EFFECTS OF ALOE VERA GEL ON MONOSODIUM GLUTAMATE INDUCED OVARIAN TOXICITY IN RATS

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ABSTRACT

Aim and Objective: Monosodium glutamate (MSG) is added to the Asian dishes as a taste enhancer. The study was formulated to assess the possible protective effect of aloe vera gel on reproductive toxicity inflicted by MSG.

Study design: It was an experimental study conducted at the Anatomy department, SZPGMI, Lahore.

Material and Method: Three groups each having 12 female Wistar albino rats were made. Group A was the control group, it was given distilled water. The experimental group B was treated with MSG 200 mg/kg b.w dissolved in 1.5 ml of distilled water, for 14 days by gastric intubation. While the experimental group C was provided with 400 mg/kg b.w. of aloe vera gel extract, along with 200 mg/kg b.w. MSG dissolved in 1.5 ml of distilled water by gastric intubation for 14 consecutive days. At the end of the experiment, all the rats were dissected and their ovaries were removed. Light microscopic examination was done to study the histological changes.

Results: The ovaries demonstrated deformed secondary and graafian follicles with vacuolization and a marked decrease in the diameters of secondary follicles (p value= 0.018) in group B. Co-administration of aloe vera gel in group C markedly improved the histopathological changes induced by MSG (p value=0.003).

Conclusion: Aloe vera gel when consumed with MSG minimizes its harmful effects.

Key words: Monosodium glutamate, taste enhancer, aloe vera, gastric intubation, follicles, vacuolization

INTRODUCTION

Artificial flavour enhancers are linked to adverse effects on the female reproductive system.¹ ² Monosodium glutamate (MSG) is a commonly added chemical in our foods. It comprises 22% sodium, 78% glutamic acid and water.³

Glutamate is a non-essential amino acid.⁴ It is added in many canned and frozen foods.⁵ MSG elicits a special taste - ‘UMAMI’ translated as pleasant savory.⁶

Increased levels of MSG cause adverse effects by acting on the glutamate receptors and resulting in increased calcium ion influx. Calcium ion influx activates enzymes like endonucleases, phospholipases, proteases causing damage to cellular structures including chromatin material, cell membranes and the cytoskeleton, culminating in cell injury.⁷ MSG causes increased formation of free acid radicals that results in oxidative stress.⁸

MSG has deleterious effects on many organs. It is destructive to the arcuate nucleus of the hypothalamus which is a vital component of the reproductive neuroendocrine axis, thus it adversely affects the reproductive capacity.⁹ MSG inflicted oxidative damage results in degenerative and atrophic changes in the ovary.¹¹

Plants and natural herbs have a widespread use in the field of alternative medicine. Aloe vera plant is being utilized in conventional medicine for healing various ailments.¹² The plant has been used in various civilizations and cultures. The Egyptians hailed it as, the eternal plant. It is also known as the first aid plant. The parenchyma of the leaves contains a transparent jelly-the aloe vera gel.¹³

The gel contains 99 to 99.5% water. The rest of 0.5-1% is a fantastic amalgam of polysaccharides, phenolic compounds, enzymes, hormones, vitamins and minerals. It contains vitamins including vitamin A, C and E.¹⁴ Minerals in the gel include manganese, zinc and selenium which are antioxidants.¹⁵

The antioxidant properties of aloe vera are due to the presence of free radical scavenging enzyme systems in the gel including glutathione peroxidase, reductase, catalase and superoxide dismutase.¹⁶

Because of its documented antioxidant property, the current research was formulated to assess the possible protective effects of aloe vera gel on the
damage inflicted by monosodium glutamate on the ovaries. In this regard, the gross morphological and histological features of the ovary were evaluated.

MATERIAL AND METHODS
It was an experimental conducted in the Anatomy department of SZPGMI, Lahore. 36 adult healthy female Wistar rats, 2-3 months old, having weight of about 200-250g were procured. Sample size of 12 animals in each group was calculated using 95% confidence level, 90% power of test through power of precision 3.0 software. The rats were housed in the cages of the animal house. They were provided free access to food and water. Monosodium glutamate (Ajinomoto), brand name Shangrila Chinese salt was used. Aloe vera plants were purchased from a local nursery in Lahore. By dissolving monosodium glutamate in distilled water, a stock solution was prepared. Leaves from aloe vera plant were rinsed with water, dissected longitudinally and mucilaginous jelly was scraped out. 96 % ethanol and distilled water (50/50, v/v) were added to homogenize the gel.18

EXPERIMENTAL PROCEDURE
After one week of acclimatization, animals were allocated into 3 groups: A, B and C. All the rats were weighed at the start of the experiment.

Group A (Control): The rats of this group were given 1.5 ml distilled water by gastric intubation.

Group B (Experimental): The rats were fed by gastric intubation with MSG 200 mg/kg b.w. mixed in 1.5 ml of distilled water, for 14 days.17

Group C (Experimental): The rats of this group were provided 400 mg/kg b.w. of aloe vera gel extract18 along with 200 mg /kg b.w. of MSG mixed in 1.5 ml of distilled water, by gastric intubation, for 14 days.

On the fifteenth day, 24 hours after the last dose, all the rats were weighed and then euthanized. They were dissected and their ovaries were removed. Later the ovaries were fixed in 10% buffered formaldehyde. Tissue processing was done. 5μm sections were made and stained using haematoxylin and eosin. Digital research photographic microscope was used to take the photomicrographs.

STATISTICAL ANALYSIS
Data was assessed using SPSS 20.0. Quantitative variables were presented using mean ± S.D and comparison among groups was done by one way ANOVA. Tukey’s test was run for posthoc assessment. Qualitative variables were presented as frequency and percentage. Chi-square test was run for comparison among groups. A p-value ≤ 0.05 was taken as significant.

RESULTS
1. Secondary Follicle: In group A, the secondary follicles were located in the ovarian cortical stroma. Spherical primary oocyte were surrounded by granulosa cells. The theca interna consisted of polyhedral cells having oval nuclei. In addition to the theca cells there were fibroblasts, collagen fibres and blood capillaries. The theca externa comprised of collagen and smooth muscle fibres. The theca externa merged with the stroma. Irregular fluid filled spaces called antrum folliculi were seen between the cells of the zona granulosa. The antrum of follicles were packed with a fluid known as the liquor folliculi (Figure 1).

Ovaries of 7 rats (58.3%) in group B revealed secondary follicles with vacuolization. The secondary follicles in group B had decreased diameters and the theca interna was displaced away from theca externa (Figure 2). No abnormal secondary follicle seen in the group A and C (Figure 3). A significant difference for the appearance of the secondary follicle among groups was observed (p- value<0.001).

![Figure 1: Photomicrograph belonging to group A, having normal Secondary follicle with Follicular Antrum (FA), Primary Oocyte (O), Zona Granulosa (ZG), Theca Interna (TI), Theca Externa (TE) (H&E, 200X).](image)

2. Diameter of Secondary Follicle:
The mean diameter of secondary follicles was recorded. One way ANOVA was applied for comparison of the diameters of secondary follicles among groups. The difference in the diameter of secondary follicles was significant (p-value = 0.038) (Table 1).

The diameters of secondary follicles belonging to group B were markedly decreased in comparison to group A (p-value= 0.018). However, there was a considerable increase in the diameters of secondary follicles seen in group C (p-value= 0.041) (Table 2).
3. Graafian Follicle:
The graafian follicles belonging to the control group were observed in the ovarian cortex. Eccentrically placed primary oocytes in these follicles were surrounded by granulosa cells present in 2-4 layers. The corona radiata was seen immediately surrounding the oocyte. The follicular antrum was markedly enlarged. Theca interna comprised of polyhedral cells with oval nuclei. The theca interna merged with the theca externa (Figure 4). In group B, 9 (75.5%) rats had abnormal graafian follicle. The graafian follicles of rats belonging to group B exhibited a decrease in their size and atrophy of the oocyte and vacuolization (Figure 5). Experimental group C had similar findings as in group A (Figure 6). There was no abnormal graafian follicle seen in group A and C. A significant difference was noted for the appearance of graafian follicle among groups (p-value<0.001).

4. Diameter of Graafian Follicle:
The mean diameter of graafian follicle in all groups were significant (p-value = 0.004) (Table 3). Post hoc analysis proved that diameters of graafian follicles in group C was significantly increased (p-value= 0.003) (Table 4).

Table 1: Diameter of secondary follicle among groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter of secondary follicle (µm)</td>
<td>346.2 ± 119.3</td>
<td>235.4 ± 84.9</td>
<td>330.2 ± 119.5</td>
<td>0.038*</td>
</tr>
</tbody>
</table>

One way ANOVA

Table 2: Pair wise comparison of diameter of secondary follicle among groups.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Groups</th>
<th>Groups</th>
<th>Mean Difference</th>
<th>Std. Error</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>B</td>
<td>110.792</td>
<td>44.553</td>
<td>0.018*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>16.000</td>
<td>44.553</td>
<td>0.722</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>C</td>
<td>-94.792</td>
<td>44.553</td>
<td>0.041*</td>
</tr>
</tbody>
</table>

Table 3: Diameter of Graafian follicle among groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter of Graafian follicle (µm)</td>
<td>485.4 ± 43.5</td>
<td>416.0 ± 76.7</td>
<td>538.1 ± 112.7</td>
<td>0.004*</td>
</tr>
</tbody>
</table>

One way ANOVA

Table 4: Pair wise comparison of diameter of Graafian follicle among groups.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Groups</th>
<th>Groups</th>
<th>Mean Difference</th>
<th>Std. Err or</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>B</td>
<td>69.375</td>
<td>33.737</td>
<td>0.115</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>-52.708</td>
<td>33.737</td>
<td>0.276</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>C</td>
<td>-122.083</td>
<td>33.737</td>
<td>0.003*</td>
</tr>
</tbody>
</table>
DISCUSSION

MSG resulted in secondary follicles with vacuolization and a decrease in their size. In group C, appearance and size of the secondary follicles were identical to the control group A. A marked reduction in the diameters of secondary follicles seen in group B in comparison those of the control group. Mustafa et al, in 2015 found that MSG results in marked reduction in the number of corpus lutei, the primary, secondary and graafian follicle count. They also documented vacuolization and an increase in number of atretic follicles. Vacuolization was triggered by oxidative stress and vacuoles form as a result of harmful substances.19

The diameters of secondary follicles in group C were protected from the effects of MSG as their diameters were greater than the diameters seen in group B. Barmak and Khaksar, in 2014 noticed a similar increase in the diameters of secondary follicles after aloe vera administration. They advocated the presence of antioxidants like saponins and phenols in the gel, protect the ovarian follicles.20

Afrough et al, in 2016 suggested that oxidative stress causes detrimental effects on the mature follicle transition from secondary to tertiary follicles. Reactive oxygen species accelerate the process of follicular atresia and granulosa cell apoptosis. Whereas aloe vera improves the follicular development and ovulation in diabetic mice by defending against the damages of oxidative stress.21

The graafian follicles of experimental group B showed decreased size, vacuolization and atrophy of the oocyte. The current research revealed that the mean diameter of graafian follicle in all groups was statistically significant (p-value = 0.004). Post hoc analysis proved that the diameters of graafian follicles in group C were significantly greater in comparison to the follicles belonging to group B.

Miskowiak et al, in 1999 suggested that perinatal injection of MSG leads to a decrease in the graafian follicle count.22 While in 2011 Shima and friends, proved that aloe vera improved and reinforced the ovarian structure. They found an increase in the count and diameters of both secondary and mature graafian follicles. Anti-oxidants in the gel protect against the ovarian atrophy.23

Oladipo et al, in 2015 indicated that the degeneration of ovarian follicles and their oocytes may result from the oxidative damage inflicted by MSG. Oxidative stress results whenever there is increased liberation of free radicals and decreased scavenging activity of antioxidant enzyme systems like glutathione.

**Figure 4:** Photomicrograph belonging to group A having normal Graafian follicle with normal Follicular Antrum (FA), Primary Oocyte (O), Corona Radiata (CR), Cumulus Oophorus (CO), Zona Granulosa (ZG), Theca Interna (TI), Theca Externa (TE) (H&E, 200X).

**Figure 5:** Photomicrograph belonging to group B showing Graafian follicle with Follicular Antrum (FA) showing vacuolization (Yellow arrows), Primary Oocyte (O) showing atrophy, Zona Granulosa (ZG), Theca Interna (TI), Theca Externa (TE) (H&E, 200X).

**Figure 6:** Photomicrograph belonging to group C showing normal Graafian follicle with normal Follicular Antrum (FA), Primary Oocyte (O), Corona Radiata (CR), Cumulus Oophorus (CO), Zona Granulosa (ZG), Theca Interna (TI), Theca Externa (TE) (H&E, 200X).
reductase, glutathione catalase, glutathione peroxidase and superoxide dismutase.\textsuperscript{17}

Ramachandraiahgari and colleagues, in 2012 suggested that aloe leaf extract greatly lowered lipid peroxidase levels, elevated the effectiveness of glutathione peroxidase and reductase. It also produced a marked increase in the levels of catalase. They concluded that free radical scavenging activity existent in aloe can be favorable against the pathological changes produced by ROS.\textsuperscript{24}

Abbasi, Rana and Anjum in 2016, found that vitamin C along with MSG protected the follicles as it supports the follicular membrane and is required for follicular growth.\textsuperscript{25} Vitamin C is present in aloe vera gel, it may be a factor ameliorating factor in our experimental group C.

The mechanism of damage created by MSG is through the production and liberation of reactive oxygen species that adversely affect the genetic information. Therefore, researchers have hypothesized that antioxidants can help revert MSG induced pathologies.\textsuperscript{26}

The present research proved that aloe vera gel when co-administered with MSG counteracted its hazard as it increases the levels of antioxidants and curtails the levels of free radicals in the body.

CONCLUSION

MSG produced a marked decrease in the diameters of ovarian follicles. Hence, this fact may be kept in minds by young ladies in their reproductive age, while consuming food containing MSG, especially Chinese food. Aloe vera gel on the other hand when co-administered with MSG minimizes the harmful effects of MSG and has a positive effect by increasing the diameters of secondary and mature graafian follicles.

REFERENCES


