

AMELIORATIVE EFFECTS OF VITAMIN C ON MONOSODIUM GLUTAMATE INDUCED CHANGES IN THE OVARY OF RATS

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ABSTRACT

Objectives: To evaluate the effect of Vitamin C on Monosodium Glutamate induced changes in ovary of adult Sprague Dawley rats.

Study Design: It was an analytical experimental randomized control trial.

Materials and Methods: In the current study, 45 female adult rats (Sprague Dawley) were used and equally divided into 3 groups. The control group (C) was kept on standard laboratory diet and plain water, while the experimental group A, was given Monosodium Glutamate (0.08mg/kg body weight/day) and experimental group B, was fed on both MSG and Vitamin C(250mg/kg body weight/day) for 4 weeks. After 4 weeks all rats were sacrificed. For the histological study, tissue processing was done by using Haematoxylin and Eosin stain and 5micrometer thick sections were taken out from the ovaries.

Results: After administration of MSG experimental group A showed increase in the weight of ovary. Histological examination of ovary showed vacuolization, granulosa cell degeneration, inflammatory cell infiltrate and decrease in the number of primary follicles in group A. In experimental group B, vitamin C was also given in addition to MSG by mixing it in drinking water and the dissected ovary of group B showed histological changes which were less severe than experimental group A.

Conclusion: Protective effect of vitamin C is proved on Monosodium Glutamate induced histological changes in ovary of rats.

Key Words: Degenerated Granulosa Cells, Infertility, Inflammatory cell infiltrate, MSG, Number of Primary follicles, Vitamin C, Vacuolization.

INTRODUCTION

Fertility is defined as the ability of a couple to conceive off springs, while infertility is defined as inability of a couple to conceive even after a period of twelve months of unprotected coitus. According to World Health Organization, infertility is defined as inability of a couple to conceive after twenty four months of unprotected intercourse¹.

The American Society of Reproductive Medicine consider infertility as a disease in which there is a disturbance in the normal structure or function of any part of reproductive system². Rough calculation showed

that in 40% to 50% of the couples, infertility is due to female problems which include reduced or abnormal production of oocyte, fallopian tube dysfunction and atypical implantation of embryo³.

Global rate of infertility is about 10–15% and out of 7 billion world population, around 140 million of people have infertility^{2, 6}. Even though, the population growth rate of Pakistan is about 2% but it also has high infertility rate that is about 21.9% out of which 3.5% are due to primary reasons and 18.4% are due to secondary causes¹.

The female reproductive system is easily outraged by harmful environmental factors which include industrial toxins, environmental chemicals and food additives⁷⁻⁹. And out of these food additives, commonly used food additive is Monosodium glutamate generally known as Ajinomoto¹⁰⁻¹².

MATERIALS AND METHODS

Present study was an experimental randomized control trial. Rats were acquired from animal house of NIH, Islamabad where they were kept under standard laboratory conditions. 45 female Sprague Dawley rats with a weight of 250-350g and age between 10-12 weeks were included in this study. Male rats, pregnant female rats and rats with any obvious pathology were also excluded.

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Rats were haphazardly divided into 3 groups and each group is comprised of 15 rats. The control group C was kept on their standard pellet diet and water. The experimental group A was given MSG (0.08mg/kg body weight/day) for a period of 4 weeks. The experimental group B was not only fed on MSG but also given vitamin C (250mg/kg body weight/day) for 4 weeks.

After 4 weeks, ovaries were dissected out and examined for gross and histological parameters.

After tissue processing embedding was done in paraffin wax. For the preparation of slides Haematoxylin and Eosin stains were used. Microscopic study was done with the help of 10x and 40x objectives. SPSS version 20.0 was used for statistical analysis. Qualitative data was expressed in percentages and compared by implementing Pearson Chi Square test. Quantitative data was interpreted as Mean \pm S.D. Means of all three groups were correlated by using ANOVA. A p -value of <0.05 was acknowledged as statistically significant.

RESULTS

Gross Assessment

Weight of ovary: The weight of ovary in control group(C) was 0.15 ± 0.02 gm. But weight of ovary in experimental group A was 0.21 ± 0.03 gm and in group B was 0.18 ± 0.05 gm. And the mean weight of ovary was greater in group A and this difference was considered statistically significant ($p < 0.001$). (Table I,II).

Control group had significantly lower mean weight as compared to experimental group A ($p < 0.001$). Control group had lower mean weight as compared to experimental group B ($p = 0.03$) which is insignificant. Similarly experimental group A had higher weight as compared to experimental group B ($p = 0.06$) which is also insignificant.

Microscopic Assessment (Qualitative parameters)

Theca cell vacuolization

None of the rat in control group showed presence of vacuolization in theca cells. In group, 80% showed vacuole formation in theca cells and in group B, only 33.3% showed vacuolization of theca cells. The difference in all three groups regarding vacuolization was significant with a p -value <0.001 .

Granulosa cell degeneration

None of the rat in control group showed degeneration of granulosa cells. In group A, 73.3% showed granulosa cell degeneration and in group B, only 40% showed degeneration of granulosa cells. Existence of granulosa cell degeneration was statistically significant (p -value <0.001) between all groups.

(Quantitative parameters)

Number of primary follicles

By using 10X objective, number of primary follicles were counted per slide. In control group C, the average number of primary follicles were 5.9 ± 1.2 . The number of primary follicles in experimental group A, was 2.7 ± 1.8 and in group B, the number of primary follicles was 3.4 ± 1.7 and this difference of mean was statistically significant (p -value < 0.001).

Experimental group A had significantly lower mean of primary follicles when compared with group C ($p < 0.001$). Again experimental group B had lower mean of number of primary follicles when compared with Control group ($p = 0.001$) which is significant. Correspondingly, experimental group A had lower mean number of primary follicles as compared to experimental group B ($p = 0.28$) which is insignificant. (fig; 1)

Inflammatory Cell Infiltrate

By using 40X, average number of inflammatory cell group A was 1.1 ± 0.7 per unit area. Group B showed 0.8 ± 0.5 per unit area and this difference of mean was considered statistically significant (p -value < 0.001). (Table 5)

When group A was compared with group C, group A significantly showed increase number of inflammatory cells ($p < 0.001$). On comparison, group B had significantly increase number of inflammatory cell infiltrate as compared to group C ($p < 0.001$). Similarly group A showed higher mean of inflammatory cell infiltrate when compared group B ($p = 0.18$) and the p -value was not significant (Table 6) (fig: 2)

DISCUSSION

Infertility is not a life threatening disease but it has catastrophic effect on both male and female partner, especially females. It can affect large number of population at any time of their reproductive age. Beside of numerous factors, statistic ratio indicate that, 20% of the infertility are due to idiopathic reasons, 80% is due to both male and female problems and out of this 40% are due to only female problems²¹.

The worldwide rate of infertility is about 10–15% affecting almost 140 million people out of 7 billion world inhabitants^{2,6}. The rate of infertility in Pakistan is about 21.9% out of which 3.5% are due to primary and 18.4%

Table 1: Showing multiple comparison of weight (gm) of ovary in three groups by ANOVA

Groups	Mean difference (gm)	p-value
A vs. C	-0.06	$<0.001^*$
B vs. C	-0.03	0.03*
B vs. A	0.03	0.06

Table 2: Showing mean number of primary follicles in three groups

Groups	Mean ± S.D	SEM	p-value
Group C	5.9±1.2	0.30	< 0.001*
Group A	2.7±1.8	0.46	
Group B	3.4±1.7	0.44	

Table 3: Showing multiple comparison of primary follicles in three groups by One Way ANOVA

Groups	Mean difference	p-value
Group A vs. Group C	3.2	<0.001*
Group B vs. Group C	2.5	<0.001*
Group A vs. Group B	-0.7	0.28

Table 4: Showing mean number of inflammatory cells in ovary of three groups

Groups	Mean ± S.D	SEM	p-value
Group C	0.0 ± 0.0	0.0	< 0.001*s
Group A	1.1 ± 0.7	0.2	
Group B	0.8 ± 0.5	0.1	

Table 5: Showing multiple comparison of inflammatory cells in ovary by One Way ANOVA

Groups	Mean difference	p-value
A vs. C	1.1	< 0.001*
B vs. C	0.8	< 0.001*
A vs. B	0.3	0.18

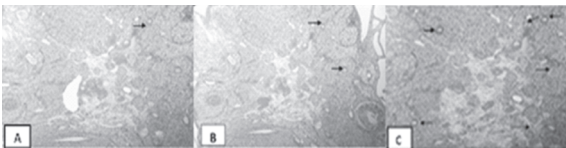


Figure 1: Showing number of primary follicles in group C, A and B

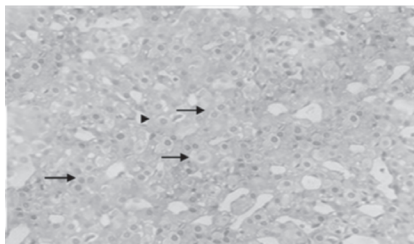


Figure 2: Showing number of inflammatory cells in group A.

are due to secondary reasons. Although, the population growth rate of Pakistan is very high that is about 2%¹¹.

In the current study, significant increase in the weight of ovaries in group A (0.21gm) was observed which is significantly different from the control group (0.15g). This result disagree with the previous report of Ilegbedion IG et al¹³ and Das¹⁶, which showed insignificant increase in ovary weight due to MSG intake for a period of 2 and 10 weeks respectively, although it was also dose dependant. In group B the average increase weight was 0.18gm which was lower than that of group A. Decrease in the weight of ovary in group B may be due to the potent antioxidating properties of vitamin C²².

In group A, 80% of the rats and in group B, 33.3% showed theca cell vacuolization which was highly significant from the control group in which none of the ovary showed vacuolization of theca cells. The results in the current study is in agreement with a study done in Egypt by Ahmed et al⁷ which showed vacuolization of granulosa cells. The present study is similar to the study carried out by Eweka et al on the effect of MSG induced histological changes in the oviducts of adult Wistar rats where vacuolization in the oviducts was attributed to the consumption of MSG¹¹.

According to Soujanya, vitamin C plays an important role in neutralizing free radicals. To maintain their stability, these free radicals will seek out for electron. Vitamin C suppress their reactivity by donating electron to free radicals²³.

In the present study, in group A 73.3 % of the rats and in group B 40% of the rats showed degeneration of granulosa cells which was highly significant from the control group in which none of the ovary showed such pathological change. Observation of this study is in harmony with the studies by Eweka A^{10,26}, Ahmed et al⁷, Veronika Husarova¹⁰, and Fayza Ahmed, in which MSG was given for a period of 2 weeks²⁰. Similar study was carried out by Saber A. Sakr, in which MSG was given for a period of 4 weeks, caused atrophic and degenerative changes in testis, Sertoli cells and Leydig cells¹⁴.

Decrease in the degeneration of granulosa cells in group B can be due to the wound repair capability of vitamin C by increasing the synthesis of collagen and extracellular matrix¹⁸.

Primary follicle is defined as, when a single or double layer of cuboidal cells surrounding an oocyte⁹. Number of primary follicles were counted in the current study, in group A average number of primary follicles was 2.7 and in group B was 3.4. However, in comparison with group C the number of primary follicle was 5.9. Similar results were reported by Naureen et al in the study of lead induced changes in ovary of mice⁸. The results of present study disagree the previous report of Das¹⁶ in which the number of primary follicles were increased in the experimental group. In group

B, folliculogenesis was improved, because vitamin C enhance the integrity of follicular membrane and it is also responsible for the synthesis of collagen which is necessary for the follicular growth¹⁹.

Ovarian tissue injury in the current study was also evident by the presence of inflammatory cells. The inflammatory cells can also cause damage to the capillaries by releasing certain chemical mediators²⁴. Number of inflammatory cells per unit area was counted in present study, in group A the average number of inflammatory cells is 1.1 and in group B is 0.8 per unit area. In comparison with the group C, the number of inflammatory cells was 0. The result of this study is an agreement with an experimental study done by Ilegbedion et al¹³ in which MSG was given for a period of 2 weeks which leads to infiltration of inflammatory cells in the oocyte and granulosa cells.

Inflammatory cells were decreased in group B, because vitamin C counteract against the process of oxidative damage and inflammation²⁵. According to Bendich et al, inflammatory cells activation can lead to rapid consumption of oxygen and generation of reactive oxygen species. These reactive oxygen species can cause damage to the inflammatory cells but high levels of vitamin C may protect these and other cells from free radical damage²⁶.

CONCLUSION

As vitamin C protects the cells against oxidative damage and inflammation. In addition it may also protect these cells from free radical damage. Hence, the results of this study have proved the protective effect of vitamin C on Monosodium Glutamate induced histological changes in ovary of rats.

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