



Effect of Monosodium Glutamate on Mice ovaries and the possible protective role of Vitamin C

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Abstract

Background: Monosodium Glutamate, commonly used food additives that produce oxidative stress causing pathogenesis in several tissues and play a role in anovulatory infertility.

Methods: This study was carried out on 24 adult female Swiss albino mice, they were divided into three equal groups each contained 8 mice: Group I (control): they were fed on standard diet. Group II: adult mice treated with MSG 4 mg / g body weight. Group III: adult mice treated with MSG 4 mg/g body weight and vitamin C 100mg/kg B.WT. administered orally for 14 days.

Results: In group II, MSG reduced number of primary, secondary, and Graafian follicles but it increased atretic follicles also it caused vacuolation of follicular cells and congestion of medullary blood vessels. However Vitamin C in group III ameliorated the mentioned histological changes caused by MSG in group III..

Conclusion: MSG causes oxidative stress affecting ovarian tissue particularly the growing follicles, maturation of follicles, and decreased ovulation. Vitamin C is an effective antioxidant agent reducing the oxidative stress effect of MSG.

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Introduction

Female infertility is a very real medical problem and the female reproductive system is very sensitive to different harmful environmental factors [1] like, chemicals, industrial pollutants and food. Most food additives act either as preservatives or as enhancers of palatability.[2] One such food additive is monosodium glutamate (MSG).[3]

Monosodium glutamate (MSG) known as AJI-NOMOTO is the sodium salt of glutamic acid. [4] MSG contains 78% of glutamic acid, 22% of sodium and water. [5] Glutamate is one of the most common amino acids found in nature and is the main component of many proteins and peptides of most tissues. Glutamate is also produced in the body and plays an essential role in human metabolism. [6] The ability of monosodium glutamate to damage nerve cells of the hypothalamus is a pointer to the fact that it may alter the neural control of reproductive hormone secretion via the hypothalamic-pituitary-gonadal regulatory axis. Such alterations in reproductive hormone secretion may adversely affect the reproductive capacity of the affected animals.[7] .The ovaries produces various steroid and peptide hormones like estrogen and progesterone, which subserve many functions in the reproductive system[3].Abnormality in ovarian function usually leads to anovulatory infertility, which constitutes a major problem.[8] Several studies were done to see the effects of MSG on the tissues concerned with the reproduction e.g. testis, ovary, uterus etc. in the neonatal animals (rats, mice).[9] The researchers, though reported reduction in weight of both testes and ovaries, they did not comment on histological changes of these organs.[10] Some workers reported few histomorphological alterations, like, increased number of atretic follicles, reduced number of graafian follicles,[11] no corpora lutea etc.[12] Fertility rate has been reported to be reduced in both sexes.[13] However, only few literatures were available detailing the changes in histomorphology following MSG administration in mice.[9]

Ascorbic acid, vitamin C is a hydrophilic antioxidant that trap free radicals in the aqueous phase thus protecting bio-membrane from oxidative damage [14] and it is the first to become depleted in the exposure to oxidative stress. [15] The aim of this study was to detect the effects of MSG on the histology of ovaries in adult mice and the possible protective role of vitamin C.

Materials and Methods

This study was carried out on 24 adult female, Swiss Webster albino mice obtained from animal facilities of the department of biology, faculty of Sciences and Education at University of Sulaimani, their ages was between 6-10 weeks and with body weight (20-24 gram) they were housed in plastic cages at the same environ-

mental conditions of light (12hrs. Light ; 12 hrs Dark), temperature (20-23°C) and humidity. The animals had free access to standard laboratory diet, prepared in animal house and water adlibitum. The mice were divided into three equal groups each contained 8 mice as follows: Group I (control) they were fed on standard diet. Group II: treated with intrapretonial injection of MSG 4 mg / g body weight for 14 days while mice in Group III were treated as those in group III plus vitamin C 100mg/kg B.WT. administered orally for 14 days. Monosodium Glutamate powder of 99% purity was obtained from Sigma Aldrich/France Company, the stock solution was prepared by dissolving MSG 4mg/kg body weight in distilled water and each mouse has been injected with accurate calculated dose of MSG according to each mouse body weight. At the end of the determined period, the mice were exposed to high doses of ethyl ether (24 hrs. after the last injection) then they were dissected, both ovaries were cleaned from external tissues and fat fixed in 10% formalin and the ovarian tissues were dehydrated in ascending grade of ethanol, cleared in xylene then embedded in paraffin lastly they were cut in 5 μ thick sections and stained with H&E then studied under light microscope.

Statistical analysis: The results were expressed as mean \pm S.D. The significance of the difference between control and treated groups were determined by the Student t-test and the results were considered as significant at $p \leq 0.05$ and highly significant when ≤ 0.01 . The paper have been approved by scientific and ethical committee of school of medicine in University of Sulaimnai.

Result

In all 24 mice, the surface of ovaries was covered by single layer of cuboidal cells the germinal epithelium (Figure 1 and 2) the cortex is made of stroma in which a connective tissue is containing follicles of different sizes and stages while the center is occupied by the highly vascularized connective tissue, the medulla. The quantitative assessment of the ovaries in all groups was done by counting follicle of different stages (Figure 3). As far as the number of different types of follicles, table 1 shows the comparison between control with group II (MSG) and group III (MSG+ Vit. C). Regarding the primordial follicles there was no statistically significant difference between the control group and group II and III as P value was > 0.05 (Table 2). While for other follicles, primary, secondary, Graafian and atretic follicles showed a statistically significant decrease in group II same is regarding the corpus luteum number, P value was < 0.05 .

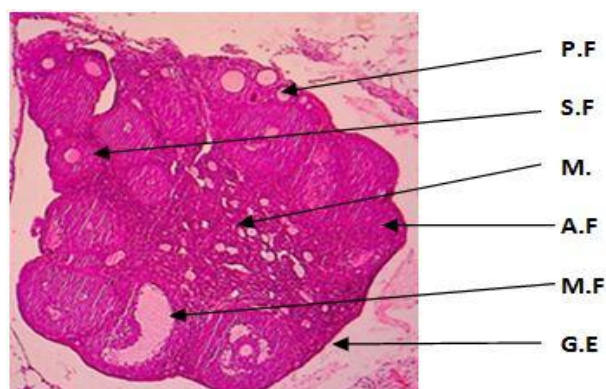


Fig.1: Ovary of control group shows. medulla (M), primary follicle (P.F), secondary follicle (S.F), antral follicle (A.F), mature follicle (M.F), germinal epithelium (G.E). 10X

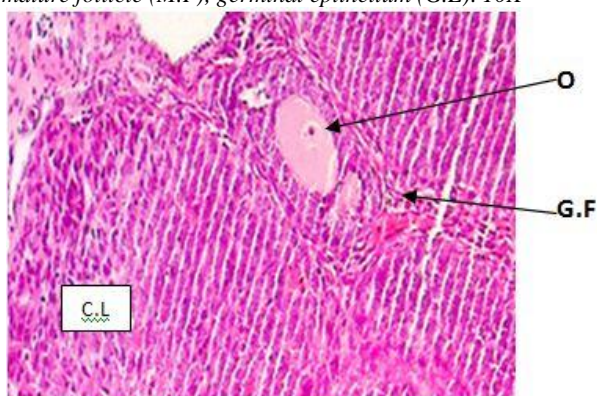
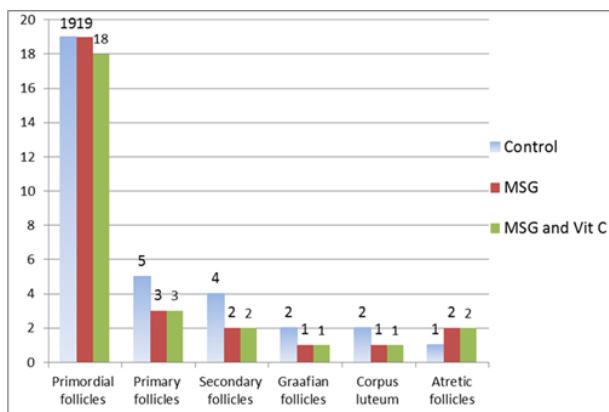


Fig. 2: Corpus luteum (C.L) of control group and a growing follicle (G.F), oocyte (O). 20X



Figures 3: Mean no. of follicles in different groups

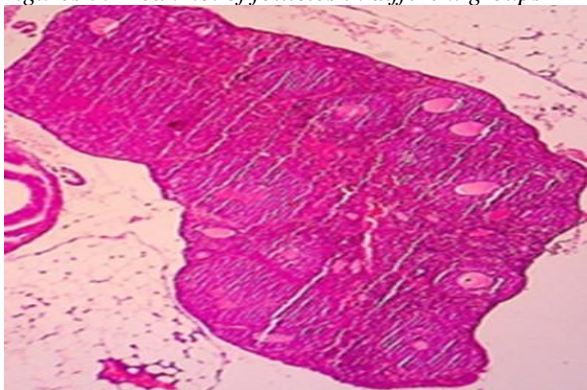


Fig. 4: Ovary of MSG treated group (II) shows generally decreased number of growing follicles & increased atretic follicles. 10X

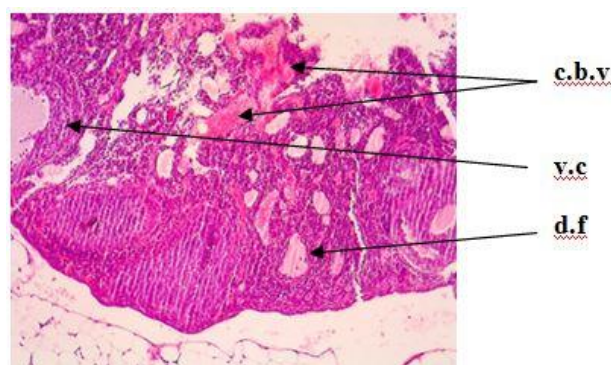


Fig 5: Ovary of Group II, shows congested blood vessels (c.b.v.) of medulla, distorted follicle (d.f), vacuolated cells (v.c). 20X

On the other hand comparing the control group with the groups II and III, the statistical evaluation for the effect of MSG in Group II and the protective role of Vitamin C in Group III has been done using t-test which was highly significant decrease in primary follicle and Graafian follicles in group II as the p-value was < 0.01 , while it was just significant for the effect of Vit .C in Group III. On the other hand the result was highly significant as p-value < 0.01 for secondary follicles and corpus luteum number in both groups II and III. The number of atretic follicles was higher in group II than group III and it was confirmed statistically as significant as p-value was < 0.05 and at the same time however the result of protective role of Vit. C in group III showed less atretic follicles but statistically it was not significant (Table 1). The result of comparison between Group II and III regarding the follicular counting has been evaluated statistically and it indicated no significant difference for all types as the p-value was > 0.05 (Table 2).

Presence of vacuolated follicular cells was also noted in all groups but it was more readily seen in group II and III than in control group, the follicular cells vacuolation, distorted follicles and congested blood vessels in the medulla all are illustrated in (Figure 5). (Table 3) shows the number of cells per selected 5 follicles per each ovary in all groups, there was a statistically significant vacuolation in group II compared to the control group, p-value was 0.012, while for group III was 0.06 which was statistically considered to be non-significant (Table 4).

Discussion

Monosodium glutamate is one of the most widely used food additives which enhance flavor of food, its toxic effect on different organs have been studied by many researchers.[16] Vitamins have noticeable role in almost all biochemical reactions and they are important antioxidants that protect tissues from oxidative stress due to their easy, rapid, and safe dietary administration in large concentration.[17] The effect of MSG on the female

Table 1: Comparison between Control Group and other Groups in regard to follicular count

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Refer- ence group	Other groups	Primordial follicles		Primary follicles		Secondary follicles		Graafian follicles		Corpus luteum		Atretic follicles	
		<i>t-test</i>	<i>P value</i>	<i>t-test</i>	<i>P value</i>	<i>t-test</i>	<i>P value</i>	<i>t-test</i>	<i>P value</i>	<i>t-test</i>	<i>P value</i>	<i>t-test</i>	<i>P value</i>
Control group	MSG	0.277	>0.05	-7.0	<0.001	-5.4	<0.01	-3.8	<0.01	-3.7	<0.01	2.7	<0.05
	MSG+vit C	-1.7	>0.05	-4.7	<0.005	-5.9	<0.01	-3.2	<0.05	-3.8	<0.01	2.2	>0.05

Table 2: Comparison between MSG group and MSG + Vit. C as regards to follicular count

Refer- ence group	Other group	Primordial follicles		Primary follicles		Secondary follicles		Graafian follicles		Corpus luteum		Atretic follicles	
		<i>t-test</i>	<i>P value</i>	<i>t-test</i>	<i>P value</i>	<i>t-test</i>	<i>P value</i>	<i>t-test</i>	<i>P value</i>	<i>t-test</i>	<i>P value</i>	<i>t-test</i>	<i>P value</i>
MSG	MSG+ vit C	-1.7	>0.05	-0.6	>0.05	0.5	>0.05	1.6	>0.05	0.0	>0.05	-2.2	>0.05

Table 3: Comparison between control group and other groups (II and III) as regards to No. of cells showed vacuolation in 5 follicles.

Groups	No. of cell	Control group	P value
MSG	11	5	0.012
MSG+vit C	7	5	0.066

Table 4: Comparison between control group and other Groups (II and III) as regards to No. of cells showed vacuolation in 5 follicles

Groups	MSG	MSG+vit C	P value
No. of cell with vacuolation in 5 follicle	11	7	0.045

reproductive has been studied by many researchers who found that it causes many pathologies in the ovaries that lead to anovulatory infertility. [1] [18] However the presence of glutamate receptors in different tissues: hypothalamus, liver, kidneys, endocrine system, ovaries, uterus, heart and lungs had been proved which explains the effect of MSG on these tissues. [19] In the current study MSG caused no change in number of primordial follicles but it reduced in primary, secondary, Graafian follicles and corpus lutei with increase in number of atretic follicles in both experimental groups but more happened with group II and these changes have been detected by others [9] they tested smaller dose on mice neonates for 75 days, those researchers blamed MSG interference with the phases of differentiation of primary follicles, but in addition to these we found an increase in number of atretic follicles in both experimental groups due to disruption of hypothalamus-pituitary-ovarian axis by toxic effect of MSG and this explanation agreed by.[10]

MSG caused histological changes in ovaries and fallopian tube, cellular hypertrophy of theca folliculi, destruction of basement membrane and stroma cells, vacuolation in ovaries.[8] [20] Vitamin C as an antioxidant in a dose of 100mg/kg B.Wt. given via orogastric tube simultaneously with MSG mixed with food for 14 days showed a protective role against nerve cells and astrocytes[17] their result confirms our result as Vit C in group III showed dramatic role in decreasing atretic follicle.

Vacuolation of varying numbers and sizes was found in all groups and this may be normal as it was seen in the control group too but it happened more with follicles in ovaries of other groups, however cytoplasmic vacuolation does occur in a wide range of cell lines either spontaneously or as induced by a variety of stimuli, the extent to which a cell becomes vacuolated depends on the cell type and it is considered as defense mechanism against harmful oxidative stress effect of these substances and vacuoles may collect these damaging substances preventing them from interfering with biological activi-

ties of these cells.[21][22] The process of vacuolation seems to follow a definite pattern with vacuolation number and size increasing gradually and cells can recover up to a certain threshold, beyond which they go to death,[23] in this study the vacuolation was triggered by MSG which is well known to cause oxidative stress effect and previously this effect has been detected by many researchers [3][24] and the appearance of vacuolation with in granulose cells has been confirmed by others.[8] MSG leads to the generation of oxygen derived free radicals and related reactive oxygen species (ROS), these substances are dangerous for biological systems as they react with DNA, proteins and lipids, leading to cellular damage as previously shown by. [25]

Furthermore in our study there was difference in the degree of vacuolation between group II and III in which both groups have been injected with the same dose of MSG 4mg/kg body weight but toxic effect of MSG was prevented to a noticeable extent by administering of Vitamin C, the antioxidant agent at dose 100mg/kg/day [17] [26] along with MSG for 14 days. Another findings detected was the congestion of blood vessels within the ovarian medulla may be due to inhibition of prostaglandin synthesis, as these compounds are known to be involved in the regulation of blood flow, this commensurate with the results reported by [27] who demonstrated congestion of the testicular blood vessels following MSG treatment of male rats. [1] [8] [20]

Conclusion

MSG causes oxidative stress affecting ovarian tissue particularly decrease number of growing follicles maturation of follicles decrease ovulation. Vitamin C is an effective antioxidant agent decreasing the oxidative stress effect of MSG.

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Competing Interests

None declared.

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