Research Article

Protective effects of Fenugreek seed on Monosodium Glutamate (MSG) Spermatozoa morphology and characteristics in male mice

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Abstract

Objective(s): This study aims to assess the protective effects of fenugreek seed on the morphology and characteristics of spermatozoa exposed to Monosodium Glutamate (MSG) in male mice. **Materials and Methods:** The experiment was performed using Albino Wistar male mice, divided into four groups, each consisting of five in number. Group C & D received 1.8 & 2.7 g kg^{-1} of MSG orally, group B received 2.7g kg^{-1} of MSG and fenugreek seed powder (1% of body weight from a 1% aqueous extract) as a supplement and group A was taken control treated with normal saline. Treatment was conducted for a period of 30 days. After the treatment period, various parameters were assessed to evaluate the morphology of spermatozoa and its characteristics. Various parameters like sperm percentage motility, sperm count, sperm morphology, Epididymal sperm viability, and testosterone level were evaluated. **Results*:*** Toxic effects were observed in MSG-treated groups, decreasing sperm motility, sperm count and increase in sperm abnormality was observed, reproductive organ-like testes weight was also found to reduced. Testosterone level also decreased. Toxic effect of MSG was found to be reduced in the group treated with fenugreek seed as a supplement. **Conclusion:** The MSG-induced toxicity effect has been restored by fenugreek seed powder.

***Keywords*:** Fenugreek, Monosodium Glutamate (MSG), Sperm Morphology, Sperm motility, Sperm abnormality

Introduction

Monosodium glutamate (MSG) is a highly contentious flavor enhancer in the realm of food additives. Renowned for its savory, meat-like taste, MSG finds widespread use in various processed foods. However, its application has been accompanied by a spectrum of toxic side effects observed in both humans and experimental animals (Durojaiye & Abolurin, 1993; Hamza & Diab, 2020). Reported symptoms include weakness, flushing, headaches, sweating, numbness, and dizziness. Furthermore, MSG has been implicated in exacerbating or instigating several medical conditions, encompassing asthma, urticaria, atopic dermatitis, ventricular arrhythmia, neuropathy, and abdominal discomfort (Geha et al., 2020).

Notably, MSG has been found to exert adverse effects on the male and female reproductive systems. Studies have demonstrated its toxic impact on the testes, leading to oligozoospermia and aberrant sperm morphology in male Wistar rats in a dose-dependent manner (Onakewhor et al., 1998). Such effects are implicated in male infertility, characterized by testicular hemorrhage, degeneration, and alterations in sperm cell population and morphology (Oforofuo et al., 1997; Das & Ghosh, 2010; Igwebuike et al., 2011; Hamza & Al-Harbi, 2014; Nosseir et al., 2012; Kianifard, 2016).

Despite being commonly regarded as safe for consumption, MSG lacks a recommended daily dosage. Excessive intake of MSG can induce toxicity, posing risks to various tissues and organs. Prior research has highlighted its adverse effects, particularly on reproductive organs, potentially elevating the risk of infertility. This underscores the importance of

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comprehending the consequences of chronic oral exposure to MSG, particularly given its prevalent use as a flavor enhancer in fast-food preparations, heightening the likelihood of male infertility.

Consequently, this investigation aims to assess the effects of chronic oral MSG exposure on rats, specifically focusing on sperm morphology and functionality. Additionally, the study seeks to evaluate the potential protective effects of fenugreek seed, a commonly utilized spice in food preparation (Oluwole et al., 2024).

Material and Methods

Chemicals

Monosodium glutamate was purchased from local market of Durgapur (West Bengal), 23.5178° N, 87.3460° E, India, which is used in food preparation. All the other chemicals and reagents are of laboratory standard.

Plant Material

Fenugreek seeds were obtained from a local market of Durgapur (West Bengal),23.5178° N, 87.3460° E, India, freed from impurities, and finely ground into powder. The resulting powder was combined with water (1 g in 100 ml), subjected to vortex mixing for 10 minutes, and then centrifuged. The resulting supernatant was employed as the aqueous extract, and this preparation was made fresh daily before feeding the mice.

Animals Required

In the present study adult Swiss albino male mice (25-30 gm body weight) of the same age group and same body weight were used. These animals were given a pellet diet and had free access to water ad libitum. The experimental work was started after a week of procuring the animals so that animals were well acclimatized to the new environment. Four groups were made (each group has five animals in no.) Normal adult male mice will be administered MSG orally once daily in the following dose levels for 30consecutive days and the effect was observed on the 31st day after completing the dosing cycle. The dose has been considered according to the study conducted by Mondal et al. (2016), in which effects of MSG were observed in the female reproductive system (Mondal et al., 2016).

Treatment groups

Group A: distilled water (control group)

Group B: 2.7 gkg^{-1} body weight (i.e. 15 % of LD_{50} of MSG) + fenugreek seed Powder (1% of body weight from1% aqueous extract)*

Group C: 1.8 gkg^{-1} body weight (i.e. 10 % of LD_{50} of MSG)

Group D: 2.7 gkg^{-1} body weight (i.e. 15 % of LD_{50} of MSG)

*Fenugreek seed was used as a supplement to treat only the group which received the higher dose was done intentionally to observe its protective effects against MSG induced toxic effects in same mice. Two groups were kept for the effects of MSG, considering MSG dose dependent effects.

The body weight of each group was determined weekly. After the end of the experimental period, all animals were sacrificed by performing cervical dislocation. Strict guidelines of the Institutional Animal Ethics Committee and CPSEA were followed to carry out the experimental work on animals; bearing approval no. BCRCP/IAEC/10/2018. The epididymis was carefully separated from the testes. The testes were removed, cleaned of accessory tissues, and weighed.

Semen Collection

After sacrificing the mice, orchidectomy was carefully done by the open castration method. The method was performed according to the protocol used by Akusu et al. (1985) and Oyeyemi et al. (2005) with some modifications. At the midline or pre- scrotal incision was made with fine precision and exposing the spermatic cord the testicles were dissected out and the cauda epididymis was separated into watch glass which was kept on an ice bath. Thereafter semen sample was collected. Analysing of the sample was performed immediately after the successful collection of the sample by evaluating percentage motility, sperm count, sperm morphology, and Epididymal Sperm Viability (Akusu et al., 1985; Oyeyemi & Ubiogoro, 2005).

Percentage Motility

On a warm glass slide, 50µl of semen were collected using a 2.9% buffered sodium citrate solution and covered with a glass slip, it was viewed under the microscope at a magnification value of x40. Evaluation was done by considering the sperm cells moving in a unidirectional motion for motility rating. While sperm cells moving in circles, in a backward direction or pendulating movement were excluded. At least 200 sperm were evaluated (Vasquez et al., 2012).

Sperm Count

Spermatozoa were counted by haemocytometer using the improved Neubauer chamber as described by Pant et al. (2003).The epididymis from one side was removed and minced in 1 ml phosphate buffered saline (PBS, pH 7.2) and 80 µ nylon mesh was used to filter the suspension. Aqueous eosin Y 1% (One drop) was added to the filtrate and was kept for 30 minutes. An aliquot of the suspension was taken in white blood cell pipette (Haemocytometer) up to 0.5 marks and then diluted in PBS up to mark 11. The dilution

was mixed thoroughly and placed into Neubauer's chamber. Then sperm count was done according to the standard procedure 16 in 8 squares of 0.1 cm^2 each except the central erythrocyte area in the grid of the neubar chamber. The total count was then multiplied by the correction factor, 5 x 10^4. For each animal, 500 sperm was screened and classified as normal and abnormal sperm according to the standard procedure (Pizzi et al., 1977; Kenjale et al., 2008; Narayana et al., 2002; Vega et al., 1988; Narayana et al., 2005).

Sperm Morphology

To evaluate abnormalities in sperm shape a part of the sperm suspension was used for preparing smears after staining with 1 % Eosin Y for 30 minutes. Smears were prepared on cleaned glass slides and air dried.

Cytological evaluation for abnormalities in spermatozoa morphology was carried out using a binocular microscope at 100 x magnification. The sperms was assessed for morphological abnormalities of sperm shape according to the Adebowale Bernard Saba et al. (2009). For screening per group thousands sperm were counted and the sperm presenting the defects in shape and structure of either head or tail or both were considered as abnormal and the data were presented as percentage incidence of total abnormalities. Were many types of abnormality parameter are shown including Double-headed, Multiple, Double tailed, Coiled, Flagellum with ansa, Bent at the cephalocaudal region, Amorphous, Hookless flagella, Coiled with microcephaly, Curved flagellum & Total sperm abnormality (Saba et al., 2009).

Epididymal Sperm Viability

Sperm suspensions of about 20 µl were mixed with 20 µl (0.05%) eosin-yellowish. Assessment of slides was assessed by a microscope with ×40 magnification following 2 minutes incubation at room temperature. Live sperms will not stain but dead sperms will appear pink in colour. In each sample, 200 sperms were counted and viability percentages were recorded (Iranpour & Valojerdi, 2013).

Statistical analysis

All the data obtained from this study were expressed as mean ±SEM. Statistical comparisons between the values obtained in control and in treated mice were evaluated by paired Student's t test / analysis of variance (ANOVA) whichever is applicable. $p \leq 0.05$ was considered as Significant using Primer Software and MS Office 2013.

Results

Mean Body Weight

The weight of treatment and control groups gradually increases during the treatment period without any significant difference. The treatment group's animals gained body weight higher than the control (Table 1).

Reproductive and other organ weights

Reproductive and other organ weights were emphasized on the effect of MSG on male mice, only group D treated mice testis significantly decreased in their weight compared to control. No effect was observed in group B and other organs also do not show any significant differences from control (Table 2).

Sperm abnormality assay

The result of the sperm abnormalities depicted in (figure 1), a significant change was observed in the treated groups with a higher % of abnormality (63.03 and 94.9) % in groups C & D respectively compared to Fenugreek seed and MSG treated Group B (14%), whereas control group A show only 7% of abnormality in total. Different % of sperm

Table 1. Observation of weekly body weight of mice

Groups	Weeks				
	0	**1st**	**2nd**	**3rd**	**4th**
Group-A	26.2±1.7	29.4±2.11	29.4±1.96	28.4±2.08	28.4±2.08
Group-B	26.4±1.6	28.6±1.5	30.4±1.2	31±0.8	32±0.8
Group-C	28.6±1.2	30±1.3	30.8±1.3	31.7±1.2	32.2±1.1
Group-D	26±1.2	27.8±1.2	28.8±1.3	30±1.3	31.4±1.07

Group A-Control, Group B-2.7 gm/kg MSG +fenugreek seed powder(5%w/w), Group C-1.8gm/kg MSG & Group D-2.7gm/kg MSG, (n number of animals per group = 5), all values are reported as mean±S.E.M, value marked with an asterisk (*) is significantly different from the control group, P<0.05 (student t-test)

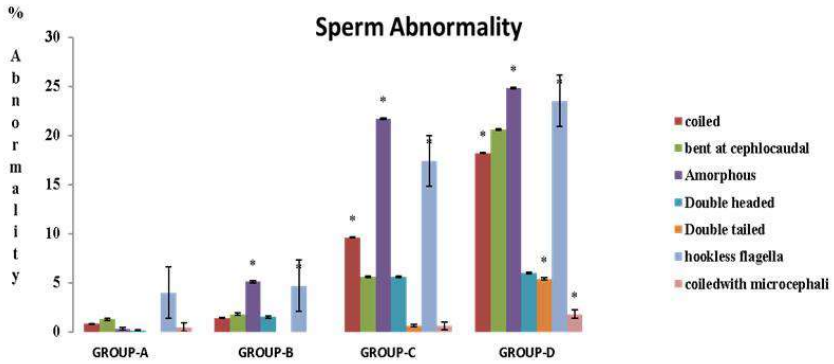

Figure 1. Percentage of Sperm Abnormality, (n number of animals per group = 5), all values are reported as mean±SE, value marked with an asterisk (*) is significantly different from the control group, P<0.05 (student t-test)

Table 2. Observation of mean organ weight of Reproductive and other organs

Groups	Reproductive & other organs							
	Testes		Kidney		Prostate	Liver	Spleen	Heart
	Right	left	R	L				
Group A	0.011±0.02	.09±.003	0.18±.04	0.18±0.05*	0.04±.003	1.4±.03	0.08±0.17	0.12±.01
Group B	0.11±0.002	0.11±.002	0.20±.008	0.20±0.04	0.05±.002	1.61±.002	0.08±0.002	0.12±.01
Group C	0.08±0.005	0.08±.003	0.17±04	0.18±0.01	0.03±.002	1.24±.04	0.8±.002	0.13±.12
Group D	0.05±0.002	0.04±.002	0.19±.004	0.19±0.02	0.05±.003	1.0±.23	0.06±.003	0.16±.002

Group A-Control, Group B-2.7 gm/kg MSG +fenugreek seed powder(5%w/w), Group C-1.8gm/kg MSG & Group D-2.7gm/kg MSG, (n number of animals per group = 5), all values are reported as mean±SEM, value marked with an asterisk (*) is significantly different from the control group, P<0.05 (student t-test)

abnormality was observed are explained as follows- ***Coiled*****,** this abnormality was observed higher in treated mice of group C (9.6 %) and group D (18.2 %). Whereas the % of abnormality observed in fenugreek seed powder and MSG-treated group B is only 1.4 % and the control group A is (0.82 %). ***Amorphous,*** the MSG-treated groups C & D were observed to have significantly (p<0.05) more spermatozoa with amorphous (21.7 & 24.8) % respectively. When compared with those of groups B (5.1 %) and A (0.32%). ***Bent at the cephalocaudal region***, the occurrence of this abnormality is significantly (p<0.05) higher in MSG-treated mice at (5.6 %) and (20.6 %) in C & D groups respectively when compared to Group B and A showing (1.8 and 1.2) % respectively. ***Hookless Flagella***, the group of mice in C & D show (17.4 and 23.5) % abnormality which is significantly different (p<0.05) from group B and A with (4.7 and 3.9) % respectively. ***Double***

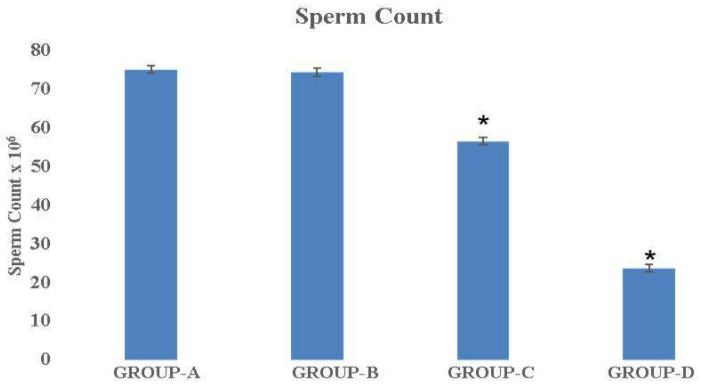

Figure 2. Sperm Count: Mean value of sperm count in control and MSG-treated mice x 106, (n number of animals per group = 5), all values are reported as mean±SE, value marked with an asterisk (*) is significantly different from the control group, P<0.05 (student t-test)

headed, was observed in group C and D of (5.6 and 6) % abnormality which is significantly different ($p<0.05$) from group B and A with (1.5 and 0.12) % of abnormality.

Sperm count

The result of the sperm count was depicted in (figure 2), the sperm count was found to significantly ($p<0.05$) decrease in groups D and C with (23.6±0.43 and 56.51±o.74) $x10^6$ respectively when compared with the group B and A.

Percentage Sperm Motility

The result of percentage sperm motility is depicted in (figure 3) The degree of motility of spermatozoa of MSG-treated mice in groups C & D was significantly ($P<0.05$) lower in comparison to group B and control group.

Hormonal Assay

Serum Testosterone level was found to decrease in groups C (276.1± 2.28 ng/dl) & D (236.46±5.7 ng/dl). Whereas group B found to be retained testosterone level as compared to control group A (304.9± 1.75ng/dl) (Figure 4).

Discussion

The study observed an increase in body weight among male mice exposed to MSG over the study duration. Suggesting MSG has an influencing effect in increase the appetite as it has been observed (Ajayi et al., 2020).

On the assessment of sperm analysis, MSG shows its toxic effect in decreasing sperm count, sperm motility, and increasing sperm abnormality, reproductive organ-like testis weight was also reduced. In hormonal assay level of testosterone also decreases in male mice. Low sperm concentration (oligospermia), poor sperm motility (asthenospermia), or abnormal morphology (teratospermia) is the indicators Infertility and problems of impaired fecundity (Kumar & Singh, 2015). Thus on overconsumption of MSG, obtained results suggest if MSG are used vigorously without any restriction in fast food preparation it can had reverse effect in the health of society leading a chance to increase infertility in male.

On supplement of powder of fenugreek seed in their normal

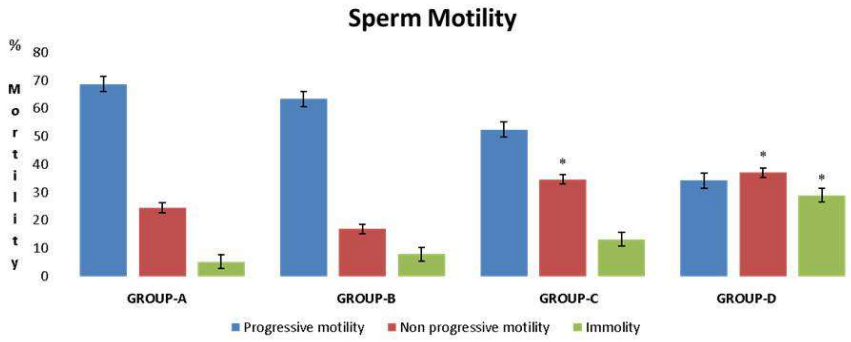

Figure 3. Sperm Motility: Mean value of percentage sperm motility, (n number of animals per group = 5), all values are reported as mean±SE, value marked with an asterisk (*) is significantly different from the control group, $P<0.05$ (student t-test)

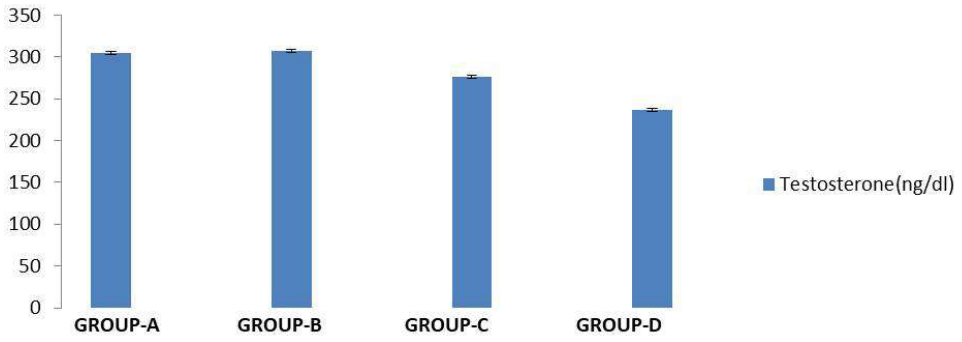

Figure 4. Serum Testosterone: Hormone Assay effects of MSG on testosterone level, Mean value of percentage sperm motility, (n number of animals per group = 5), all values are reported as mean±SE, value marked with an asterisk (*) is significantly different from the control group, $P<0.05$ (student t-test)

diet, MSG-induced toxic effects are not observed in group where fenugreek was given along with MSG. All the indicators of testicular damage was found to remain similar to control groups without any decrease in sperm count, sperm motility and testosterone level, percentage of sperm abnormality was also not affected. Suggesting shielding effects of fenugreek against MSG induced toxic effects.

Conclusion

Thus, from this observational study, it may be concluded that MSG at higher doses may affect male fertility and the administration of fenugreek seed with MSG confirms the protective effects of a commonly used spice "Fenugreek "as the shield to prevent the toxic effects of MSG. Within the limitation of the study the mode by which MSG would have induced the testicular damage and how fenugreek seed protect those changes needed to explore further. At the end we can conclude that fenugreek which is a common spice use as an ingredient in food preparation can be a mandatory inclusion in our daily food preparation.

Declarations

Author contribution statement

Singhmura S: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Writing of the manuscript.

Kumari A: Performed the experiments

Dey R: Contribute in writing the paper.

Pradhan P: Contribute in writing the paper.

Banik S: Contribute in writing the paper.

Mandal A: Contribute in writing the paper.

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Competing interest statement

The authors declare no conflict of interest.

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