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RESEARCH ARTICLE

The effects of chronic intraperitoneal monosodium glutamate in social interaction and on rotating rod in adult Swiss albino mice

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ABSTRACT

Background: Short-term use of monosodium glutamate (MSG) induces anxiety in animals. Due to the lack of stringent regulations for food supplements and increase in the use of MSG in the past decade, the long-term effects of MSG need to be evaluated. There is a scarcity of the literature on the chronic effects of MSG on anxiety, hence the present study. Since anxiety models require an intact motor function, motor function was also evaluated. **Aims and Objectives:** The aim of the study was to evaluate the long-term effects of intraperitoneal (i.p.) MSG on mice models of anxiety (social interaction) and motor function (accelerating rota rod). **Materials and Methods:** The present prospective interventional study was conducted on 40 adult male Swiss albino mice. Mice were randomly divided into four equal groups to receive, distilled water, and MSG at doses of 40, 60, and 80 mg/kg/day for 3 months. Parameters were assessed at baseline, 1 month, 2 months, and 3 months of daily MSG administration. Statistical tests of significance were the Wilcoxon signed-rank test and Friedman test (within group) and Kruskal–Wallis Test (between groups), $P < 0.05$ was considered statistically significant. **Results:** Social interaction time was reduced in all the MSG-treated groups ($P < 0.05$) without any change in motor function ($P > 0.05$). Anxiety was evident from 40 mg/kg/day MSG from the 1st month till 3 months compared to baseline and controls. Duration-dependent change in social interaction was observed with MSG at doses of 40 mg/kg/day and 60 mg/kg/day. Dose-dependent change in social interaction was not observed among treatment groups. **Conclusion:** Long-term administration of MSG produced anxiogenic effects at doses of 40 mg/kg, 60 mg/kg, and 80 mg/kg without impairing the motor functions in the mice.

KEY WORDS: Anxiety; Motor Function; Albino Mice; Monosodium Glutamate

INTRODUCTION

Anxiety is a neuropsychiatric condition that manifests as a future-oriented mood state which consists of complex cognitive, affective, psychological, and behavioral system in anticipation of a threatening situation or on anticipation of events.[1] Anxiety is one of the most common neuropsychiatric disorders and with an annual global incidence of 45 million, a prevalence of 300 million, and a contribution to disability-adjusted life years of 28 million.[2] Over the past three decades, there has been an absolute increase in anxiety disorders by 50%.[2] Social anxiety is the second-most common anxiety disorder with a 12-month prevalence rate of 7.4%.[1] The causes of anxiety have not been clearly elucidates, but the established causes include substance abuse, childhood trauma, and medications. However, the link of anxiety with food and food additives has not been clearly defined or studied.

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Monosodium glutamate (MSG) is a widely accepted food additive and is considered as a generally regarded as a safe compound. Urbanization and fast-food culture have led to excessive consumption of MSG in the past decade. The taste sensation it produces is considered to be other than the normal five tastes that we usually perceive and this is named as UMAMI. The average consumption of MSG ranges between 0.3 and 1 g daily in industrialized countries while the amount is still higher in impoverished nations.[3] The lack of stringent regulations on food additives and the exploding fast-food culture has led to a dramatic increase in the consumption of MSG with minimal or no scientific data on the various neuropsychiatric effects of MSG. MSG is addictive since it increases in hedonic perception, appetite stimulation, and conditioned preference to its own taste perception.[4] MSG has been linked to a lot of diseases including anxiety in the past few years. There is evidence of an increase in glutamate in the central nervous system with increased consumption of MSG.[5] Higher levels of glutamate in the central nervous system alter brain plasticity and striatal dopaminergic release which would induce reward behavior, especially reward memory and reward consolidation.[5] Alterations in reward behaviors have been implicated in both anxiety and depression.[6]

Human studies on MSG consumption are lacking since the amount consumed daily cannot be measured accurately and human studies with MSG would be unethical. The only evidence available is from animal studies and these studies are of short duration. Our question is "Can a commonly used food additive be a contributor of the increasing global burden of anxiety?." There is a dearth of information on the chronic effects of MSG at doses equivalent to human consumption on models of anxiety. Since models of anxiety require an intact motor function, the present study evaluates whether MSG produces impairment in models of anxiety and motor function in adult Swiss albino mice.

MATERIALS AND METHODS

The present study was a prospective interventional animal study which was conducted in accordance with ARRIVE and CCSEA guidelines at a licensed small animal holding facility of Sree Gokulam Medical College and Research Foundation, Venjaramoodu, Trivandrum (1065/PO/Re/S/07/CPCSEA). The research protocol was approved by the Institutional Animal Ethics Committee (002/08 aM/M2/2018). A minimum number of 10 animals in each group was necessary to detect any statistically significant difference between the groups and the sample size was calculated assuming an α of 0.05, power of 95%, and a 10% attrition rate. A total number of 40 adult Swiss albino mice of age 3 months or above weighing 20–30 g were used in the study. Experimental animals were procured from CCSEA licensed vendor (Mahaveera Enterprises, D. No. 2-2-647/258, Srinivas Nagar Colony, Bagh Amberpet, Hyderabad; License No: 500 013146/99/CPCSEA) and were initial quarantined and allowed to acclimatize for initial 14 days in the quarantine area following which they were moved to the mice holding area. Animals were randomly divided into groups of 10 and were caged in groups of 4–6. Animals were caged in polycarbonate cages, at temperatures ranging between 24°C and 28°C and a relative humidity of <55%. All animals were fed standard animal feed (laboratory pellet chow) and were provided water *ad libitum*. 12-h light-dark cycle was followed in the facility.

Mice were randomly divided into groups of 10, Group 1 (n = 10) served as the negative control and received standard laboratory diet, and reverse osmosis (RO) water *ad libitum* along with 0.3 ml distilled water i.p. using standard technique daily for 3 months. Group 2 (n = 10), Group 3 (n = 10), and Group 4 (n = 10) received daily MSG at doses of 40 mg/kg, 60 mg/kg, and 80 mg/kg, respectively, for 3 months i.p. along with standard laboratory diet and RO water *ad libitum*. Dilution was calculated based on a maximum volume of 0.3 mL to be administered. Anxiety in mice was evaluated by a social interaction test and motor function was evaluated using an accelerating rota rod apparatus for mice. The tests were conducted at baseline, 3 days of administration of the drugs, 1 month, 2 months, and 3 months of drug administration.

Experimental Designs

Social interaction test

Pairs of mice were allowed to freely interact in a novel environment (standard mice cage without the covering lid and with new corncob bedding material), and time spent interacting with each other was recorded as the dependent measure. A reduction in anxiety increases social interaction time and an increase in anxiety will decrease the social interaction time. Recording equipment was mounted above the cage at a distance that provides complete coverage of the test area but does not interfere with the test environment. Test pairs were mice receiving the same dose of the test drug. Two days before the social interaction test, test mice were acclimated in the testing cage for 10 min and the test was conducted on the 3rd day. On day 3, the testing pair of mice was placed in the test cage for the 10 min test session.

Accelerating rotarod test

Mice were placed on rotating rod in an automated apparatus with grooved rotating rod having 3–7 cm diameter. The rotation speeds can be ranged between 0 and 30 rotations/min, falling of the mice will trigger a lever, and this will stop the automated timer allowing to observe the time taken to fall down. On the test, day mice were allowed to acclimatize the room for 15 min following which animals were placed in the rotating rod and the speed was gradually increased from zero to thirty rotations per minute. To avoid learning of balancing

on the rotating rod, mice were not trained for the experiment, and mice who could stay in the rotating rod for more than a period of 3 min at 30 rpm at baseline were excluded from the study. After each test, the rod and base plate were wiped with spirit to eliminate the smell of the previous animals. The latency to fall recorded as the endpoint measurement for further analysis.

Analysis was conducted using R and the values were rounded off to a single decimal and are expressed as median (interquartile range). The normality of distribution was done using Shapiro–Wilk test. Within group comparison was done using the Friedman test (more than 2 values) and the Wilcoxon signed-rank test (2 values). Between groups comparison was done using the Kruskal–Wallis test and $P < 0.05$ was considered statistically significant.

RESULTS

The present prospective interventional study assessed the effects of MSG at doses equivalent to human consumption (40 mg/kg/day, 60 mg/kg/day, and 80 mg/kg/day) on the model of anxiety (social interaction test). Since models of anxiety require an intact motor system, motor function was assessed using the rota rod apparatus. Within group comparison showed a significant difference in social interaction in MSG treated group [$P < 0.05$, Table 1and Figure 1] compared to baseline while the control group did not show any difference in social interaction ($P = 0.3$).

There was significantly lower social interaction among animals treated with MSG at 40 mg/kg/day at 1 month compared to baseline ($P = 0.01$). Compared to 1st month, 2 months of MSG administration resulted in significantly lower social interaction ($P = 0.004$). There was no significant change in the social interaction between 2 and 3 months of administration of MSG. MSG at 60 mg/kg/day administration showed no significant change in social interaction at 1 month compared to baseline ($P = 0.05$). Compared to 1st month, there was no significant reduction in social interaction at 2 months of treatment ($P = 0.1$) and compared to 2 months of treatment, there was no significant change in social interaction at 3 months ($P = 0.05$). At a dose of 80 mg/kg/day, there was significantly lower social interaction at 1 month of administration compared to baseline ($P = 0.01$). There was no significant difference in the social interaction at 2 months compared to 1 month ($P = 0.1$) and no significant difference between 3 months and 2 months of administration ($P = 0.1$). Compared to the baseline, all MSG-treated groups showed significantly lower social interaction at 3 months of treatment [Table 2].

Between groups, there was no significant difference in social interaction at baseline ($P = 0.5$). There was a significant difference in social interaction between groups at 1 month ($P < 0.001$), 2 months ($P < 0.001$), and 3 months ($P < 0.001$) [Table 3]. The effect of doses of MSG on the social interaction at various doses and at time intervals is demonstrated in Figure 1.

Within group, the comparison showed no significant change in rota rod fall time among controls and MSG treated groups using the Friedman test [Table 4].

DISCUSSION

The present prospective international study was conducted on 40 Swiss albino mice to receive distilled water

Table 1: Within group comparison of social interaction time at various time intervals

Group	Median social interaction time (IQR) at baseline	Median social interaction time (IQR) at 1 month	Median social interaction time (IQR) at 2 months	Median social interaction time (IQR) at 3 months	*P*-value
Control	3.4 (3.3–3.4)	3 (2.7–3.1)	3.1 (2.5–3.5)	3.1 (2.7–3.5)	0.3
MSG 40 mg/kg/day	3.3 (3.1–3.6)	2.7 (2.5–3.3)	2.3 (2.1–2.9)	2.3 (1.7–3)	<0.001*
MSG 60 mg/kg/day	2.8 (2.3–2.9)	2.1 (1.9–2.4)	1.9 (1.7–2.2)	1.3 (1.1–1.5)	<0.001*
MSG 80 mg/kg/day	3 (2.6–3.1)	1.9 (1.5–2.2)	1.7 (1.2–2)	1.2 (1.1–1.7)	0.001*

*Indicates significant difference within group using Friedman test; All groups treated with MSG had significantly lower social interaction at 3 months of the administration. IQR: Interquartile range, MSG: Monosodium glutamate

Table 2: Comparison of social interaction time at baseline and 3 months within groups

Group	Median Social interaction time (IQR) at baseline	Median Social interaction time (IQR) at 3 months	*P*-value
Control	3.4 (3.28–3.43)	3.1 (2.7–3.45)	0.334
MSG (40 mg/kg/day)	3.2 (3.08–3.48)	2.3 (1.73–2.95)	0.01*
MSG (60 mg/kg/day)	2.8 (2.25–2.9)	1.3 (1.18–1.48)	0.005*
MSG (80 mg/kg/day)	2.9 (2.45–3.03)	1.2 (1.05–1.73)	0.01*

*Indicates a significant difference between the groups using the Wilcoxon signed-rank test, IQR: Interquartile range, MSG: Monosodium glutamate

Table 3: Between group comparison of social interaction time

Parameter	Control	MSG 40 mg/kg/day	MSG 60 mg/kg/day	MSG 80 mg/kg/day	*P*-value
Median social interaction (IQR) at baseline	3.4 (3.3–3.4)	3.2 (3.1–3.5)	2.8 (2.3–2.9)	2.9 (2.5–3)	0.5
Median social interaction time (IQR) at 1 month	3 (2.6–3.1)	2.8 (2.6–3.3)	2.1 (1.9–2.4)	1.9 (1.5–2.2)	<0.001*
Median social interaction time (IQR) at 2 months	3 (2.7–3.2)	2.2 (2–2.5)	1.9 (1.7–2.2)	1.7 (1.2–2)	<0.001*
Median social interaction time (IQR) at 3 months	3.1 (2.7–3.5)	2.3 (1.7–3)	1.3 (1.2–1.8)	1.2 (1–1.7)	<0.001*

*Indicates a significant difference between groups using the Kruskal–Wallis test. IQR: Interquartile range, MSG: Monosodium Glutamate

Table 4: Within group comparison of rota rod fall time

Group	Median rota rod fall time (IQR) at baseline	Median rota rod fall time (IQR) at 1 month	Median rota rod fall time (IQR) at 2 months	Median rota rod fall time (IQR) at 3 months	*P*-value
Control	253 (124–300)	164 (105–219)	166 (104–265)	108 (86–216)	0.3
MSG 40 mg/kg/day	185 (137–250)	236 (185.5–290)	180 (144–270)	180 (110–263)	0.3
MSG 60 mg/kg/day	158 (121.8–300)	195 (155–285)	179 (133–262)	107 (95–187.5)	0.1
MSG 80 mg/kg/day	240 (163–300)	246 (195–300)	260 (174–290)	210 (154–-270)	0.5

No significant difference was observed within the groups. IQR: Interquartile range, MSG: Monosodium glutamate

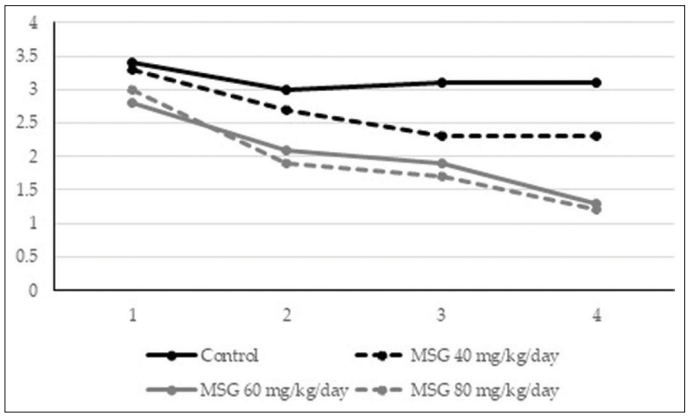

Figure 1: Social interaction time change in treatment groups

(controls) and MSG at serially increasing doses which is equivalent to human consumption. Among controls, there was no significant difference in social interaction time from baseline, 1 month, 2 months, and 3 months (P = 0.3). Within the MSG treated groups, a significant reduction in social interaction time was observed among groups treated with MSG 40 mg/kg/day (P < 0.001), MSG 60 mg/kg/day (P < 0.001), and MSG 80 mg/kg/day (P = 0.001) at 1 month, 2 months, and 3 months of treatment compared to baseline. In the MSG-treated group at the dose of 40 mg/kg/day, the animals showed significantly lower social interaction time at 1 month compared to baseline (P = 0.01), at 2 months compared to 1 month of administration (P = 0.004), and no significant difference at 3 months of treatment compared to 2 months. Animals which received MSG at a dose of 60 mg/kg/day showed significantly lower social interaction time compared to baseline, but did not show any significant changes at each time point compared to the previous. MSG at 80 mg/kg/day showed significantly lower social interaction time at 1 month of administration compared to baseline (P = 0.01), whereas there was no other significant difference between other time points. Compared to baseline, all MSG-treated groups showed significantly lower social interaction time at 3 months of treatment, (MSG 40 mg/kg/day [P = 0.01], MSG 60 mg/kg/day [P = 0.005], and MSG 80 mg/kg/day [P = 0.01]). Compared to controls, there was significantly lower social interaction among the mice treated with MSG at 40, 60, and 80 mg/kg/day (P < 0.05).

Higher doses of MSG, that is, 80 mg/kg, 160 mg/kg, and 320 mg/kg for 21 days have been shown to be associated with anxiety-like behavior in the open field and anxiety-related place preference behavior in mice.[4] In rats with treadmill exercise tests, it has been demonstrated that MSG induces cortical spreading depression and induces anxiety-like behavior and exercise provides protection against this event.[7] Our findings suggest MSG induced anxiety-like behavior in adult Swiss albino mice and the effect was not consistently dose-dependent. These findings have been observed in treadmill exercise in rats,[7] and also in spontaneous locomotor activity, contextual fear conditioning, and forced swimming test in rats.[8] Similar effects were also observed in open field tests in newborn Wistar rats which were given subcutaneous MSG along with partial hepatectomy.[9] Since the liver is the major metabolizing organ of MSG, partial hepatectomy would increase the central nervous system levels of glutamate and subsequent excitotoxicity which results in anxiety-like behavior.[9] The study also demonstrated that pretreatment with escitalopram would possibly ameliorate these effects.

The strengths of the study are being the first of its kind which uses social interaction test for anxiety in mice. Limitation is the non-demonstration of dose-dependent increase in these effects which would require larger samples.

CONCLUSION

MSG treated animals showed anxiety-like behavior in adult Swiss albino mice which was seen in all doses.

REFERENCES

1. Chand SP, Marwaha R. Anxiety. In: StatPearls. Treasure Island, FL: StatPearls Publishing; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/nbk470361 [Last accessed on 2023 Aug 05].
2. Yang X, Fang Y, Chen H, Zhang T, Yin X, Man J, *et al.* Global, regional and national burden of anxiety disorders from 1990 to 2019: Results from the Global Burden of Disease Study 2019. Epidemiol Psychiatr Sci 2021;30:e36.
3. Zanfirescu A, Ungurianu A, Tsatsakis AM, Nițulescu GM, Kouretas D, Veskoukis A, *et al.* A review of the alleged health hazards of monosodium glutamate. Compr Rev Food Sci Food Saf 2019;18:1111-34.
4. Onaolapo OJ, Aremu OS, Onaolapo AY. Monosodium glutamate-associated alterations in open field, anxiety-related and conditioned place preference behaviours in mice. Naunyn Schmiedebergs Arch Pharmacol 2017;390:677-89.
5. Onaolapo OJ, Onaolapo AY. Acute low dose monosodium glutamate retards novelty induced behaviours in male swiss albino mice. Glob J Neurol Neurosurg 2013;1:1-6.
6. Burkhouse KL, Kujawa A, Kennedy AE, Shankman SA, Langenecker SA, Phan KL, *et al.* Neural reactivity to reward as a predictor of cognitive behavioral therapy response in anxiety and depression. Depress Anxiety 2016;33:281-8.
7. Vitor-de-Lima SM, de Brito Medeiros L, Benevides RD, Dos Santos CN, da Silva NO, Guedes RC. Monosodium glutamate and treadmill exercise: Anxiety-like behavior and spreading depression features in young adult rats. Nutr Neurosci 2019;22:435-43.
8. Quines CB, Rosa SG, Da Rocha JT, Gai BM, Bortolatto CF, Duarte MM, *et al.* Monosodium glutamate, a food additive, induces depressive-like and anxiogenic-like behaviors in young rats. Life Sci 2014;107:27-31.
9. Zhao BB, Chen LL, Long QH, Xie GJ, Xu B, Li ZF, *et al.* Preventive effects of escitalopram against anxiety-like depressive behaviors in monosodium glutamate-teated rats subjected to partial hepatectomy. Front Psychol 2019;10:2462.

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