental barrier (Shosha et al.,2023), and chronic use of MSG is reported to induce severe injury to the male reproductive system by inducing oxidative stress (Jubaidi *et al*., 2019). Hence, a study on the effects of MSG on the development of the fetus or embryo is important, as it can be crucial in deciding the diet during pregnancy or during the neonatal stages. Zebrafish, a member of the Cyprinidae family, have been used as a model organism for biological research since the 1930s. They offer several advantages for conducting *in vivo* studies related to developmental biology and toxicology. In addition to the genetic homology of zebrafish with higher vertebrates, they share a common developmental process and embryogenesis, with a similar basic chordate body plan (Bauer et al.,2021). Zebrafish embryos are remarkably permeable, allowing small molecules added in water to diffuse easily into the embryo. The gross morphological effects of a toxicant can be easily visualized due to the transparency of the embryonic and larval stages and the availability of fluorescent reporter molecules, finally giving the possibility of correlating and determining the affected molecular pathway (De Esch *et al*.,2012). In addition, the data acquired for fish embryo toxicity (FET) can be extrapolated to adult fish toxicity (AFT) testing, as both are found to be comparable. It has also been noted that if FET could be regarded as an alternative to AFT, then FET will provide nearly equivalent predictions of hazards while improving overall animal welfare (Belanger *et al*.,2013). Suthamnatpong *et al*.,2017 reported that exposure of zebrafish embryos at 48hpf to MSG at the concentrations of 15, 150 and 1,500 ppm led to abnormal embryo cardiac function and tachycardia, appearance of abnormal body curvature, pericardial edema, yolk edema, or abdominal edema. Reversible responses, such as lateral recumbency, head tilt, and upside-down position of the hatching larva, were also observed. The mortality rate in this study was determined at a higher concentration of MSG, i.e. between 5000ppm and 160,000ppm, whereas the present study is done using lesser concentrations of 300ppm and below, and the concentration of MSG, which decreased the hatching percentage of the embryos to 50% was determined.

To address these concerns, the present study was undertaken to evaluate the effect of MSG on the development of zebrafish embryos and to assess morphological changes in embryos. This could be an indication of the effect of exposure to MSG during gestation and the neonatal stages of growth.

MATERIALS AND METHODS

Chemicals

Monosodium glutamate (L-glutamic acid monosodium salt monohydrate (MSG), extra pure, 99%) was purchased from SRL Chemicals, India, and used in this study. The stock solution was prepared by solubilizing MSG in double-distilled water at concentrations of 100 mg/L and 300 mg/L. The solution was prepared fresh when the experiment was conducted.

Preparation of test solutions of MSG

A stock solution of 300 mg/L was prepared and two-fold diluted to 150 mg/L, 75 mg/L, 37.5 mg/L, 18.75 mg/L, 9.38 mg/L, 4.687 mg/L, 2.34 mg/L. Another concentration, 100 mg/L, was used for the experiment. Six wells containing 2mL of reverse osmosis (RO) water were used as controls. All the test solutions were prepared at 26°C in a temperature-controlled room.

Maintenance of zebra fish and embryo collection

The experiment was performed at the Laboratory Animal Medicine unit of the Tamil Nadu Veterinary and Animal Sciences University, Madhavaram, Chennai, with the IAEC approval number 14/SA/IAEC/2023, dated 07.01.2023. Adult wild zebra fish (*Danio rerio*), 6 months of age, were maintained in a static aqua culture system supplied with filtered and reverse osmosis-purified (RO) water in a 14:10 h light: dark cycle at 26±1°C. Water was aerated continuously using an aquarium air pump. The fish were fed twice per day with adult zebrafish commercial fish feed. Eggs were collected from the spawning group at a male: female ratio of 1:2 and kept in a separate breeding tank. After spawning, the embryos were collected and transferred into clean Petri dishes containing RO water and allowed to grow until 4 h post-fertilization (hpf). The embryos were carefully washed to remove debris, while unhealthy and dead embryos (appearing as white, coagulated, opaque eggs) were removed by aspiration using a disposable plastic Pasteur pipette. Microscopic observation of the collected eggs was performed at 4 hpf to observe the embryonic development prior to treatment. Eggs that reached the blastula stage were selected for experiments. These maintenance and selection criteria were based on OECD guideline 236 for the acute toxicity study of fish embryos.

Zebrafish Embryotoxicity Test

Fertilized embryos were selected at the blastula stage and transferred into 24-well plates. Two sets of plates were used in the study, with six control wells in each plate. The test was initiated at 4 hpf and ended at 96 hpf, which covers the entire period of organogenesis in zebrafish embryos. The 24-well plates were kept in an incubator at 26.5 ±1°C with a light/dark cycle of 14-hour light/10-hour dark. The exposure was performed at the start of the light cycle. The total volume of each well was 2mL of test solution of the corresponding concentration, with the first well of 100 mg/L and the second well of 300 mg/L, which was then serially two-fold diluted to 150 mg/L, 75 mg/L, 37.5 mg/L, 18.75 mg/L, 9.38 mg/L, 4.687 mg/L, 2.34 mg/L. Each well was duplicated and the control wells contained 2mL of RO water. All test solutions were renewed every 24 h, and dead eggs were removed.

Evaluation of embryos for morphological changes

The health status of embryos was recorded at 4, 6, 24, 48, 72, and 96 hpf during the exposure period. Survival and sublethal end points were assessed at these time points. Sets of developmental events such as tail detachment, somite formation, eye development, embryo movement, heartbeat, blood circulation, pigmentation of the head and body, pigmentation of the tail, pectoral fin development, and protruding mouth were assessed. Mortality and hatchability rates were also determined. The stages of fish embryo development were studied according to OECD 236 and Kimmel *et al.*,1995. The five main observation points were mortality rate, detachment of tail and resorption of yolk sac, development of somites and eye, melanin pigmentations over the body, pericardial and yolk sac oedema.

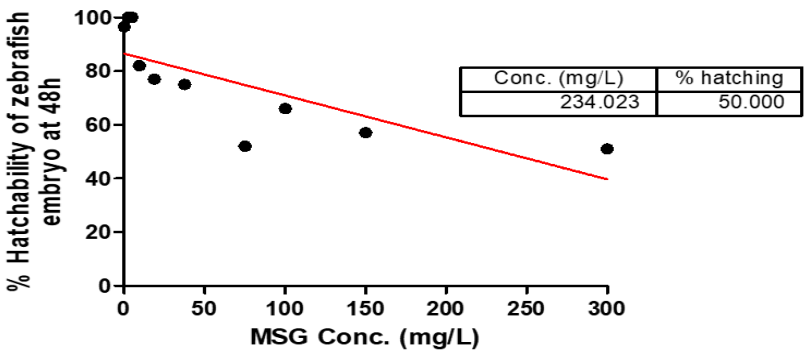
STATISTICS

The percentage hatchability of the embryos was calculated for all the tested concentrations. By using the linear regression method, the concentration required to delay 50% of the hatchability was calculated.

RESULTS

Effect of Monosodium Glutamate on the hatching process in zebrafish embryos

The hatching rate of embryos at 48 h was delayed in the treatment groups exposed to concentrations of 300 mg/L, 150 mg/L, 100 mg/L, 75 mg/L, 37.5 mg/L, 18.75 mg/L, and 9.38 mg/L, as compared to the control group. At 48h, the concentration required to cause a 50% delay in hatching was calculated as 234 mg/L based on the linear regression model. (Figure 1). Embryos continued to develop inside the chorion but were unable to hatch out. When the exposure time was prolonged to 96 h, the hatching rate of the treatment group with concentrations of 300 mg/L, 150 mg/L and 75 mg/L increased to 90%. In addition, over the previous 24 h the movement of embryos inside the chorion was lower in the treatment group than in the control group. Moreover, the hatching rate at 96 hpf was higher than that at 72 hpf in all the groups.



Concentration of the test solution	Percentage of embryos hatched at 48hrs(mean)
300 mg/L	51%
150 mg/L	57%
100 mg/L	66%
75 mg/L	52%
37.5 mg/L	75%
18.75 mg/L	77%
9.38 mg/L	82%
4.687 mg/L	100%
2.34 mg/L	100%
Control group	96%

Figure 1: Linear regression graph of Monosodium glutamate concentration (X axis) and percentage hatchability of zebrafish embryo at 48hpf (Y axis). Table depict the mean hatching rate of embryos at different concentrations of test solution (MSG).

Effect of Monosodium glutamate on the Mortality Rate in Zebrafish Embryos

The mortality rate was analyzed at 24 and 48 h post-fertilization, and it was found that there was not much difference between the mortality rates of the control and treatment groups. A natural mortality rate of 2-5% was observed in both the control and treatment groups, and the mortality rate was not dependent on the MSG exposure concentration. Coagulation of the embryo (dead embryo) (Figure 2B) was mainly considered at 24 hours post-fertilisation (hpf) as a mass of unorganized cells in the middle of the egg. No difference was observed between the control and MSG-exposed zebrafish eggs until 96 hpf in the mortality rate.

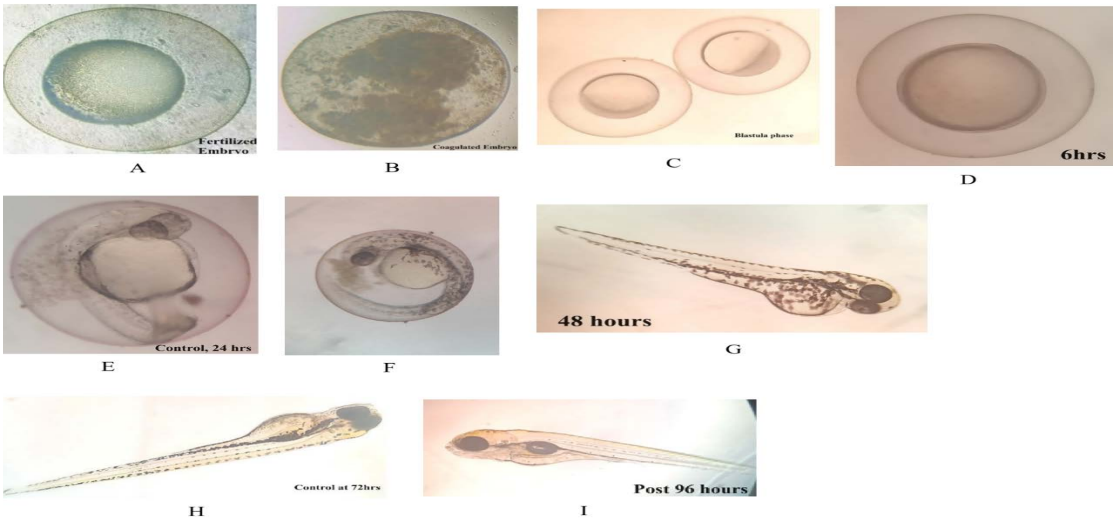


Figure 2:Microscopic images of zebrafish embryo at different time points in control group (A)fertilized embryo, (B) coagulated or dead embryo, (C) blastula phase, (D) 6hours of fertilization,(E)24hours, (F) 30hours,(G)48hrs, (H)72hrs, (I)96hrs.

Effect of Monosodium Glutamate on the phenotype of the embryo

In the treatment group with concentrations 300 mg/L, 150 mg/L, and 75 mg/L, approximately 80% of the embryos showed no detachment of the tail from the yolk ball even after 24 h (Figure 3). At 72 hpf, embryo yolk sac extension/ resorption was delayed in the treatment groups of concentrations 300 mg/L, 150 mg/L compared to that in the control group (Figure 4B). The absorption of the yolk begins at

17 h, and the length of the yolk extension should be more than half the diameter of the yolk ball. Detachment of the tail was observed at 24 h in all the embryos in the control group. The treatment groups (300 mg/L, 150 mg/L, and 75 mg/L) embryos showed a non-regressed yolk ball even at 96 h. A detailed comparison of the control and treatment groups at a concentration of 300 mg/L is presented in Table 2. In some of the embryos at 72 h post-fertilization, pericardial edema was observed, which was not much appreciated at 96 hpf. At 24, 48, 72, and 96 hpf, no major observable abnormalities were detected in the development of notochord, tail, head, or mouth formation. However, the development of the different stages lagged behind compared to the control group.

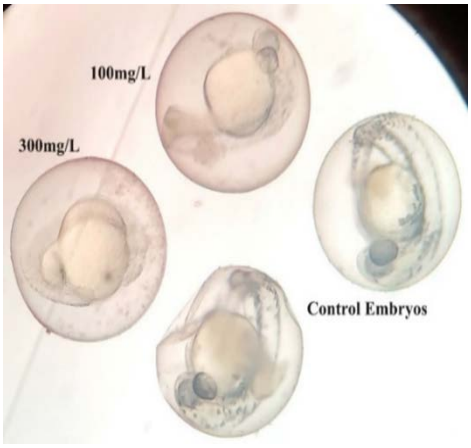


Figure 3: Comparison of embryos of the treatment and control group at 24-30 h. It can be noted that the melanin deposition and development of eye buds were delayed in embryos treated with 300 and 100 mg/L of MSG.

Effect of Monosodium glutamate on Melanin pigmentation.

Melanophore deposition began to occur from 24-48 hours in the control group and started with pigmentation in the retina and fin. Melanin pigmentation was not uniform in the treatment groups compared with that in the control group at 24-48 hpf. Melanophores developed as lateral strips in the control at 48 h, with xanthophores in the head. At concentrations of 300, 150, and 75 mg/L, the embryos displayed typical albinism with no melanin observed at 48 hpf. It was observed that in the treatment groups with higher concentrations (300, 150, and 75 mg/L), there was less deposition of melanophores on the skin and eye; hence, the defect in melanin deposition seems to be concentration-dependent. While in groups exposed to lesser concentration, viz., 37.5, 18.75, and 9.38 mg/L, the pigmentation began developing by 72 hpf. In addition, the iridophores that gave yellow pigmentation to the head and body were delayed, as seen in the treatment group, and were inversely related to the concentration of the solution.

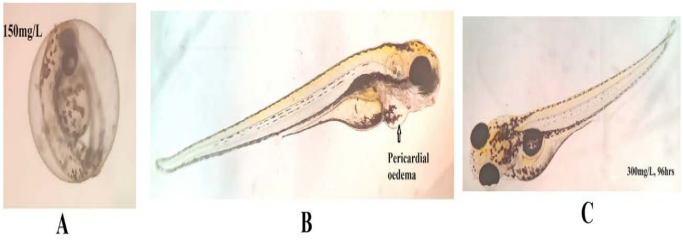
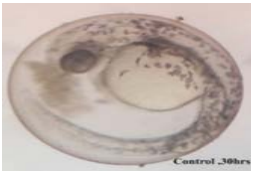
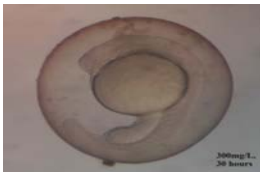
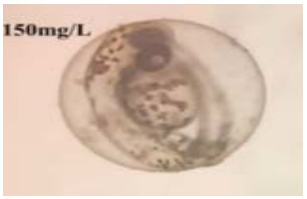
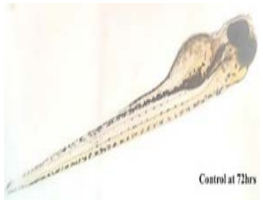


Figure 4: Microscopic images of zebrafish embryos at (A) 48 hpf, (B) 72hpfand (C)96hpf. The embryo at 72hrs showed a delay in the development of mouth parts, resorption of yolk and pericardial oedema at 150 mg/L when compared to the control.

Table 2: Comparison between the developmental stages of embryos observed at 24 -30, 48, 72, 96 hours in the control and treatment groups (300 mg/L).

Time after fertilisation	Control embryos		Treatment (300mg/L)	
24 hrs	Detachment of tail Somite formation not clear Eye bud formation and pigmentation started;	 Movement of the embryo could be observed	 Movement of the embryo not observed Yolk sac normal	Somite formation is slow Eye bud formation can't be appreciated

48 hrs	Melanin pigmentations can be appreciated. Xanthophores deposition in the head region More than 70% larvae hatched.	 Growth of pharyngeal region with small open mouth.Yolk ball approximately same size of head	 Xanthophores are very less in head region Only 50%larvae hatched, but they were fully grown inside the chorion.	No growth in pharyngeal region. Resorption of yolk is slow. Melanin pigmentations were very meagre
72 hrs	Melanin deposition near to swim bladder making the region darker. Pectoral fin is developed.		 Melanin deposition near swim bladder can be appreciated	Fin development incomplete Lesser xanthophore deposition.
96 hrs	Regression of yolk ball into yolk band Inflation of Swim bladder Swim actively Movement of the jaw is appreciated.		 Movement of larvae is less Jaw growth is not appreciable.	Regression of yolk is slow Inflation of swim bladder can be seen

Effect of monosodium glutamate on the development of somites and the eye

Somite development was observed in both control and treated embryos, and unhatched embryos also showed somite formation. The eye bud was not clearly developed in the treatment groups with higher concentrations (300, 150, and 75 mg/L) at 30 hpf compared to the control group.

DISCUSSION

Monosodium glutamate (MSG) has long been used as a taste enhancer in various food products. Several studies have been conducted on the harmful effects of long-term consumption of MSG on the health and well-being of individuals. MSG is reported to cause obesity, hyperglycemia, and neurological symptoms such as nausea, migraine, hyperactivity, and Alzheimer's disease (Niaz *et al.*,2018). Hence, the present study was undertaken to understand the effects of MSG on the development of zebrafish embryos from 4 to 96 h post-fertilization.

Zebrafish embryos were exposed to concentrations of 300, 150, 100, 75, 37.5, 18.75, 9.38, 4.687, and 2.34 mg/L monosodium glutamate from 4 hpf to 96 hpf. The embryos showed a large suite of abnormalities in the developmental stages at higher concentrations, which could recover after 96 h of fertilization. The mortality rate in the treatment groups was comparable to that of the control, and a natural mortality rate of 2-5% was observed in almost all wells. MSG did not cause lethality in embryos, even at the highest concentration of 300 mg/L. These results are in agreement with the findings of Suthamnatpong *et al.*,2017, where abnormal heart function was observed at 24hpf along with the appearance of abnormal body curvature, pericardial edema, and yolk sac edema at concentrations of 15, 150, and 1,500 ppm. The LC_{50} of MSG was determined as 15,200ppm at 48hpf and 10,300ppm at 96hpf.Bölükbaş and Öznurlu (2023) reported a mortality of 80.41% and 78.68% at the concentrations of 0.6 and 1.2 mg/g of MSG administration *in-ovo* in chicken egg. Nnadozie *et al.* (2019) also reported that chronic administration of MSG would lead to neonatal death in Wistar rats.

Hatching was delayed in the treatment group, and by the end of the test, all surviving embryos had hatched. Zebrafish embryos in the control group showed a mean hatching rate

of 96%. In the treatment groups, the calculated concentration required to cause a 50% delay in hatching was calculated as 234 mg/L. No observed effect of concentration (NOEC) on hatching was observed at 4.7 mg/L. Embryo hatching involves the presence of hatching enzymes and the movement of embryos. Although the embryos did not hatch, they developed inside the chorion. The egg envelope is composed of polymers of e-(c-glutamyl) lysine isopeptide crosslinks produced by transglutaminase. The egg envelope is cleaved by the enzyme ZHE1 (Wisenden *et al*., 2022). As hatching requires movement of the embryo, which was found to be less in the treatment group, this could be due to the neurological impact of MSG.

In 40% of the embryos, somite formation was not observed in the test groups treated with 150, 100, and 300 mg/L at 24-30 hours. All other developmental stages were slower in treatment groups at concentrations of 37.5 mg/L and above. The toxicity rate was lower in embryos treated with lower concentrations, viz., 18.75, 9.38, 4.687, and 2.34 mg/L of MSG. This implies that the toxicity observed was concentration-dependent, and the growth rate was inversely proportional to the concentration of MSG. In addition, 30% of the embryos showed no detachment of the tail bud or regression of the yolk sac at 72 h. Although the rate of development was slower, the embryos could survive the effect and hatch out by 96-120 hours of fertilisation.

Embryos exposed to 100 and 300 mg/L displayed typical albinism, with no melanin deposition observed at 48 hpf. As incubation continued, there was mild deposition of melanin in embryos exposed to 100 mg/L. In two-fold serially diluted treatment groups (300, 150, 75, 37.5, 18.75, and 9.38 mg/L, showed lesser melanin pigmentation. Delayed and less melanin deposition was observed in the treatment groups than in the control group. Although all melanophores in zebrafish are generated from the embryonic neural crest, their distribution and signalling pathways are unclear. cAMP plays a major role in the distribution and control of melanoblast and melanocyte numbers (Jin & Thibaudeau, 1999). Glutamate, an NMDA receptor ligand, is involved in signalling pathways during development and regulates the proliferation, migration, and survival of neuronal progenitors and immature neurons (Sheldon & Robinson, 2007). The NMDA receptor-mediated pathway is thought to be involved in melanosome transfer, calcium influx, and melanocyte filopodia formation (Ni *et al*., 2016). This suggests that Glu may be involved in melanophore deposition in zebrafish embryos. Further studies are required to investigate the mechanisms involved. Additionally, the density of superficial melanophores increases or decreases based on differentiation and apoptosis, respectively, in zebrafish (Sugimoto *et al*.,2005).

Pericardial edema is an after-effect of improper fluid excretion and circulation in the body, which may be an indication of developmental anomalies of the heart, kidney, and skin. This condition can be caused by circulatory failure, ionic imbalance, kidney failure, and permeability defects (Hill *et al*., 2004). MSG at a concentration of 300 mg/L induced pericardial edema in ZFE at 72 hpf, indicating that embryos are more vulnerable to the effects of MSG immediately after hatching. After subcutaneous administration of MSG to neonatal rats, the animals were found to be hypertensive, and baroreflex sensitivity was increased in the MSG obese rats, and parasympathetic alterations may have originated from central areas of cardiovascular control (Karlen-Amarante *et al*.,2012). Moreover, MSG was found to alter ECG values with an increased heart rate and decreased force of cardiac muscle contraction (Hazzaa *et al*.,2020).

Subcutaneous administration of MSG to pregnant and foetal rats showed that MSG could drastically damage acetylcholinesterase-positive neurons in the area of the prostrema of the brain. Foetal rats were found to be more sensitive to glutamate. Similarly, accumulation of glutamate in foetal brains could occur and damage the brain cells by transplacental exposure of foetuses from mothers exposed to glutamate-rich food (Tóth *et al*.,1987). Even though the present study revealed that somite formation in all the treatment groups was completely developed in 60% of cases, further detailed analysis should be performed on neuronal toxicity in embryos, as many studies have revealed the deleterious effect of MSG in brain cells. MSG could cause an imbalance in glucose metabolism and lead to obesity in neonatal rats when exposed *in utero*, as MSG could cross the placental barrier in pregnant rats. In addition, it can induce neuroendocrine dysfunction in rats exposed to MSG at earlier stages (Collison *et al*., 2012).

Considering these results, monosodium glutamate induced toxicities such as delayed development, reduced melanin pigmentation, and pericardial edema in zebrafish embryos at concentrations of 100 mg/L and above.

CONCLUSION

MSG did not cause lethality in embryos, even at the highest concentration of 300 mg/L. The mortality rate in the treatment groups was comparable to that of the control, and a natural mortality rate of 2-5% was observed in almost all wells. MSG caused a retarded growth from 24 h to 72 h, but growth by 96 h could recover with developmental delay. Embryos exposed to 100 and 300 mg/L displayed typical albinism, with no melanin deposition observed at

48 hpf. As incubation continued, there was mild deposition of melanin in embryos exposed to 100 mg/L. MSG at a concentration of 300 mg/L induced pericardial edema in Zebra Fish Embryos at 72 hpf, indicating that embryos are more vulnerable to the effects of MSG immediately after hatching. Thus, it can be concluded that monosodium glutamate can induce developmental toxicity in zebrafish embryos at concentrations of 100 mg/L and above.

ETHICAL APPROVAL

IAEC No. 14/SA/IAEC/2023, 07.01.2023 (IAEC, Tamil Nadu Veterinary and Animal Sciences University)

AUTHOR CONTRIBUTION

Megha Madhusudhanan performed this experiment and initial data analysis and wrote the paper. M R Srinivasan, Jalantha P, and Azhahianambi P supervised this work, curated the data, gave suggestions while performing the experiment, and in writing the article.

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DISCLOSURE STATEMENT

The authors declare that they have no competing interest in publishing the data.

REFERENCES:

Bauer B, Mally A, Liedtke D (2021). Zebrafish embryos and larvae as alternative animal models for toxicity testing. *Int. J. Mol. Sci*.22(24):13417.

Belanger SE, Rawlings JM, Carr GJ (2013). Use of fish embryo toxicity tests for the prediction of acute fish toxicity to chemicals. *Environ. Toxicol. Chem*.32(8):1768-83.

Beyreuther K, Biesalski HK, Fernstrom JD, Grimm P, Hammes WP, Heinemann U, Kempski O, Stehle P, Steinhart H, Walker R (2007). Consensus meeting: monosodium glutamate–an update. *Eur. J. Clin. Nutr*.61(3):304-13.

Bölükbaş F, Öznurlu Y (2023). Determining the effects of in ovo administration of monosodium glutamate on the embryonic development of brain in chickens. *NeuroToxicology*.94:87-97.

Collison KS, Makhoul NJ, Zaidi MZ, Al-Rabiah R, Inglis A, Andres BL, Ubungen R, Shoukri M, Al-Mohanna FA (2012). Interactive effects of neonatal exposure to monosodium glutamate and aspartame on glucose homeostasis. *Nutrition and metabolism*.9:1-3.

Das D, Banerjee A, Bhattacharjee A, Mukherjee S, Maji BK(2022). Dietary food additive monosodium glutamate with or without high-lipid diet induces spleen anomaly: A mechanistic approach on rat model. *Open Life Sciences*.17(1):22-31.

De Esch C, Slieker R, Wolterbeek A, Woutersen R, de Groot D (2012). Zebrafish as potential model for developmental neurotoxicity testing: a mini review. *Neurotoxicol. teratol.* 34(6):545-53.

Hazzaa SM, El-Roghy ES, Abd Eldaim MA, Elgarawany GE (2020). Monosodium glutamate induces cardiac toxicity via oxidative stress, fibrosis, and P53 proapoptotic protein expression in rats. *Environ. Sci. Pollut. Res*. 27:20014-24.

Hill AJ, Bello SM, Prasch AL, Peterson RE, Heideman W (2004). Water permeability and TCDD-induced edema in zebrafish early-life stages. *Toxicological Sciences*. 78(1):78-87.

Insawang T, Selmi C, Cha'on U, Pethlert S, Yongvanit P, Areejitranusorn P, Boonsiri P, Khampitak T, Tangrassameeprasert R, Pinitsoontorn C, Prasongwattana V (2012). Monosodium glutamate (MSG) intake is associated with the prevalence of metabolic syndrome in a rural Thai population. *Nutrition and Metabolism*.9:1-6.

Jin EJ, Thibaudeau G (1999). Effects of lithium on pigmentation in the embryonic zebrafish (Brachydanio rerio). *Biochim Biophys Acta Mol. Cell Res*.1449(1):93-9.

Jubaidi FF, Mathialagan RD, Noor MM, Taib IS, Budin SB (2019). Monosodium glutamate daily oral supplementation: Study of its effects on male reproductive system on rat model. *Syst. Biol. Reprod. Med*.65(3):194-204.

Karlen-Amarante M, Da Cunha NV, De Andrade O, De Souza HC, Martins-Pinge MC (2012). Altered baroreflex and autonomic modulation in monosodium glutamate-induced hyperadipose rats. *Metabolism*.61(10):1435-42.

Kazmi Z, Fatima I, Perveen S, Malik SS (2017). Monosodium glutamate: Review on clinical reports. *Int. J. food Prop.* 20(2):1807-15.

Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF(1995). Stages of embryonic development of the zebrafish. *Developmental dynamics*.203(3):253-310.

Ni J, Wang N, Gao L, Li L, Zheng S, Liu Y, Ozukum M, Nikiforova A, Zhao G, Song Z (2016). The effect of the NMDA receptor-dependent signaling pathway on cell morphology and melanosome transfer in melanocytes. *J. Dermatol. Sci*.84(3):296-304.

Niaz K, Zaplatic E, Spoor J (2018). Extensive use of monosodium glutamate: A threat to public health? *EXCLI J*.17:273.

Nnadozie JO, Chijioke UO, Okafor OC, Olusina DB, Oli AN, Nwonu PC, Mbagwu HO, Chijioke CP (2019). Chronic toxicity of low dose monosodium glutamate in albino Wistar rats. BMC Res. Notes.12:1-7.

Rhodes J, Titherley AC, Norman JA, Wood R, Lord DW (1991). A survey of the monosodium glutamate content of foods and an estimation of the dietary intake of monosodium glutamate. *Food Addit. Contam*.8(3):265-74.

Sheldon AL, Robinson MB (2007). The role of glutamate transporters in neurodegenerative diseases and potential oppor tunities for intervention. *Neurochem. Int*.51(6-7):333-55.

Shosha HM, Ebaid HM, Toraih EA, Abdelrazek HM, Elrayess RA (2023). Effect of monosodium glutamate on fetal development and progesterone level in pregnant Wistar Albino rats. *Environ. Sci. Pollut. Res*.30(17):49779-97.

Sugimoto M, Yuki M, Miyakoshi T, Maruko K (2005). The influence of long-term chromatic adaptation on pigment cells and striped pigment patterns in the skin of the zebrafish, Danio rerio. *J. Exp. Zool. A Comp. Exp. Biol.* 303(6):430-40.

Suthamnatpong N, Ponpornpisit A (2017). Effects of monosodium glutamate on heart beat and the embryonic development of zebrafish. *The Thai Journal of Veterinary Medicine*.47(4):523-30.

Toth L, Karcsu S, Feledi J, Kreutzberg GW (1987). Neurotoxicity of monosodium-L-glutamate in pregnant and fetal rats. *Acta neuropathol*.75:16-22.

Williams AN, Woessner K (2009). Monosodium glutamate 'allergy': menace or myth? *Clin Exp Allergy*.39(5):640-6.

Wisenden BD, Paulson DC, Orr M (2022). Zebrafish embryos hatch early in response to chemical and mechanical indicators of predation risk, resulting in underdeveloped swimming ability of hatchling larvae. *Biology Open* 11(12):059229.

Zanfirescu A, Ungurianu A, Tsatsakis AM, Nițulescu GM, Kouretas D, Veskoukis A, Tsoukalas D, Engin AB, Aschner M, Margină D (2019). A review of the alleged health hazards of monosodium glutamate. *Compr. Rev. Food Sci. Food Saf*.18(4):1111-34.