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Research Article
Potential Aphrodisiac Effect of *Ginkgo Biloba* in Monosodium Glutamate Induced Reproductive Behavior Changes in Mice

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Abstract

Background and Objective: Monosodium glutamate (MSG) used as taste enhancer is known to cause disease conditions including the reproductive system defects. Present study was aimed to evaluate the effect of *Ginkgo biloba* in monosodium glutamate induced changes on the reproductive behavior and organs in mice. **Materials and Method:** Albino mice of either sex were used in the study. MSG at 4 mg g^{-1} was treated to experimental mice for 4 weeks followed by two-weeks of *G. biloba* administration at 50,100 and 150 mg kg^{-1}, orally. The influence of the MSG as well as the treatment with *G. biloba* extract on reproductive behavior was recorded in a closed camera circuit (CCTV) attached to a computer. Sperm analysis was done to determine the effect of MSG and *G. biloba* extract on total sperm count and sperm shape abnormalities, besides determining the relative weight of testis. The data obtained was analyzed statistically to find the significance. **Results:** The data from the study indicated that 4-weeks of MSG treatment significantly ($p<0.05$) reduced the sexual activity behavior and altered the total sperm count, sperm shape and relative weight of testis compared to control animals. The highest tested dose of *G. biloba* (150 mg kg^{-1}) produced significant ($p<0.05$) reversal in defective sexual behavior activities and improved the total sperm count and reduced the sperm shape anomalies. However, none of the tested dose of *G. biloba* showed significant inhibition on the elevated relative testes weight in MSG mice. **Conclusion:** The data suggested that *G. biloba* at 150 mg kg^{-1} improved the sexual behavior and activity diminished by MSG, besides correcting the quantitative and qualitative defects in spermatozoa. These actions can be related to the neuronal and local tissue protective and antioxidant properties of *G. biloba*.

Key words: *Ginkgo biloba*, monosodium glutamate, reproductive behavior, sperm count, sperm shape, relative testis weight

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Monosodium glutamate (MSG) is a sodium salt of glutamic acid used frequently in food industry to enhance the taste. MSG is commonly used in processed and packaged fast foods and can also be found in raw foods such as apple, chicken, onion, eggs, mild, etc[1]. According to food and drug administration (FDA), consumption of MSG is considered safe if the daily intake is less than 3 g. However, long-term usage of MSG is reported to cause several deleterious effects on health, characterized as 'Chinese Restaurant Syndrome'[2].

In the body, MSG gets metabolized to glutamine, glutamate and aspartate and activates several receptors located in every part of the cell[3]. MSG is reported to be a neurotoxic agent and is known to cause various neurobehavior changes such as anxiety, depression, migraine and Alzheimer's disease[2,3]. The metabolic defects associated with MSG include hyperlipidemia, obesity and diabetes mellitus[4]. Long-term exposure of MSG is also linked to higher incidences of cancers[5].

The reproductive organ dysfunction has been reported with the chronic intake of MSG. In males, it is found to damage the spermatogenesis, Leydig cell and testosterone production[6]. The female organ effects include damage to follicle maturation, loss of regular menstruation, abortion and still-births[7]. The neuroendocrine damage produced by MSG disturbs the normal physiology of arousal, sensitization and libido activities[8].

Ginkgo biloba (G. biloba) or maidenhair, is a tree native to China that has been grown for thousands of years for a variety of uses. Because it is the only surviving member of an ancient order of plants, it is sometimes referred to as a living fossil[9]. While its leaves and seeds are often used in traditional Chinese medicine, modern research primarily focuses on ginkgo extract, which is made from the leaves. *Ginkgo* supplements are associated with several health claims and uses, most of which focus on brain function and blood circulation[10].

G. biloba reported to benefit the patient from several neurological diseases such as dementia, cognitive impairment and Alzheimer's disease[11]. The extract available commercially as tablets, tea, capsule and is frequently used for the aphrodisiac property. The extract is reported to be beneficial in arousal dysfunction, diminished libido and defective sensitization in both and female patients[12]. The important components identified in the extract are quercetin, isorhamenetin, bilobetin, ginkgetin and sciadopitysin, etc[13]. This study was designed to evaluate the role of *Ginkgo biloba* against MSG-induced reproductive behavior defects in mice.

MATERIALS AND METHODS

Duration of the study: The present research was conducted during the months of September, 2019 and December, 2019 in the research labs of Pharmacology and Toxicology, College of Pharmacy, Qassim University.

Drugs and solvents: Commercially available *Ginkgo biloba* extract and monosodium glutamate was procured from the authorized chemical and drugs' supplier of the college. The solvents and other reagents were of analytical grade and were obtained from the college's storehouse.

Animals: Six albino mice of either sex weighing 30-35 g were obtained from the central animal house after approval from the Institutional Animal Ethics Committee (Approval ID 2019-CP-9). The animals were housed in 12-h bright/dark environment supplied with pellet food and water *ad labitum*. The animals were maintained in standard laboratory condition at the temperature 22±2°C.

Grouping of the animals: Six animals in each group were randomly selected as group-1 [(Control-normal saline, 0.5 mL 100 g^{-1}, body weight (b.wt.)], group-2 (Positive control, MSG-4 mg g^{-1}, b.wt.)[14], group-3 (Treatment-1, MSG+*G. biloba*, 50 mg kg^{-1}, b.wt.)[15], group-4 (Treatment-2, MSG+*G. biloba*, 100 mg kg^{-1}, b.wt.), group-5 (Treatment-3, MSG+*G. biloba*, 150 mg kg^{-1}, b.wt.).

Preparation of drug solutions and duration of treatment: Animals were daily treated with monosodium glutamate at 4 mg g^{-1}, b.wt., orally for four weeks. The salt was dissolved in drinking water at the dose needed for that group after calculating their daily water consumption. The *G. biloba* extract was dissolved in distilled water. Three doses of 50, 100, 150 mg kg^{-1} were tested to animals daily for two weeks by oral route. Towards the end of the treatment, animals were sacrificed under light-ether anesthesia after recording their productive behaviors' parameters.

Reproductive behavior: The study was done on sexually active adult male and female mice. To verify the sexually receptive stage of female mice, a vaginal smear was prepared and observed under light microscope using 10X objective. The presence of large number of cornified irregular shaped cells suggests that the female mice are now in sexually receptive di-estrus phase[16]. The di-estrus phase female mice and sexually experienced male mice were allowed in separate cage in a dim-light and calm atmosphere. The sexual activity of the

pair was recorded overnight in a close circuit camera (CCTV) attached to a computer. The aphrodisiac properties of various treatments were studied by recording the orientation behavior and the sexual activity of mice.

The parameters for orientation behavior prior to sexual activity include licking, anogenital sniffing, genital grooming and climbing. In this, each activity was recorded as 1-score and the average number in the overnight duration was calculated. The sexual behavior was evaluated by[17]:

$$\text{Libido index (\%)} = \frac{\text{Number mated}}{\text{Number paired}} \times 100$$

$$\text{Intromission ratio (\%)} = \frac{\text{Number of intromission}}{\text{Number of mount} + \text{Number of intromission}}$$

$$\text{Copulatory efficiency (\%)} = \frac{\text{Number of intromission}}{\text{Number of mounts}} \times 100$$

$$\text{Intercopulatory interval} = \text{Average time between intromission}$$

Sperm analysis: For the total sperm count, the cauda epididymis of the mice was harvested and added to phosphate buffered glucose saline (PBGS). The debris was removed to obtain a clear suspension. To calculate total sperm count a Neubauers' haemocytometer was used. In brief, a drop of the suspension was added to either side of the haemocytometer and count was recorded[18]. The total number of sperms present in the four chambers were taken and represented as cubic millimeter after multiplying with dilution factor (50,000).

The sperm shape analysis was done as per the procedure described by Wyrobek *et al.*[19]. To the suspension of sperm, 2-3 drops of aqueous Eosin-Y stain was added. A smear was prepared on a clean glass slide. Different types of sperm shape abnormalities such as double-headed, double body, irregular head, tailless, headless and giant head were analyzed among 100 sperms counted for each slide. The average number was recorded to calculate the percentage sperm abnormality.

Relative weight of testis: The relative weight of testis was calculated by finding the weight of the testis with respect to the body weight[20].

Statistical analysis: The data obtained from the study was statistically evaluated by one-way ANOVA. The $p<0.05$ was be considered to indicate the significance of the result. All the values were represented as Mean±SEM.

RESULTS

Effect of *G. biloba* on the orientation behavior prior to sexual activity in MSG treated mice: The orientation behavior prior to sexual activity is represented in Table1. Administration of MSG (4 mg g^{-1}) was found to significantly alter the orientation parameters such as licking ($p<0.001$), anogenital sniffing ($p<0.001$), genital grooming ($p<0.05$) and climbing ($p<0.001$) compared to control animals. *G. biloba* at the lowest tested dose produced a significant ($p<0.05$) increase in the climbing character but did not alter significantly other parameters when compared with MSG-treated group. *G. biloba* at 100 mg was found to significantly increase licking ($p<0.05$) and climbing ($p<0.001$) character. On the other hand, *G. biloba* at the highest tested dose (150 mg) increased significantly licking ($p<0.05$), anogenital sniffing ($p<0.01$) and climbing ($p<0.001$) characters. However, none of the tested doses of *G. biloba* altered significantly the genital grooming parameter in MSG treated mice.

Effect of *G. biloba* on the sexual behavior in MSG treated mice: The sexual activity of the experimental animals was measured by recording the percentage libido index, intromission ratio, copulatory efficiency and intercopulatory interval. MSG administration for 4 weeks was found to decrease significantly the percentage libido index ($p<0.05$), intromission ratio ($p<0.05$), copulatory efficiency ($p<0.01$) and increased the intercopulatory interval ($p<0.001$) when compared to the normal animals. The lowest dose of *G. biloba* (50 mg) did not alter significantly the sexual activity parameters, while the medial dose of *G. biloba* (100 mg) showed a significant enhancement in the intromission ratio ($p<0.05$) and suppression of intercopulatory interval ($p<0.05$) compared to the MSG group. The highest dose of *G. biloba* (100 mg) was found to enhance the % libido index ($p<0.05$), intromission ratio ($p<0.01$), copulatory efficiency ($p<0.05$) and reduced the intercopulatory interval ($p<0.001$) compared to the challenge group (Table 2).

Effect of *G. biloba* on total sperm count in MSG treated mice: Total sperm count analysis revealed that exposure of MSG reduced the number significantly ($p<0.05$) compared to the normal saline treated animals. The lower and medial doses of *G. biloba* (50 and 100 mg) did not showed significant variation, however, the highest tested dose of *G. biloba* (150 mg) produced significant elevation ($p<0.05$) in the total sperm count compared to the MSG treated animals (Fig. 1a).

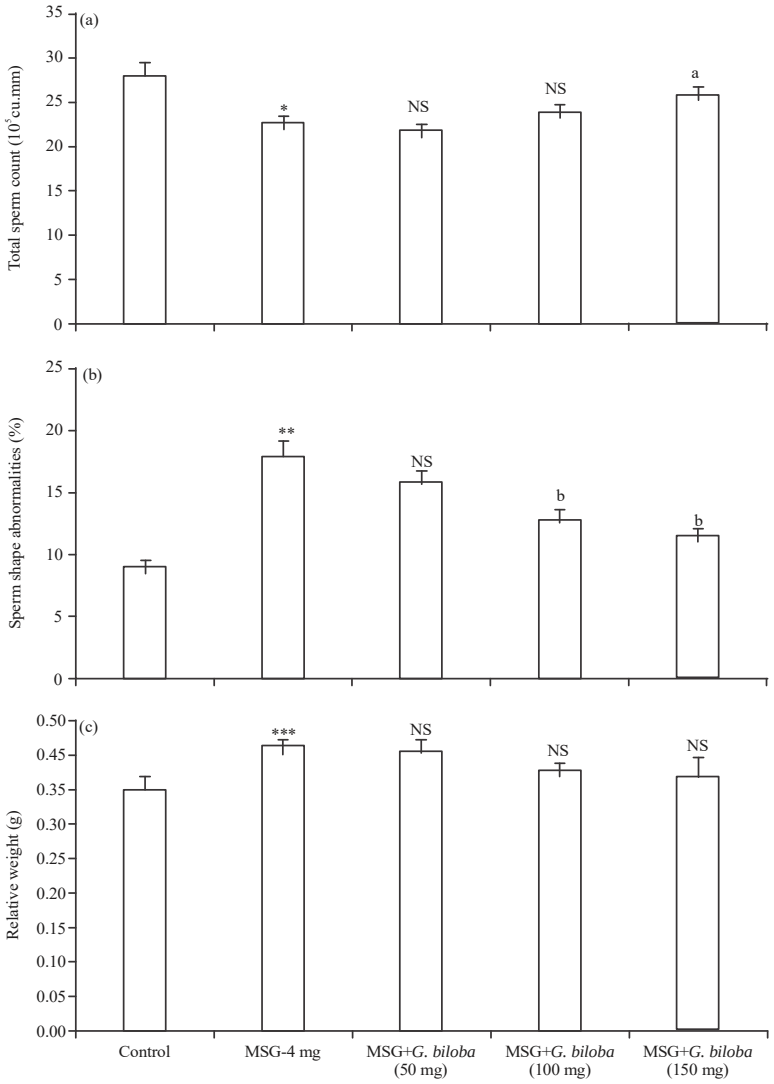

Fig. 1(a-c): Effect of *G. Biloba* on: (a) Total sperm count, (b) Sperm shape abnormalities and (c) Relative testis weight in MSG treated mice

NS: Not significant, n = 6, one-way ANOVA followed by Turkey, *p<0.05 compared with control, [a]p<0.05 compared with MSG group, **p<0.01 compared with control, [b]p<0.01 compared with MSG group, ***p<0.05 compared with control, values are represented as Mean±SEM

Table 1: Effect of *G. biloba* on the orientation behavior prior to sexual activity in MSG treated mice

	Scores			
Treatments	Licking	Anogenital sniffing	Genital grooming	Climbing
Control	9.34±0.21	8.42±0.52	12.82±0.71	6.48±0.47
Monosodium glutamate (MSG-4 mg)	6.24±0.18**	4.19±0.39**	09.41±0.86*	2.64±0.12**
MSG+*G. biloba* (50 mg)	6.91±0.35^{NS}	4.15±0.32^{NS}	08.94±1.06^{NS}	3.85±0.37^{a}
MSG+*G. biloba* (100 mg)	7.54±0.38^{a}	5.16±0.48^{NS}	09.67±0.74^{NS}	4.98±0.26^{c}
MSG+*G. biloba* (150 mg)	7.84±0.41^{a}	6.47±0.58^{b}	10.02±0.93^{NS}	6.97±0.46^{c}

Values are represented as Mean±SEM, NS: Not significant, n = 6, One-way ANOVA followed by Tukey, *p<0.05, **p<0.001 compared with control, [a]p<0.05, [b]p<0.01, [c]p<0.001 compared with MSG group

Effect of *G. biloba* on sperm shape abnormalities in MSG treated mice: The sperm shape analysis is represented in Fig. 1b. MSG treatment for 4-weeks was found to increase the percentage abnormality significantly (p<0.001) compared to the normal animals. The lowest tested dose of *G. biloba* (50 mg) did not showed significant difference but the medial (100 mg) and higher doses (150 mg) exhibited significant (p<0.01) diminution in

Table 2: Effect of *G. biloba* on the sexual behavior in MSG treated mice

Treatments	Libido index (%)	Intromission ratio (scores)	Copulatory efficiency (%)	Intercopulatory interval (sec)
Control	88.71±9.34	0.31±0.03	93.71±7.38	456.12±10.83
Monosodium glutamate (MSG-4 mg)	62.80±6.46*	0.22±0.01*	54.23±4.92**	692.54±08.21***
MSG+*G. biloba* (50 mg)	59.24±7.22^{NS}	0.26±0.03^{NS}	61.88±5.69^{NS}	677.23±07.56^{NS}
MSG+*G. biloba* (100 mg)	69.48±5.39^{NS}	0.29±0.03^{a}	68.91±5.09^{NS}	623.97±08.54^{c}
MSG+*G. biloba* (150 mg)	79.81±4.02^{a}	0.30±0.02^{b}	72.39±6.08^{a}	587.64±09.88^{c}

Values are represented as Mean±SEM, n = 6, NS: Not significant, One-way ANOVA followed by Tukey, *p<0.05, **p<0.01, ***p<0.001 compared with control
^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001 compared with MSG group

the percentage sperm abnormality compared to the positive control group.

Effect of *G. biloba* on the relative testis weight in MSG treated mice: The data from the relative weight of testis study indicated that administration of MSG (4 mg) for 4-weeks significantly (p<0.05) increased the weight compared to the control group. The tested doses of *G. biloba* (50, 100 and 150 mg) when administered to the MSG-treated mice although reduced the relative testis weight but the change was found to be insignificant compared to the MSG group (Fig. 1c).

DISCUSSION

The observations from the present study indicated that four weeks of MSG treatment to mice significantly (p<0.01) decreased the orientation behavior and the sexual activity parameters in comparison with the normal animals (Table1, 2). Earlier studies indicated that MSG administration can produce neuronal toxic effects on CNS including Hypothalamic-pituitary axis (HPA). Damage to HPA is reported to affect the reproductive behavior and the sexual activity[8].

There are studies that suggested that MSG mediated HPA damage can alter the level of gonadotropic hormones (FSG and LH) and testosterone due to damage to Leydig cells[21]. Alteration in the serum concentration of testosterone is known to affect the sexual motivation and libido characteristics in both males and females[22]. The diminished orientation characters and the sexual activity behavior observed in the present study (Table1, 2) could be linked to these mechanisms.

The present study findings on the total sperm count, sperm abnormalities and relative testis weight are in agreement with these data (Fig. 1a-c). The observations are in agreement with the previous research where MSG administration to the experimental rats significantly (p<0.05) reduced the total sperm count, besides increasing the percentage sperm abnormalities and relative testis weight[23]. In these studies, it was reported that administration of MSG has modulatory effect on the testicular morphology. Chronic MSG treatment has been reported to damage the spermatogenesis by getting concentrated in the vital reproductive organs such as epididymis and vas deferens. The mechanism suggested is the hyper-activation of metabotropic glutamate receptor that caused excess influx of calcium leading to damage of male reproductive organs and severely affecting the spermatozoa[23,24]. MSG also reported to induce the inflammatory process by activating the various chemical mediators such as interleukins, TNF-α and chemokines. The significant increase in the relative testis weight suggested the possible cellular damage induced by the MSG and the associated inflammatory process that followed afterwards[25].

Observation of the present study is that administration of *G. biloba* exhibited a dose-dependent reversal in the reproductive behavior diminished by MSG. The lowest tested dose of *G. biloba* (50 mg kg^{-1}) produced significant increase in the climbing character, the medial dose (100 mg kg^{-1}) enhanced licking, intromission ratio and intercopulatory interval besides improving the climbing behavior. The highest tested dose (150 mg kg^{-1}) produced further improvement in the activities mentioned for the medial and lower dose of *G. biloba* and also exhibited significant augmentation in anogenital sniffing, percentage libido index and copulatory efficiency (Table 1, 2).

As reported, *G. biloba* extract contains several active ingredients known to possess the therapeutic activity. The components are categorized in to two groups, viz., flavonoids (kaempferol, quercetin, isorhamnetin) and terpenes (ginkgolides, bilobalide)[9,13]. These components are reported to exhibit multiple actions such as vaso-regulation (dilation, decreasing the blood viscosity and elasticity) that can improve the blood circulation to the genitals, leading to sexual arousal response[26]. Further, a non-ginkgolide non-flavonoid component of *G. biloba* was reported to cause the relaxing effect on the vascular smooth muscle[27]. Studies also indicated that *G. biloba* can modulate the synthesis of nitric oxide considered to be important endogenous substance in the genital organ performance[26,27].

Another important finding of this study is that *G. biloba* reversed the abnormalities in the total sperm count and shape induced by MSG (Fig. 1a, b). One of the mechanisms suggested for the damage of reproductive organ/cells is the excess accumulation of calcium ions leading to tissue damage and generation of free radicals[23]. *G. biloba* in the earlier studies has been reported to possess the antioxidant potential[15] that could have prevented the quantitative and qualitative defects in the spermatozoa induced by MSG. However, the tested dose of *G. biloba* in this study did not produce significant variation in the relative weight of testis. The doses, although reduced the relative testis weight but the effect was found to be insignificant when compared to the MSG treated mice (Fig. 1c). Further studies are suggested involving higher doses and/or longer duration of *G. biloba* to confirm the action on relative weight of reproductive organs.

The present research suggested that chronic exposure of MSG can produce defects in spermatozoa, reproductive behavior and organ in mice. The salt is commonly ingredient in various food products. Since it is difficult to avoid MSG in our daily life, one of the best options is to contract its adverse actions. *Ginkgo biloba* being a natural substance has protected the reproductive defects induced by MSG. Increasing the dietary intake of *Ginkgo biloba* might provide protection against several health complications induced by common food additives.

CONCLUSION

The results from the present study indicated that administration of Monosodium glutamate (MSG) commonly used to enhance the taste of food can produce adverse effects on the sexual behavior, besides altering the total sperm count, sperm shape and relative weight of testis. *G. biloba* at 150 mg kg^{-1} was found to exhibit a significant reversal in the damages induced by MSG on the sexual behavior parameters, total sperm count and shape. These actions could be linked to the phytoconstituents that were known to cause vaso-regulation and antioxidant effects. However, the non-significant improvement in the relative testis weigh might require further studies with higher doses and/or longer duration of treatment to determine the precise role of *G. biloba* in the MSG induced reproductive organ damages.

SIGNIFICANCE STATEMENT

This study discovered the possible aphrodisiac potential of *Ginkgo biloba* against the monosodium glutamate induced reproductive defects in mice. *Ginkgo biloba* being a natural substance *perse* is devoid of side effects. The regular consumption of the *Ginkgo biloba* extract could benefit in minimizing the neurological, metabolic and reproductive defects induced by monosodium glutamate. The research could open new avenues to study the role of food additives on the incidences of new and existing diseased conditions and, the potential action of naturally occurring substances in reducing these complications.

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