International Journal of Biomolecules and Biomedicine (IJBB)
ISSN: 2221-1063 (Print), 2222-503X (Online)
http://www.innspub.net
Vol. 17, No. 3, p. 7-12, 2023

RESEARCH PAPER OPEN ACCESS

The excitotoxic effect of monosodium glutamate on Zebra Fish (*Danio rerio)*

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Key words: Sub-lethal concentration, Toxicity assay, Morphology, Protein, Carbohydrate

Article Published: 12 December 2023

Abstract

The sodium salt of glutamic acid is monosodium glutamate (MSG), also referred to as sodium glutamate is one of the most frequently used food additives in processed foods. In addition to its flavour-enhancing properties, MSG has been linked to several toxicities like Obesity, metabolic issues, Chinese Restaurant Syndrome, neurotoxicity and detrimental effects on the reproductive system. The current study is geared up to shed light on the effect of monosodium glutamate on *Danio rerio,* a fresh water fish. The objective is to study the lethal and sub-lethal concentration of MSG and its effects on morphology and biochemical parameters like total protein and carbohydrate in Zebra fish. The toxicity assay was conducted for 96hrs and the dose of 23.98 ppm was taken as LC_{50}. The tissues were taken from exposed and control fish, they are used for estimation of total carbohydrate and protein. The organisms showed statistically significant difference between exposed and control group. In this present study MSG has affected fish morphologically by discoloration, protrusion of eyes and the levels of proteins and carbohydrates decrease with increasing concentration of MSG, therefore it has effects on metabolism of the organism.

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Introduction

There is an emerging trend in the world with the altered lifestyles of human towards the fast food which contain lot of food additives such as Monosodium Glutamate (Al-Qudsi and Al-Jahdali, 2012). Among the food additives monosodium Glutamate is highly controversial. Though they are profoundly added as food additive they express excitotoxicity and are involved in several neurological diseases, which makes us to question the reliability when consuming (Augustine *et al.,* 2019). MonoSodium Glutamate (MSG) is a form of glutamate and is the sodium salt of glutamic acid; an amino acid, which exist as white crystals. It does not give unique taste, but it is able to provide distinct flavor to food when it is added as an additive (Abdelkader *et al.,* 2016). MonoSodium Glutamate has a particular taste that falls outside the locale of the four exemplary tastes: sweet, sour, salty, and bitter. This taste is designated "Umami". Due to this unique taste, numerous food makers use MSG (David Tin Win, 2008). MSG is considered as an excitotoxin which can alter the normal functions of neurotransmission in animal biological systems. Glutamate is a naturally available excitatory neurotransmitter in the central nervous system of mammals including human (Storto *et al.*, 2000). Previous investigations that have been carried out to demonstrate the effect of MSG on animal models have shown that different biological effects, especially on the early developmental stages. The toxicity and teratogenicity of MSG have been investigated using different laboratory animals. The toxicity test is conducted to study the response elicited by the foreign substance in a living organism. The median lethal concentration (LC_{50}) and the end point (death) is determined through toxicological studies or bioassays (Swetha and Kiran, 2019). The test organism used in this experiment is a fresh water fish, *Danio rerio*, native to south Asia, belonging to the family *Cyprinidae*. The Zebra fish is a significant and broadly utilized vertebrate model living being in logical examination, for instance in drug improvement, specifically pre-clinical development, since it has certain likenesses to human science. There have been many studies on the effect of Monosodium Glutamate on embryonic development. Very few works have been done to study the effects of MSG on fish and its biochemical effects. The biochemical parameters are significant as they are best indicators of fish health. This work narrows down to study the toxic effects on fresh water fish and its impact on biochemical parameters like total carbohydrate and protein.

Materials and methods

Acute toxicity

Different concentrations of MSG were prepared (10ppm, 25ppm, 50ppm, 75 ppm) and to each of them ten fishes of uniform size (2g) were exposed separately and the per cent mortality was recorded at 96 hours of exposure. The experimental design contains three replicates of ten fish each in given sampling. Fishes were not fed during the 96 hours of experimental period and were left undisturbed. The responses along with the mortality were recorded every 6 hours, and the dead fishes were removed. The control was also maintained. Cessation of visible movements including gills and loss of reaction on application of external stimuli to the caudal peduncle were considered signs of mortality following OCED. The median lethal concentration (LC_{50}) was calculated with Probit Analysis (Finney, 1978).

Water quality analysis

The estimations of p^H, Total hardness, dissolved oxygen and dissolved carbon di oxide was analysed.

Hardness test (EDTA method): It was performed according to APHA, 1998.

P^H: The p^H of the control and test water samples was measured before and after experiment using p^H meter.

Dissolved oxygen: The amount of oxygen dissolved is estimated using Winkler's method

Chemical oxygen demand: It was performed following Sec.5220 COD, 2017 protocol.

Estimation of biochemical constituents

Estimation of protein in muscle: Protein content of the tissue was estimated by the method of Lowry *et al.* (1951).

Estimation of carbohydrates content in muscle: Carbohydrates content of the tissue was estimated adopting Anthrone method (Nicholas *et al.,* 1956).

Results and discussion

Table 1. depicts the water quality of the control and treated tanks. The minerals that are dissolved in the aquatic system affect the pH of the water, which affects the solubility of substances. As a result, the existence of these elements affects the water quality (Malathi *et al.*, 2020). The p^H of the control was measured to be 8.56. After 96hrs of exposure to MSG the test sample water was measured and it was found to be declining. The fish and other vertebrates have an average p^H of about 7.4, as the water comes in close contact to blood through the blood vessels in gills and skin, therefore the ideal water p^H ranges from 7.0 to 8.0 and is desirable up to 9.0 (William and Robert, 1992). Increasing COD also supports the decline in oxygen level, as the chemical oxygen demand increases it leads to reduced dissolved oxygen level which in turn causes anaerobic reactions in the system, which is harmful to the organisms. The oxygen content decreases with increase in concentration of MSG whereas the Carbon di oxide increases with increase in concentration. This explains the decline in p^H value as CO_2 increases the carbonic acid in the water. Claude *et al.* (2016) explained that water with low alkalinity shows greater p^H fluctuations due to dissolved CO_2, released during the respiration when compared to water with higher alkalinity. The hardness of the water decreased in comparison with control tank. In addition to the toxic effects of MSG on the fish, the level of dissolved oxygen could also have affected the wellbeing of the organisms in water.

Table 2. Shows the lethal and median lethal concentrations of the monosodium Glutamate in *Danio rerio*. In the acute toxicity test the higher concentration shows increased mortality than the lower concentrations. Discoloration was observed in the higher concentration, and fishes were lethargic. They showed abnormal ventilatory movements like gulping of air at the surface of water and reduced opercular movements. Protrusion of eyeball was observed in the high concentration along with other mentioned signs of illness. Later the fishes showed difficulty in swimming and the fishes settled at the bottom. There was no death in the control group, the fishes were healthy, active and did not show any kind of illness. Whereas in the test group the cumulative mortality increased with increase in the concentration of the MSG. The 96hrs LC_{50} value was found to be 23.98 ppm. In the treatment tank the fishes showed decrease in their activity each day. Sometimes erratic movement was observed. These indicate body work weaknesses like catalyst hindrances, postponement and disturbances in neural transmission, blockage of apprehensive transmission between the sensory system and effector destinations, alongside aggravations in metabolic pathways (Rand, 1985). MSG is also related to neurological and neuropsychiatric disorders and also endocrine disorders as they are excitotoxins (Ikonomidou and Turski, 1995). Excess fluid build-up leads to protrusion of eyes in the Zebra fish.

Table 1. Effect of MonoSodium Glutamate on water quality

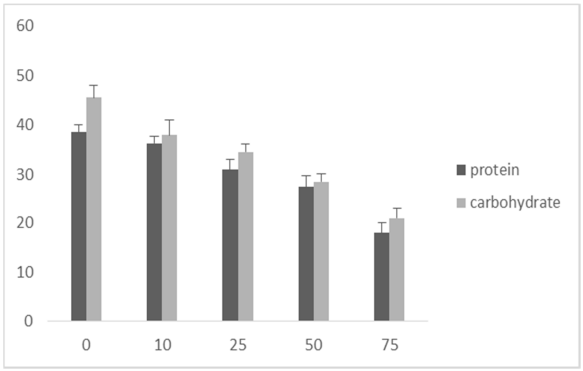
Concentration of MSG(ppm)	p^H	Hardness test (mg/ml)	Dissolved O_2 (ppm)	COD (ppm)
Control	8.56	75	5	0.2
10	8.18	60	3	0.3
25	8.26	50	2.6	0.5
50	8.29	40	1.9	0.6
75	8.32	40	0.9	0.7

Table 2. Determination of the 96h LC_{50} of MonoSodium Glutamate in *Danio rerio*

Concentration of MSG (ppm)	No. of fishes dead			No. of fish in each trial	Mean % mortality	Probit value
	Trial 1	Trial 2	Control			
0	0	0	0	10	0	0
10	2	1	0	10	15	3.96
25	2	3	0	10	25	4.33
50	5	6	0	10	55	5.13
75	10	10	0	10	100	8.09

Loss of pigmentation of the skin is due to Melanocyte Stimulating Hormone (αMSH) and Melanin-Concentrating Hormone (MCH), which are known to be up controlled during stress reaction. Decrease in pigmentation of Zebra fish treated with MSG may either result from pressure at the cell, organ or individual level or modify the capacity of αMSH and MCH, they are the stress induced response (Mahaliyana *et al.,* 2016). The increased intake of MSG can cause biological disturbances in the system metabolically.

The biochemical alterations in organisms are considered as most sensitive and earliest events of any pollutant damage (Tripathi and Singh, 2002). The total carbohydrate decreased significantly with increase the concentration of monosodium Glutamate when compared to the control. Carbohydrate metabolism is one of the most important biological processes in *Danio rerio* metabolisms. Studies showed that dietary carbohydrate levels had an impact on the growth and development of sexually mature adults, such as body weight gain and composition and hepatic gene expression(Qian *et al.,* 2019) Carbohydrates are the first also, prompt fuel source to be used undeniably especially in the event of stress. Presentation to any sort of toxins brings about pressure which eventually brings about a decrease of complete carbohydrate content in different tissues. Under stress conditions, sugar saves are drained to meet energy necessity by all tissues (Kauser and Yasmeen, 2019). The decreasing carbohydrate trend also shows active glycogenolysis and glycolytic pathway to meet impending energy (Tripathi and Yadav, 2015). The decreasing trend of carbohydrate is due to great utilization of energy to mitigate the stress caused by monosodium glutamate. After carbohydrate the next source of energy is protein. The treated fishes were analyzed for total protein in the wet tissue. The results are depicted in Fig. 1. After 96hrs of exposure the total protein content was progressively depleted in the organisms. Protein is most characteristic organic compound found in the living cell while the protoplasm of the cell is composed of protein (Magar and Shaikh, 2012). They are the building blocks of the organisms. The reason for decline in protein might be due to rapid proteolysis or due to reduced synthesis.



Values are expressed as mean ± standard error. *p<0.05.

Fig. 1. Changes in protein and carbohydrate

Kumar and Gopal (2001) showed that degradation of proteins into amino acids is used for various metabolic activites during the stress conditions. During stress the protein is utilized for converting ketoacids to gluconeogenesis pathway for glucose synthesis (Veeraiah *et al.,* 2013). Jagadessan and Mathivanan (1999) concluded that the consumption of protein level instigates to enhancement of energy, to meet the energy requests during toxic stress. They expressed that the liver synthesized a more noteworthy measure of protein, which is required apparently fix the damage of organelle and tissue regenerator. In order to meet their energy needs, stressed fish also displayed a decrease in protein content and an increase in total amino acids (Beslin, 2023). Thus, it can be concluded that monosodium glutamate affects fish metabolism and body functions. Only lethal effects and few biochemical changes in fresh water fish was investigated in the study and this can be further used for studies in the monosodium glutamate and its effects on organisms.

Acknowledgement

The author would like to thank FCAA (Fatima College Alumnae Association) for providing seed money to pursue this research till the end and also, we extend our heartfelt thanks to the management for extending the infrastructure and instrumentation facilities to carry out this project

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