

## AMELIORATIVE EFFECT OF DILTIAZEM ON THE MONOSODIUM GLUTAMATE TREATED STROMA OF FALLOPIAN TUBE AND SERUM ESTROGEN LEVELS

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### ABSTRACT

**Objective:** To study the effect of diltiazem on monosodium glutamate induced histomorphological changes in the fallopian tube.

**Study Design:** Randomized controlled trial.

**Material and Methods:** In this experimental study 30 adult female Sprague Dawley rats of average weight of 500g were randomly divided into three groups A (control group), B and C with 10 rats in each. The experimental group B was given 0.08 mg/kg of monosodium glutamate (MSG) orally and experimental group C was given 0.08 mg/kg of MSG and 10 mg/kg of diltiazem in distilled water for 14 days. The control group (A) received only laboratory diet prepared at National Institute of Health (NIH) Islamabad with liberal water intake. Blood was taken from each animal through intracardiac route. The rats were sacrificed on 15th day of the experiment. The fallopian tubes were carefully dissected out and fixed in 10% formalin for routine histological procedures.

**Results:** The fallopian tubes of the control group A showed normal histological features, a well-defined tubal wall consisted of three distinct layers. Oviducts of group B also showed three distinct layers. The cells forming the external serosal layer were flat with centrally located nuclei giving the appearance of simple. The cells forming the intermediate muscular layer were fusiform in appearance with centrally placed nuclei giving the appearance of smooth muscle cells. The cells in the inner mucosal layer were taller than broader with oval nuclei giving the appearance of simple columnar epithelium resting on the basement membrane. There were marked vacuolations appearing in the stroma cells. The blood vessels in stroma were dilated. The histological sections of oviducts of group C showed three layers: serosa, myosalpinx, endosalpinx the external serosal layer were flat with centrally located nuclei. At the inner side of this layer, there were lying irregularly scattered thin fibers, blood vessels and irregular shaped cells. The stromal cells were loosely arranged and in all animals of this group there was no vacuolations in stroma of this group. The mean serum estrogen level for group A was  $83.53 \pm 9.87$  pmol/l. The mean serum estrogen level in group B was  $136.97 \pm 12.51$  pmol/l. The mean serum estrogen level in group C was  $64.79 \pm 17.85$  pmol/l.

**Conclusion:** The findings obtained from our study after administration of MSG and diltiazem showed stromal vacuolations in the fallopian tube of rat leading to change in its functional aspect and impairing the female fertility while treatment with diltiazem prevents these effects.

**Keywords:** Diltiazem, Fallopian tube, Monosodium Glutamate, Stroma, Vacuolations.

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### INTRODUCTION

Monosodium glutamate is a white crystalline powder which looks similar to table salt or sugar. It is composed of glutamate and sodium<sup>1</sup>. Glutamate is a non-essential most abundant amino acid found in nature and is found in both free form and bound with other amino acids in

protein. Animal proteins contain 11 to 22% by weight of glutamate and the plant proteins have up to 40% glutamate. Glutamate is found in a wide variety of foods where it has a flavor enhancing effect. It is also present in relatively high concentration in foods such as tomatoes, mushrooms, peas and certain cheese. As a result of its flavor enhancing effects glutamate is often deliberately added to foods usually as the purified monosodium salt. MSG is available in open market in Pakistan manufactured by

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National Foods and Shangrilla Foods. MSG increases the appetite by stimulating appetite center in the hypothalamus and improves the taste of food<sup>2</sup>. There are certain reports indicating the toxicity of MSG to experimental animals and human beings<sup>3</sup>. In testicular tissue ascorbic acid content is reduced by MSG<sup>4</sup>. The degenerative and atrophic changes in the fallopian tubes are induced by MSG when administered in higher dosage and for prolonged period<sup>5</sup>. It induces fibroids in rats by increasing the levels of total protein, cholesterol and estradiol (estrogen)<sup>6</sup>. The fallopian tubes or oviducts are two muscular tubes which extend from uterus to the ovaries. The wall of the oviduct consists of folded mucosa, a thick muscularis with circular and longitudinal layers and a thin serosa. The mucosa is composed of simple columnar epithelium on a lamina propria of loose connective tissue. The epithelium contains two types of cells: ciliated cells and secretory cells or peg cells.

Diltiazem is a member of the class of drugs known as calcium channel blockers, used in the treatment of hypertension, angina pectoris, and arrhythmias. It is also an effective preventive medication for migraine. Diltiazem prevents the toxic effects of MSG by reducing the intracellular calcium overload. Diltiazem blocks the calcium channels in the cell membrane, so reducing permeability of cell membrane for calcium ions. Studies have shown that pretreatment with diltiazem prevents the effects of MSG on hypothalamus of rats<sup>7</sup>. The effects of MSG on the fallopian tubes have been studied however the role of diltiazem in prevention of these effects has not been investigated.

## **MATERIAL AND METHODS**

Thirty adult female Sprague Dawley rats weighing 490 to 510 grams were taken from the animal house of National Institute of Health (NIH), Islamabad. The rats were kept in cages at standard room temperature maintained on 12 hour light/dark cycle. The rats were randomly divided into three groups A (control group), B and C with 10 rats in each group. The Group A

was fed laboratory diet for 14 days. The group B was fed normal lab diet and was given 0.5 ml of distilled water by oral gavage tube containing Monosodium Glutamate 0.08 mg/kg body weight/day at 10 am daily for 14 days. The group C was fed on laboratory diet and was given 1 ml of distilled water containing diltiazem 10 mg/kg body weight/day and 0.5 ml distilled water by oral gavage tube containing Monosodium glutamate 0.08 mg/kg body weight/day at 10am daily for 14 days. Blood was drawn for hormonal analysis through cardiac puncture. The animals were sacrificed by an over dose of ether anesthesia on 15th day. The uterine horns, fallopian tubes and ovaries were identified and right sided uterine tube of each rat (group A, B, C,) was dissected out and put into 10% formalin for fixation. The tissue was further processed for paraffin embedding. The staining of sections was done with Haematoxylin and Eosin (H&E). Motic image plus microscope Model DMB3:223 was used for histological study.

For photomicrography the Motic image plus microscope Model: DMB3: 223 using the Motic Images plus 2.0 ML software on the computer was used.

The data was entered for analysis in the computer software SPSS version 20. The quantitative variables included height of epithelium of fallopian tubes and serum estrogen levels of control and experimental groups. The means were compared for significance using the ANOVA followed by post-hoc Tuckey test for inter-group comparison at a confidence limit of 95 percent. A *p*-value <0.05 was considered as significant.

## **RESULTS**

This study included thirty animals divided into 3 groups with 10 in each group. The fallopian tubes of the control group A showed normal histological features, a well defined tubal wall consisted of three layers: the internal mucous membrane, the middle muscular layer (myosalpinx) and the external serosa. The cells forming the external serosal layer were flat with

centrally located nuclei giving the appearance of simple squamous cells. At the inner side of this layer, there were lying irregularly scattered thin fibers, blood vessels and irregular shaped cells. The cells forming the intermediate muscular layer were fusiform in appearance with centrally placed nuclei giving the appearance of smooth muscle cells. The cells in the inner mucosal layer were taller than broader with oval nuclei giving the appearance of simple columnar epithelium. Epithelium was composed of ciliated and nonciliated cells. The nuclei were stained blue whereas the cytoplasm was stained pink with H&E stain. The basal surface of cells rested on basement membrane. Stroma was lying outside the basement membrane, consisted of stromal cells, connective tissue fibers and numerous blood vessels (fig-1).

The histological sections of oviducts of group B showed three layers: serosa (external layer), myosalpinx (intermediate layer) and endosalpinx (inner layer). The cells forming the external serosal layer were flat with centrally located nuclei giving the appearance of simple squamous cells. At the inner side of this layer, there were lying irregularly scattered thin fibers, blood vessels and irregular shaped cells. The cells forming the intermediate muscular layer were fusiform in appearance with centrally placed nuclei giving the appearance of smooth muscle cells. The cells in the inner mucosal layer were taller than broader with oval nuclei giving the appearance of simple columnar epithelium resting on the basement membrane. The nuclei were stained blue whereas the cytoplasm was stained pink with H&E stain. The basal surface of cells rested on basement membrane.

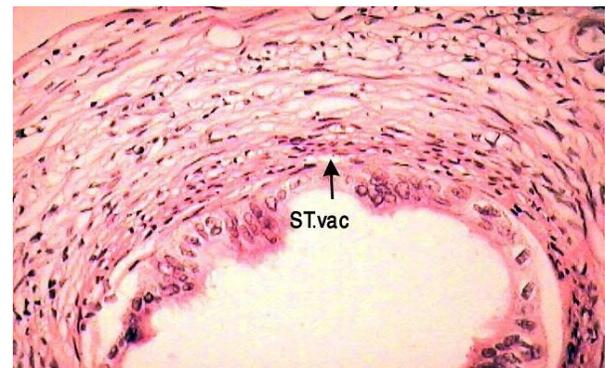
The layer external to the basement membrane consisted of stromal cells, connective tissue fibers and numerous blood vessels. The stromal cells were loosely arranged and in most of animals. There were marked vacuolations appearing in the stroma cells. In most of animals of this group, the blood vessels in stroma were dilated (fig-2).

The histological sections of oviducts of group C showed three layers: serosa (external layer), myosalpinx (intermediate layer) and endosalpinx (inner layer). The cells forming the external serosal layer were flat with centrally located nuclei giving the appearance of simple squamous cells. At the inner side of this layer,



**Figure-1: Photomicrograph showing cross section of uterine tube of rat from control group A showing ciliated cells (CC), non-ciliated cells (NCC), stroma (St.) and serosa (S) H&E stain 4X.**

there were lying irregularly scattered thin fibers, blood vessels and irregular shaped cells. The cells forming the intermediate muscular layer were fusiform in appearance with centrally placed



**Figure-2: Photomicrograph of a cross section from uterine tube (fallopian tubes) of rat from experimental group B, showing stromal vacuole (St. Vac.) and epithelium (Epi). H&E stain. X40.**

nuclei giving the appearance of smooth muscle cells. The cells in the inner mucosal layer were taller than broader with oval nuclei giving the appearance of simple columnar epithelium resting on the basement membrane. The nuclei were stained blue whereas the cytoplasm was

stained pink with H&E stain. The basal surface of cells rested on basement membrane. The stroma was lying external to the basement membrane which consisted of stromal cells, connective tissue fibers and numerous blood vessels. The stromal cells were loosely arranged and in all animals of this group there was no vacuolations in stroma of this group (fig-3).

### Hormone Analysis

The mean serum estrogen level for group A was  $83.53 \pm 9.87$  pmol/l.

The mean serum estrogen level in group B was  $136.97 \pm 12.51$  pmol/l. The difference in the mean of estrogen levels between the group A and group B was found highly significant ( $p < 0.05$ ).

The mean serum estrogen level in group C was  $64.79 \pm 17.85$  pmol/l. The difference was found highly significant statistically when compared with control group A ( $p < 0.05$ ). The difference between the mean serum estrogen levels of group B and group C was also found statistically significant ( $p < 0.05$ ). The serum estrogen level in group B was higher than group A and group C. The serum estrogen level in group C was closer, but significantly different

All the animals in control group A and experimental group C had normal histological features of oviduct stroma whereas most of rats in experimental group B treated with MSG alone had evident vacuolations in stroma of their oviducts. Our finding are similar to many



**Figure-3: Photomicrograph of a cross section of uterine tube of rat from experimental group C, showing the normal stroma. H&E stain.40X.**

earlier reports where the MSG treatment caused vacuolations in the stroma of many body organs including liver tissue<sup>8</sup>, lateral geniculate body<sup>9</sup>, medial geniculate body, testes<sup>10</sup>, superior colliculus<sup>11</sup>, oviduct<sup>12</sup>, seminiferous tubules<sup>13</sup> and interstitial connective tissue in testes<sup>14</sup>. The oviducts showed intense increase of follicular

**Table: Comparison of serum estrogen levels (pmol/l) between the control and experimental groups.**

| Control (A)<br>Mean $\pm$ SD | Experimental (B)<br>Mean $\pm$ SD | Experimental (C)<br>Mean $\pm$ SD | <i>p</i> -value |
|------------------------------|-----------------------------------|-----------------------------------|-----------------|
| $83.53 \pm 9.877$            | $136.97 \pm 12.509$               | $64.79 \pm 17.853$                | <0.001          |

*p*-value: A&B <0.001, A&C=0.014, B&C <0.001

from group A (table).

### DISCUSSION

MSG is the sodium salt of the non-essential amino acid glutamic acid and has long been used due to its flavor enhancing properties as it improves the taste of food by stimulating the appetite center as well as debated for its safety and harmful effects. Increased use of elements due to advanced proficiency can badly damage female fertility. A great hazard is unseen in the increased use of different food additives such as MSG. It is generally believed that more than 70 million couples suffer from infertility worldwide<sup>7</sup>.

atresia, vacuolated cells. Leukocyte infiltration and congestion of blood vessels were observed. These results were in accordance with those of who reported similar follicular damage in rat ovaries post-MSG-treatment. Al-Mosaibih<sup>3</sup> attributed the MSG-related degeneration of ovarian follicles to the increase of oxidative stress. Vacuolations of the granulosa cells post MSG-treatment may represent a type of cellular defense against its toxicities as well as a source of accumulating toxic agents interfering with its biological interactions in cell metabolism<sup>15</sup>.

These findings may result from enhancement of vascular inflammation and increase leakage

of leukocytes from the blood vessels into the tissues. Congestion of ovarian blood vessels may be resulted from the inhibition of MSG to prostaglandin synthesis maintaining blood flow. Also, the drastic effects of MSG on ovarian tissues were confirmed by a marked depletion of serum estrogen level which consequently induced severe histopathological lesions in endometrium<sup>16</sup>.

The vacuolations observed in the stroma of the fallopian tubes in this experiment may be due to MSG interference with higher of MSG. As a result of the alteration in the lumen of the fallopian tubes, the ciliary action and other functions can get affected as a result of probable toxic effect of MSG. It may be inferred from the present results that higher dose and prolonged administration of MSG resulted in changes observed in the fallopian tubes. MSG is a cytotoxic substance that may enhance autophagy of the cells resulting in apoptosis<sup>17</sup>.

MSG might have exerted these effects through increasing the permeability of cell membrane for calcium ions. Diltiazem reduces the permeability of calcium ions through the cell membrane and decreasing the intracellular load of calcium and thus preventing the cell changes induced by the MSG<sup>18</sup>.

Although preventive effects of diltiazem against MSG induced effects on various body parts have been demonstrated however this has not been tested on fallopian tubes. The diltiazem prevents the effects of MSG on the ovary of adult rat. During the neonatal period diltiazem prevented the toxic effects of MSG on the hypothalamus and on the ovary in rat when administered during the neonatal period<sup>19</sup>.

Our findings determined the significance of diltiazem prevention in the toxicity induced by MSG in Fallopian tubes.

The mean serum estrogen levels were significantly higher in MSG treated group B ( $136.97 \pm 12.5$  pmol/l) compared to control group A ( $83.53 \pm 9.8$  pmol/l) ( $p < 0.05$ ) and the group C ( $64.79 \pm 17.8$  pmol/l) ( $p < 0.05$ ). This implies that

MSG increases serum estrogen levels and Diltiazem protects the body from MSG induced increase in estrogen level.

The increase in the levels of estrogen found by 17 was 119.2% but in our study it was 63.9%. The MSG causes activation of enzyme aromatase which catalyzes the conversion of testosterone to estradiol, therefore resulting in increased estradiol synthesis<sup>18</sup>. This study was different in two aspects, dosage of MSG in this study was 100 mg per kg of bw and duration of this study was 60 days but in our study dosage was 0.08 mg MSG per kg of bw and duration was 14 days. This difference explains the differing results of the investigation. Disruption of estrogen level may contribute to the development of disorganized vaginal mucosa of MSG treated rats<sup>20</sup>. There was a comparative reduction and thinning of vaginal mucosa and decreased glandular function assessed by depleted its glycogen content. Several studies declared that declined blood estrogen level is accompanied by atrophic vaginitis with decreased glycogen secretion<sup>21</sup>.

## CONCLUSION

The findings obtained from our study after administration of MSG and diltiazem showed stromal vacuolations in the fallopian tube of rat leading to change in its functional aspect and impairing the female fertility while treatment with diltiazem prevents these effects.

## CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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