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MONOSODIUM GLUTAMATE DEPRESSES THE FUNCTION OF FEMALE REPRODUCTIVE SYSTEM IN RAT BY PROMOTING OXIDATIVE STRESS INDUCED CHANGES IN THE STRUCTURE OF UTERUS

MUKTI MONDAL, KAUSHIK SARKAR, PARTHA PRATIM NATH AND GOUTAM PAUL*

Molecular Neurotoxicology Laboratory, Department of Physiology, University of Kalyani, Kalyani,West Bengal- 741235, India.

ABSTRACT

The aim of the present study was to examine the effects of MSG on the function of the uterus. We have observed significant degenerative changes in the endometrial and myometrial layers of the uterus in MSG exposed rats in a dose dependent manner compared to wall structure of the uterus of control rats. To elucidate the causes of MSG induced necrosis of the uterine wall structure, the activities of the cellular oxidative stress indices in MSG exposed uterus have been studied. We have found significant decrease in the activities of SOD, CAT, GR. and GPx, and significant increase in the activity of GST in MSG exposed uterine tissue homogenate. Besides, the level of MDA in MSG exposed uterine tissue homogenate has been increased significantly. The results suggest that the degeneration of MSG induced uterine tissue might be due to enhanced oxidative stress and increased lipid peroxidation in uterine tissue cells. In conclusion, MSG depresses the function of female reproductive system in rats probably by promoting oxidative stress induced changes in the wall structure of uterus.

KEY WORDS: **Monosodium glutamate, uterine tissue, necrosis, antioxidant enzymes, lipid peroxidation.**

GOUTAM PAUL
**Professor and Head, Department of Physiology, University of Kalyani, Kalyani,
West Bengal-741235, E-mail: goutampaul.ku@gmail.com**

INTRODUCTION

Monosodium glutamate, MSG (popularly known as Ajinomoto) is a controversial food additive and is used indiscriminately to prepare different food products in restaurants and food industries as a flavor enhancing chemical all over the World. Studies show that different food products like chips, jelly, pastry, candy, pizza, noodles and even protein-rich food products like meat, fish, milk and some vegetables contains enormous amount of MSG[1,2]. The toxicity of MSG due to eating of MSG added foods has not been identified until the expression of some clinical symptoms in Chinese people in 1968 those who have been exposed to MSG through foods for many years [3]. Subsequently, the toxic hazards of MSG on the functions of some organ systems have been established through toxicological experiments in animal models [4, 5, 6, 7, 8]. The effect of MSG on the function of male reproductive system in rat model has also been reported [9, 10]. Ignoring the probable toxic hazards on humans, MSG is used heedlessly by the industries and restaurants to expand the market of the products in India and abroad [11]. Till date, the effects of MSG on female reproductive system have not been reported. So, the present study was designed to examine the probable toxic effects on uterus, one of the primary female reproductive organs, and to elucidate the basis of toxicity at the cellular level in rat models. The results of our study can be extrapolated to bridge the gap between some clinical symptoms of women related to reproductive abnormalities and prolonged MSG exposure due to consumption of MSG added foods.

MATERIALS AND METHODS

Reagents and Chemicals

All the reagents used were of analytical grade. MSG (≤99%) was purchased from Sigma-Aldrich, USA and 5, 5'-dithiobis-2-nitrobenzene (DTNB), oxidized and reduced glutathione, NADPH.Na_4 were procured from SRL Pvt. Ltd., India. Eosin and Hematoxylin were procured from Merck, India.

Animals

Studies were performed on 3-4 months old female virgin albino rats of Charles Foster strain weighing about 110-120 gm. Animals were maintained in animal house as per recommendations of the Kalyani University Animal Ethics Committee. The animals were kept in equal light-dark cycle (12L: 12D) and fed standard laboratory chow and water *ad libitum.* The animals were sacrificed by cervical dislocation on the 24th hour after the completion of last dosage.

Animal exposure and grouping

After one week of acclimatization to the laboratory environment the animals were randomly distributed into following groups each containing ten animals for chronic MSG treatment. The different doses of MSG were selected in this study according to the graded percentage of LD_{50} value (15,000–18,000 mg/kg) of MSG in rat model mentioned by JECFA 1988 [12, 13]. The animal grouping for the chronic study is as follows.

Group I	Received no test elements for 30 days and 40 days (**Control**)
Group II	Received 0.8gm MSG/kgBW/Day (i.e., approximately 5% of LD_{50} of MSG), by oral gavage mode, for 30 days and 40 days (**Treated I**)
Group III	Received 1.6gm MSG/kgBW/Day (i.e., approximately 10% of LD_{50} of MSG), by oral gavage mode, for 30 days and 40 days(**Treated II**)
Group IV	Received 2.4gm MSG/kgBW/Day (i.e., approximately 15% of LD_{50} of MSG), by oral gavage mode, for 30 days and 40 days(**Treated III**)

Sample collection

The animals were sacrificed by cervical dislocation on the 24th hour after completion of the last dosage of both treatment durations (i.e., 30 and 40 days), the uterine tissue was removed and a segment of the uterine tissue was washed in ice cold phosphate buffer solution immediately, dried and stored in -20°C for further biochemical studies. The rest of the uterine tissue was washed in buffer and kept in Neutral buffer formalin for further histological study.

Homogenate preparation

Uterine tissue segments from all the experimental groups were excised separately and minced in ice-cold saline. A known weight of tissue was homogenized in 10ml buffer (0.1M phosphate buffer, pH 8.0) with 2 mM EDTA and 0.5% Triton-X-100 by a tissue homogenizer on ice. The tissue homogenate was then centrifuged at 8000 rpm for 10 minutes. The pellet was discarded and supernatant was re-centrifuged at 12000 rpm for 10 minutes then the supernatant was collected and stored at -20°C for further study.

Biochemical assay

Superoxide dismutase (SOD) activity was measured according to the method of Marklund and Marklund, 1974. One unit of SOD activity was defined as the enzyme activity that inhibits the auto-oxidation of pyrogallol by 50%[14]. The catalase (CAT) activity was measured followed by the method of Sinha et al, 1972[15]. Further, the glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione-s-transferase (GST) activities were measured by using the methods of Staal et al, 1969, Rotruck et al, 1973 and Habig et al, 1974 respectively with slight modifications [16, 17, 18]. Moreover, the amount of malondialdehyde (MDA) as marker of lipid peroxidation was estimated according to the protocol of Devasagayam and Tarachand, 1987[19]. The protein content of uterine tissue homogenate was measured by using the method of Lowry et al, 1951 [20].

Histological staining technique for morphological study of the uterine tissue

Neutral buffered formalin (NBF) fixed and paraffin impregnated uterine tissue sections were stained with normal hematoxylin-eosin stain according to the method of Bancroft et al, 2002 with slight modifications[21]. Briefly, 5μm paraffin section of uterine tissue was kept sequentially in xylene and graded ethanol and stained with hematoxylin for 2 minutes. After removing the excess color the slide was counterstained with eosin and then the stained slides were dehydrated with graded ethanol, cleared with xylene and mounted with DPX and were observed under the microscope (100X

magnification). Images were obtained by digital SLR Olympus Camera (E-620) fitted with Olympus light microscope (CH20i).

Statistical Analysis

All the data obtained from this study were expressed as mean ± SEM. Statistical comparisons between the values obtained in control and in treated rats were evaluated by paired Student's t test or analysis of variance (ANOVA) whichever is applicable. $p \leq 0.05$ was considered as significant.

RESULTS

Histological study in order to examine the effect of MSG on the structure of uterus

We observed a significant degeneration of the wall structure of uterus in a dose dependent manner in both 30 and 40 days treatment durations in MSG exposed female rats compared to control female rats (Figure 1). MSG produced distinguishable necrotic changes in the endometrial and myometrial layers of the uterus. Necrosis of the endometrial epithelium, including the epithelium of endometrial glands, was observed. Numerous lacunae were observed in the endometrial layer. Moreover, the diameter of the endometrium was also reduced in MSG treated uterus. Necrosis of the uterine smooth muscle cells was also revealed by the presence of spaces of lesions in the myometrial layer of the MSG treated uterus. We also observed a significant degenerative change in the perimetrium of the uterus (Figure 1).

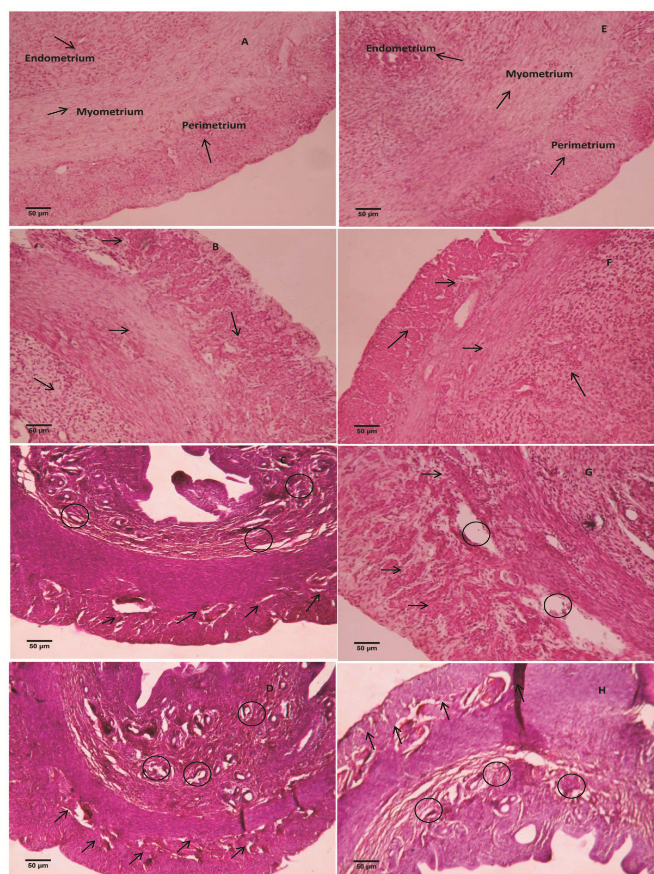

Figure 1

Microphotographs of fixed transverse sections of the wall of the uterus stained with hematoxylin and eosin showing the histological alterations in the wall structure of uterus in rats exposed to different dosages of MSG for two exposed durations (100X magnification). A and E- Control groups of rats for 30 and 40 days durations, B and F- Treated I groups of rats for 30 and 40 days durations, C and G- Treated II groups of rats for 30 and 40 days duration, D and H - Treated III groups of rats for 30 and 40 days durations. Arrows indicate the spaces of lesions in the wall structure of uterus. Circles indicate presence of lacunae in the uterine wall structure. Images were obtained by digital SLR Olympus Camera (E-620) fitted with Olympus light microscope (CH20i).

The oxidative stress variables of MSG exposed uterine tissue

We observed a significant decrease in the activities of SOD, CAT, GR, and GPx and increase in the activity of GST in a dose dependent manner in MSG exposed uterine tissue homogenate in comparison with the activity of SOD, CAT, GR, GPx, and GST in control groups of rats (Figure 2). Further, the quantity of MDA was increased in MSG exposed uterine tissue homogenate in dose dependent manner compared to control samples (Figure 2).

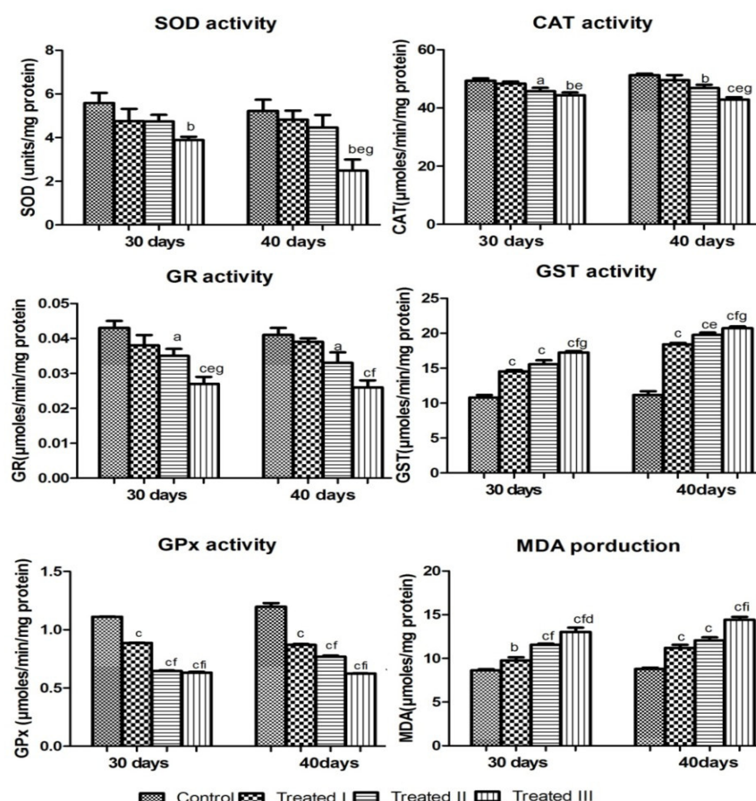

Figure 2

Graphical representations of the activities of antioxidant enzymes- SOD, CAT, GR, GST GPx, and MDA content in uterine tissue homogenate of MSG exposed animal groups for 30 days and 40 days treatment durations. Values are represented as mean±SEM (n=8), [a,b,c] p<0.05, 0.01, 0.001vs.Control, [d,e,f] p<0.05, 0.01, 0.001vs.Treated I, [g,h,i] p<0.05, 0.01, 0.001vs. Treated II.

DISCUSSION

Monosodium glutamate (MSG) is indiscriminately used in restaurants and food industries in India and abroad as flavor enhancer food additive to make the prepared foods more attractive. The probable toxic effects of MSG on some organ systems, including on male reproductive system, have been reported in animal models [22, 23, 24, 25, 26, 27, 10]. The effects of MSG on female reproductive system function have not been reported till date. The present study was designed to examine the probable toxic effects of MSG on the function of uterus, the organ responsible for implantation, housing and growth of embryo, and parturition of fetus, in rats treated with three dosages of MSG for two sub-chronic exposure durations. In order to examine the effect of MSG on uterine wall structure, we have studied the structural changes, if any, in paraffin impregnated transverse section of the uterus by using the standard protocol of eosin-hematoxylin staining method in MSG exposed and control rats. We found significant degenerative changes in the uterine wall structure in MSG exposed female rats compared to control rats. Significant architectural changes in the endometrium and myometrium were observed in MSG exposed uterine sections. Severe necrosis of the endometrial and glandular epithelium in endometrium was observed in MSG exposed rats. We have found the occurrence of numerous lacunae within the tissue matrix of endometrium (Figure 1). Besides, the endometrium was also shrinked in MSG treated rats. The myometrium showed significant degenerative features including the lesions spaces. Individual smooth muscle cells were segregated and numerous lacunae were developed within the muscle layers. Perimetrium of uterus also showed a bit necrotic changes. The results of the histopathological studies suggest that MSG might depress the function of the uterus by inducing the structural degenerations in the wall structure. Thus, it can be hypothesized that MSG induces the process of degeneration of the uterus probably by inducing the oxidative stress and lipid peroxidation in uterine tissue cells. We have found a significant decreased in the activities of SOD, CAT, GR, and GPx; and increase in the activity of GST in MSG exposed uterine tissue homogenate in comparison with the activity of control tests. Moreover, the level of MDA was increased in MSG exposed uterine tissue homogenate compared to control test (Figure 2). Superoxide dismutase (SOD) is an antioxidant enzyme which converts superoxide anions into hydrogen peroxide; and catalase (CAT) and glutathione peroxidase (GPx) convert hydrogen peroxide into water and oxygen in the cells. Glutathione reductase (GR) helps to maintain the cyclic activity of GPx by providing the level of reduced glutathione [28, 29, 30]. In our study the activities of SOD, CAT, GPx and GR have been decreased. So, the results suggest that MSG might induce the cellular oxidative stress in uterus by inhibiting the antioxidant defense mechanisms which

are responsible for the scavenging of the accumulated superoxide anions from the cells. These results support the hypothesis that MSG induces the oxidative stress induced degenerative processes probably by inhibiting the antioxidant defense mechanisms in uterine tissue cells. In conclusion, MSG depresses the function of uterus probably by promoting the oxidative stress induced structural alterations in the uterine wall architecture.

CONCLUSION

MSG depresses the function of female reproductive system probably by impairing the functions of uterus.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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