Protective effect of Aqueous Garlic Extract on Monosodium Glutamate Induced Toxicity on Johnson's Scoring of Infertility in Adult Wistar Rats

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Abstract

Objective: To observe the effect of aqueous garlic extract (AGE) on the MSG-induced toxicity on the Johnson score of infertility in adult Wistar rats.

Method: This study was conducted at the University of Health Sciences, Lahore. A total of 24 adult male Wistar rats weighing 150-175 g and aged 6-8 weeks were collected and divided into 4 groups (6 rats each). Group A acted as a control, with 6 ml / kg of distilled water given orally by gavage and intraperitoneally for 14 days. Group B received 4 g / kg MSG dissolved in 6 ml of distilled water intraperitoneally for 14 days. Group C orally administered 200 mg / kg AGE dissolved in 0.4 ml of distilled water for 14 days by forced oral administration, and Group D first intraperitoneally administered 4 g / kg of MSG dissolved in 6 ml of distilled water. Subsequently, AGE 200 mg /kg dissolved in 0.4 ml of distilled water was given for 14 days by forced oral administration. All doses were administered once daily.

Results: At the end of study, one way ANOVA and Post hoc Tukey test were found to be significant (0.000) proving toxicity induced by MSG and protection rendered by AGE in B and D groups respectively.

Conclusion: Aqueous garlic extract improves and protects against the deleterious effects of MSG on Johnson's Scoring of infertility of adult Wistar rats.

Keywords: Monosodium glutamate (MSG), Johnson's Score, testes, Aqueous garlic extract (AGE), Wistar rats.

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Introduction

Monosodium glutamate (MSG), a salt of glutamic acid (an amino acid), is a well-known flavor enhancer and a typical ingredient in foods such as canned vegetables, soups, processed meats, and traditional flavorings. It is also found in many Chinese dishes. Glutamate is a nonessential amino acid synthesized by the human body and processed in almost all natural edible substances, especially protein-rich foods such as dairy products, meat and many vegetables.[1]

MSG disrupts the typical histology of testes. This fact becomes important as infertility is estimated to affect 15% of couples, a total of 48.5 million couples worldwide.[2] Continued use of MSG induces oxidative stress in liver and heart tissues as well as testes of experimental animals due to metabolic disturbances. Oxidative stress increases the formation of free radicals and reactive oxygen species (ROS), leading to oxidative damage to biomolecules that cannot be neutralized by antioxidant resistance frameworks.[3] High oxidative stress causes lipid and protein changes in cell membranes, leading to the initia-

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tion of dysfunctional metabolic pathways.[4] Allium sativum, commonly known as garlic, is an invigorating and exceptional scent that adds aroma, flavor and nutrition to daily dinner.[5] It contains a variety of potent sulfur compounds contributing to its medicinal efficacy. Ajoene is the best of the compounds explored. Allicin (diallylthiosulfinate) and S-modified cysteines are the basic thiosulfinates, approximately 60-80% of which is allicin.[6,7] Alliin is converted to its metabolites. Allicin, pyruvate and alkali by the activity of the garlic button enzyme allinase.[8] AGE can lower cholesterol levels by up to 75%. It has the effect of reducing calcium transfer in the coronary arteries. The addition of garlic plays a crucial role in supporting the structure and validity of endothelial cells along with their endothelial capacity. AGEs are thought to lower homocysteine levels, increase micro-circulation, and protect endothelial cells from oxidative stress. Garlic reduces the risk of prostate tumors and various malignancies.[9] Rationale of current study is to observe the protective effect of AGE on MSG induced toxicity leading to male infertility.

Materials & Method

The present research was conducted at the Department of Anatomy, University of Health Sciences Lahore after ethical approval was obtained. A total of 24 Wistar rats weighing 150-175 g, aged 6-8 weeks were purchased from the animal facility of the University of Health Sciences, Lahore. These 24 rats were split into 4 groups. A, B, C, D, each having 6 rats. Experimental animals were maintained at a controlled temperature of 23±2 °C, 55 ± 5% humidity and a 12 h light-dark cycle each. Rats received standard rat chow and water ad libitum and were acclimated two weeks before beginning of the experiment. The rat's body weight was measured at its first and final day (day 15) of the experiment. Group A served as a control and was administered with distilled water daily by gavage and intraperitoneally at a dose of 6 ml/kg for 14 days. Group B received 4g/kg MSG dissolved in 6 ml distilled water intraperitoneally daily for 14 days. Group C was gavaged daily with 200 mg/kg of AGE dissolved in 0.4 ml of distilled water for 14 days. Oral gavage daily for 14 days. MSG with a concentration of ninety nine percent was purchased from the local market. The 4 gm/kg of MSG was dissolved in 06ml of distilled water.Local garlic was obtained from a new crop to produce AGE. Garlic cloves were separated, peeled, washed with distilled water and left to dry at room temperature for 1 hour. 50 grams of these cloves were taken, cut into small pieces, and finally she was ground with a pestle and pestle containing 100 ml of distilled water and filtered using a cotton cloth. The final concentration of garlic in this filtrate was 500 mg/ml. AGE weighing 200 mg / kg body weight was prepared daily to treat the rats and orally administered by nasogastric tube feeding. The scoring was performed with the help of an X40 objective. In the event of uncertainty regarding the spermatozoa some tissues were checked at greater magnification also. Tubules in one field at the corner were chosen, their score recorded and the slide was shifted in figure of 'S' to take in the neighboring zone within the field while the scoring continued. Impaired tubules at the borders of the area were rejected. At least ten seminiferous tubules from every slide were examined and scored. Three slides from every animal were analyzed and an aggregate of 720 measurements were made. Following is the scale of scoring (Johnson et al., 1970):

10. Normal tubules with complete spermatogenesis and many spermatozoa present
9. Many spermatozoa (>5) present but disorganized seminiferous epithelium with sloughing or obliterated lumen
8. A few spermatozoa (less than 5 per tubule)
7. No spermatozoa in lumen but many (>5) spermatids
6. No spermatozoa but a few spermatids (less than 5 per tubule)
5. No spermatozoa or spermatids but many spermatocytes
4. A few spermatocytes (less than 5 per tubules), no spermatozoa or spermatids
3. Only spermatogonia and Sertoli cells present
2. Sertoli cells present, no spermatogenic cells

Table 1: *Effect of MSG (4g/kg body weight) given intraperitoneally for 14 days on the mean Johnson's score of rats. Values given are mean ± standard deviation of 6 animals. Statistics according to one way ANOVA for comparing mean Johnson score among all groups A, B, C & D (n=6).*

Parameter	Group A (n=6)	Group B (n=6)	Group C (n=6)	Group D (n=6)	*p – value
Mean Johnson's score	9.56±0.21	5.7±0.33	9.0±0.322	8.9±0.25	0.000

*p ⩽ 0.05 is considered as statistically significant.

1. No cell in the section of tubule

Results

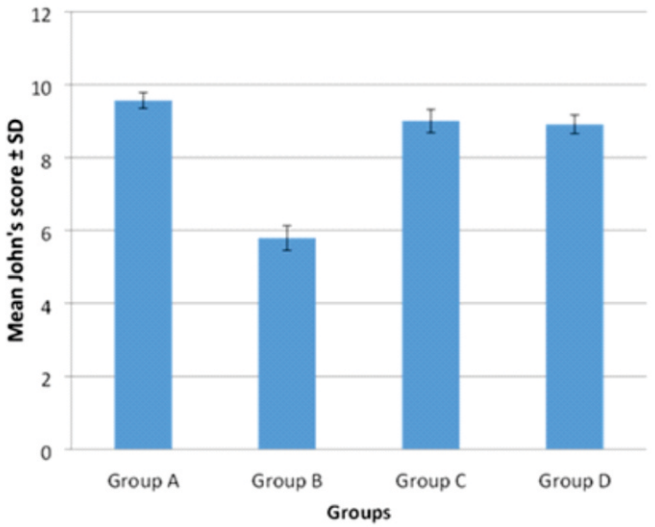

Fig-1: *Bar chart depicting the mean Johnson's score among groups (n=6).*

Table 2: *Effect of MSG (4g/kg body weight) given intraperitoneally for 14 days on the mean Johnson's score of rats. Each value is the mean of 6 animals. Statistics according to Post hoc Tukey for multiple comparison of mean Johnson's score among all groups A, B, C & D.*

Group I	Group J	Mean Difference (I-J)	Standard Error of Mean	*p-value
A	B	3.77	0.172	0.000
	C	0.565	0.164	0.013
	D	0.648	0.164	0.004
B	A	-3.77	0.172	0.000
	C	-3.2	0.172	0.000
	D	-3.12	0.172	0.000
C	A	-0.565	0.164	0.013
	B	3.20	0.172	0.000
	D	0.0833	0.164	0.956
D	A	-0.64	0.164	0.004
	B	3.12	0.172	0.000
	C	-.0833	.164	.956

Discussion

MSG consumption is associated with a variety of health hazards, including reproductive toxicity. It is worth looking for a phytochemical strategy with effective protection and a wide range of safety profiles. Previous studies have shown the presence of functional glutamate transporters and receptors in the testes of rats and mice.[11] Compared with control rats, rats treated with monosodium glutamate had a significantly reduced caudal epididymal sperm reserve.In the present study, Group B which was administered with MSG showed statistically significant results with low scoring in Johnson's score of infertility. Group C exhibited near normal results and Group D showed the protective effects of AGE against toxic effects of MSG. The deteriorating changes in germ and somatic cells of seminiferous tubules may have emerged from the interactivity of MSG with proteins and enzymes disrupting the antioxidant defense mechanism prompting collection of free radicals leading to inflammatory reaction and mitochondrial damage.[12]

Conclusion

Aqueous Garlic extract protected against the MSG induced low levels in Johnson's scoring of infertility in adult Wistar rats.

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Conflict of Interest *None*

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Authors Contribution

HSA, FR: Conceptualization of Project
SF, FR: Data Collection
SN, FR: Literature Search
FR, HSA: Statistical Analysis
AS, HSA: Drafting, Revision
FI, HSA: Writing of Manuscript