




Fenugreek Seed Extract Mitigates MSG-Induced Uterine Dysfunction in Rats through Enhanced Antioxidative Defense Mechanisms



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Article History:

Received: 09th Jun., 2023

Accepted: 15th Mar., 2024

Published: 30th Mar., 2024

Keywords:

Monosodium glutamate, Fenugreek seed extracts, histo-architectural changes, antioxidative defense

How to cite this Article:

Mukti Mondal, Kaushik Sarkar and Goutam Paul (2024). Fenugreek Seed Extract Mitigates MSG-Induced Uterine Dysfunction in Rats through Enhanced Antioxidative Defense Mechanisms. *International Journal of Experimental Research and Review*, 37(Spl.), 139-148.

DOI:

<https://doi.org/10.52756/ijerr.2024.v37spl.012>

Abstract: To assess the mitigating effectiveness of fenugreek seed extract in MSG-induced suppression of uterine function in rats, the effects of MSG in combination with fenugreek seed extract on the oxidative stress variables in the uterus and histo-architectural changes in the uterus have been studied and compared with the results obtained in the uterus in MSG-treated and control groups of rats. The study investigated the impact of MSG in conjunction with fenugreek seed extract on uterine function and oxidative stress. Female virgin albino rats were distributed into seven groups (Control, Treated I, Treated II, Treated III, Treated I+ Fenugreek seed extract, Treated II+ Fenugreek seed extract, Treated III+ Fenugreek seed extract) and subjected to thirty-day and forty-day of treatment via oral gavage. Despite no significant changes in mean body weight observed in MSG and fenugreek seed extract combination groups compared to the control, a notable counteraction was observed in the results obtained from rats exposed to MSG alone. This implies a possible protective function of fenugreek seed extract against oxidative stress in the uterus caused by MSG. Rats that received MSG in combination with fenugreek seed extract in their uterine tissue homogenate showed negligible changes in the activities of SOD, CAT, GR, GPx, GST, and MDA production when compared to the control group. Additionally, when comparing the MSG-exposed groups compare to control groups, a noteworthy decline in the activities of SOD, CAT, GR, and GPx and an increase in the activity of GST and MDA production were noted. When MSG was applied in conjunction with fenugreek seed extract for both treatment durations, no discernible histo-architectural alterations in the uterus were seen compared to the uterine tissues of the control groups of rats. Thus, it can be inferred from the findings that fenugreek seed extract significantly mitigated the oxidative stress and histo-architectural changes in rat uterine tissues caused by MSG.

Introduction

MSG (Monosodium glutamate) is a sodium glutamate salt stored naturally in our daily food and a tiny amount is added in food products. Glutamic acid is a non-essential amino acid as our body synthesizes glutamates (the brain chemical) from other sources naturally present in the body. It is responsible for the metabolism of crude nutrients and is very significant in the rebuilding section of body protein, energy metabolism and synaptic

neurotransmission (Lölinger, 2000). The MSG is characterized as the fifth taste, umami, which is just as unique as the other four (Kwok, 1968). MSG (C₃H₈NO₄Na) contains 78% of glutamic acid, 22% of sodium and water (Samuel, 1969; Yamaguchi and Ninomiya, 2000; Jinap and Hajeb, 2010; Erb, 2006; Neely, 2013; Moskin, 2008; Wijayasekara and Wansapala, 2017). It is a flavor enhancing food additive used in Asian cooking, fast foods and commercial

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packaged foods. It actually augments the appetite and produces a salient umami taste sensation in humans for a portion of food to which it is added (Zanfirescu et al., 2019; Fernstrom, 2018). The probable toxicity of MSG on human health has been reported discretely. MSG has been used heedlessly in food processing to attract consumers without considering its probable toxicity. It has been reported that several fast food products such as chips, jelly, pastry, candy, pizza and noodles; many protein-rich food products such as meat, fish, milk, and some vegetables contain MSG above the maximum permissible limit (Geha et al., 2000). MSG is an accumulation of sodium glutamate, which is a natural ingredient in our everyday food list. Also, a small amount of MSG is added to food products. Glutamic acid is referred to as a non-essential amino acid because our body can synthesize this amino acid from other readily available substances already present in the body in the form of a known chemical called glutamate (Pepino et al., 2010; Bayram et al., 2023; Evbuomwan et al., 2023). The glucose circulates in the blood and eventually diffuses through the cell membrane to initiate glycolysis. Based on numerous scientific research reviews and experiments, MSG has a variety of harmful effects on various organ systems in animal models. (Husarova and Ostatnikov, 2013; Das and Ghosh, 2010; Das and Ghosh, 2011; Okediran et al., 2014; Eweka et al., 2011; Miskowiak and Partyka, 2000; Collison et al., 2012; Alao et al., 2010; Kasozi et al., 2018; Kouzuki et al., 2019; Morita et al., 2021; Lima CB, 2013; Atef and El-moris, 2019; Mohamed et al., 2021).

Control	Only distilled water
Treated I (5% of LD ₅₀ of MSG)	: Received 0.8gm of MSG/kgBW/Day
Treated II (10% of LD ₅₀ of MSG)	: Received 1.6gm of MSG/kgBW/Day
Treated III (15% of LD ₅₀ of MSG)	: Received 2.4gm of MSG/kgBW/Day
Treated I + Fenugreek seed extract	: Received TI+ Fenugreek seed extract
Treated II + Fenugreek seed extract	: Received TII+ Fenugreek seed extract
Treated III + Fenugreek seed extract	: Received TIII+ Fenugreek seed extract

According to reports, in some animal models exposed to various toxic substances, fenugreek (*Trigonella foenumgraecum* Linn.) seed extract (Fenugreek, family Fabaceae) exhibits protective action against oxidative stress in the liver (Kaviarasan et al., 2006; Shah et al., 2019; Zia et al., 2001). In their report, Kumar and Bhandari (2013) also reported on the antioxidant defence activity of fenugreek in MSG-induced necrosis of hepatic cells (Kumar and Bhandari, 2013). The objective was to find out if the fenugreek's ability to decrease the uterus's oxidative stress and trophic function, which was recently interpreted as MSG, causes stress in the uterus. The rats in this study were given a combination of thirty-day and forty-day of exposure to MSG and fenugreek. The

outcomes were then compared to the consequences of the rats in the MSG-exposed groups, as well as the control groups.

Methodology

Reagents and Chemicals

Analytical-grade chemicals and reagents were utilised for this experiment. MSG was obtained from Sigma-Aldrich, USA. Pyrogallol, TCA, TBA, HCl, EDTA, BSA, Triton-X-100, H₂O₂, sodium azide, K₂Cr₂O₇ were procured from EMerck, India. Nicotinamide adenine dinucleotide phosphate hydrogen (reduced), reduced glutathione, Elman's reagent, CDNB, and oxidised glutathione, Tris, Folin Ciocalteu's phenol reagent, hematoxylin and eosin, and other materials were acquired from LOBA CHEME Pvt. Ltd., HiMEDIA laboratories Pvt. Ltd., and Sisco Research Laboratories (SRL) Pvt. Ltd., India respectively.

Animals

Rats, weighing between 110 and 120 gm and 90-120 days old (female virgin albino, Charles Foster strain), were used as an animal model for this study. Rats were kept in the Animal House in accordance with recommendations made by the national guidelines and by the Animal Ethics Committee of Kalyani University. As per standard protocol, rats were maintained in the departmental animal room. Test animals were grouped for chronic exposure to various dosages of MSG and fenugreek seed extract after acclimatization. The MSG doses were chosen based on the various percentages of the LD₅₀ value, as reported by Leung and Foster (2003);

Walker and Lupien (2000) and JECFA (1988). Additionally, the dosages of fenugreek seed extracts were selected based on an experiment done by Kumar et al., 2014. On the 24th hour following the last dose, the rats were sacrificed by cervical dislocation and used in further additional tests. The following are the animal groupings:

Received Fenugreek seed extract: 1gm/kgBW/Day. Test animals received the MSG and fenugreek seed extract dosages (oral gavage route) for thirty-day and forty-day treatment periods.

Preparation of crude extracts from fenugreek seeds

Fenugreek seeds powder (FSP) extract was prepared in our laboratory. Before extraction, all of the fenugreek seeds were carefully cleaned with distilled water to

remove any dirt or other foreign objects and they were then dried for twenty-four hours at 40°C in a hot air oven. FSP was then produced by grinding the dried seeds in a lab grinder. This FSP was then used as the raw material for preparing crude extracts, whereas distilled water was used as the extraction solvent (i.e., aqueous extract). Due to the high viscosity of FSP in water mixture, a higher solids: solvent ratio of 1:20 was used for aqueous extraction. At first, the aqueous mixture was continuously stirred with the help of a magnetic stirrer hot plate for 15 mins. Therefore, the mixture was then extracted by evaporating solvent using a Soxhlet extractor and supernatant was filtered. At last, a rough water extract was produced. Afterwards, using the following equation, the extract's final concentration was determined as yield (%): Yield (%) is equal to $[W_f - W_i] \times 100$. W_i is the raw material's beginning weight and W_f is the final weight of the crude extract powder (Kumar et al., 2014).

Measurement of Mean Body Weight

Mean body weight of the rats in the trial was measured on the first, second, and subsequent days of the same treatment and at every ten alternate days from the first to the last day of that treatment period or more accurately. The initial body weight of the rats was determined by measuring their weight on the day of the first dose application, and the final body weight was determined by measuring their weight on the day of sacrifice by our standard laboratory protocol (Mondal et al., 2014).

Biochemical Assay for Uterine Tissue Homogenate

Segments of uterine tissue from each experimental group were removed individually and minced in ice-cold saline for the biochemical study by using a tissue homogenizer (RQ-127A, REMi, India). The tissue homogenate was then centrifuged using a cooling centrifuge (C-24BL, REMI, India), and the supernatant was stored at -20°C (Mondal et al., 2016).

Biochemical Assay

Estimation of different antioxidant enzymatic activities

The following protocols have been used to measure the activities of various antioxidant enzymatic-

Estimation of total protein for the calculation of enzymatic activities

The protein content of uterine tissue homogenate was measured by using the Lowry method (Lowry et al., 1951).

Method of Histological Staining for Morphological Examination of the Uterine Tissue

Hematoxylin-eosin stain was applied to uterine tissue sections that had been neutral buffered formalin (NBF) fixed, paraffin impregnated by the methodology described by Bancroft et al., 2002. Image was captured by a digital SLR Olympus camera (E-620) equipped with an Olympus light microscope (CH20i).

Statistical Analysis

The data received from the study were all presented as mean \pm SEM. ANOVA or the Student's "t" test, whichever was more appropriate, was used to statistically compare the values obtained in the treated and control groups of rats. GraphPad Prism 5.03 (GraphPad Software, Inc.) was used to draw the graphs. The level of significance was set at $p \leq 0.05$.

Results

Effect of MSG; MSG and fenugreek seed extract in combination on mean body weight of female rats

The mean body weight exhibited a significant increase across all groups of rats exposed to varying doses of MSG, namely 5%, 10%, and 15% of the LD₅₀ of MSG. This increase occurred in a dose-dependent manner when compared to the control groups. Notably, rats exposed to a combination of fenugreek seed extract and MSG did not show significant changes in mean body weight compared to the control groups (refer to Table 1).

Effect of MSG on oxidative stress variables in uterine tissue homogenate of control; MSG exposed; and fenugreek in combination with MSG exposed groups of rats and the role of fenugreek in the mitigation of MSG induced effect

Enzymatic activity	Method
Superoxide dismutase (SOD)	Marklund and Marklund, 1974
Catalase (CAT)	Sinha, 1972
Glutathione reductase (GR)	Staal et al., 1969
Glutathione peroxidase (GPx)	Rotruck et al., 1973
Glutathione-s-transferase (GST)	Habig et al., 1974
Malondialdehyde (MDA)	Devasagayam and Tarachand, 1987

Rats exposed to MSG showed a significant decrease in the activities of SOD, CAT, GR, and GPx against the control group. Moreover, the activity of GST and MDA generation increased significantly in the MSG-exposed groups. However, the above enzymes, such as SOD, CAT, GR and GPx did not fall and therefore, the GST and MDA content was not increased significantly in rats injected with MSG and fenugreek seed extract in combination as compared to control (refer to Figure 1, 2 and 3).

Histo-architectural study of the uterus

Remarkable degenerative changes of the uterine wall structure in both MSG exposure durations of rats were observed compared to control. MSG produced marked necrotic changes in the endometrial and myometrial layers of the uterine wall structure. Necrosis of the endometrial epithelium, including the epithelium of endometrial glands, was observed.

Table 1. Represent the mean body weight of control; MSG exposed; and fenugreek seed extract in combination with MSG exposed groups of rats. Data are expressed as Mean±SEM, ten observations, ^ap<0.05 vs. control and ^bp<0.05 vs. MSG

Exposure Duration	30-day exposure duration		40-day exposure duration	
Exposure groups	Mean Body Weight (Gm.)			
	Initial	Final	Initial	Final
Control	110±2.58	119.2±2.29	100±2.58	118.6±1.93
Treated I (5% of LD ₅₀ of MSG)	110±3.65	122±2.44 ^a	110±3.33	119.4±2.68 ^a
Treated I + Fenugreek seed extract	110±2.58	119.8±1.69	110±2.98	118.6±1.71
Treated II (10% of LD ₅₀ of MSG)	110±3.33	123±3.13	110±3.4	120.4±3.3 ^a
Treated II + Fenugreek seed extract	110±3.33	120.1±2.04	110±2.58	119.2±2.49
Treated III (15% of LD ₅₀ of MSG)	110±4.3	125.8±3.91	110±2.58	125.8±1.5 ^a
Treated III + Fenugreek seed extract	109±2.33	120.8±1.75 ^b	109±2.33	121.1±1.06 ^b

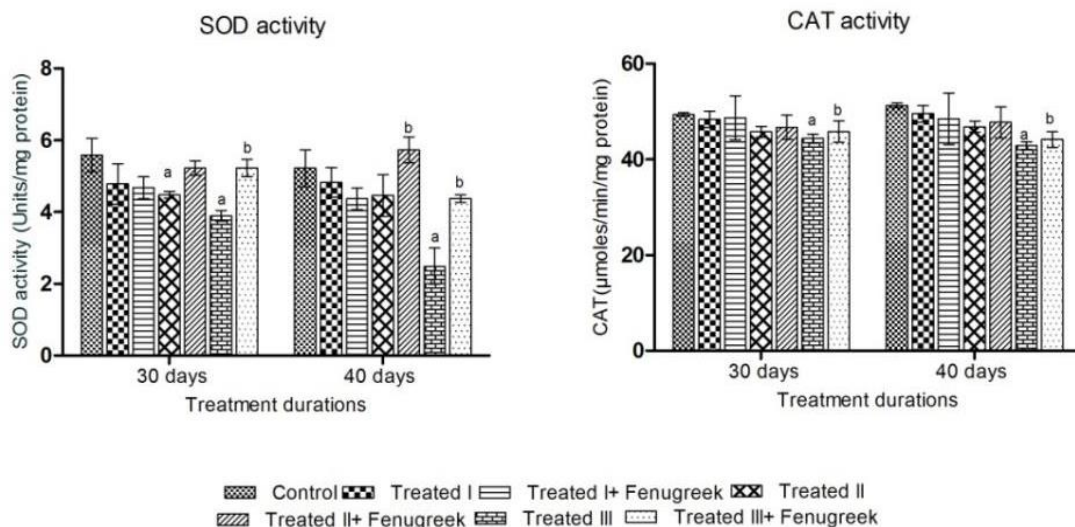


Figure 1. Bar diagram shows the activities of superoxide dismutase and catalase in uterine tissue homogenate of control; MSG exposed and MSG in combination with fenugreek seed extract groups of rats for thirty-day and forty-day treatment periods. Data expressed as Mean±SEM, seven observations, ^ap<0.05 vs. control and ^bp<0.05 vs. MSG.

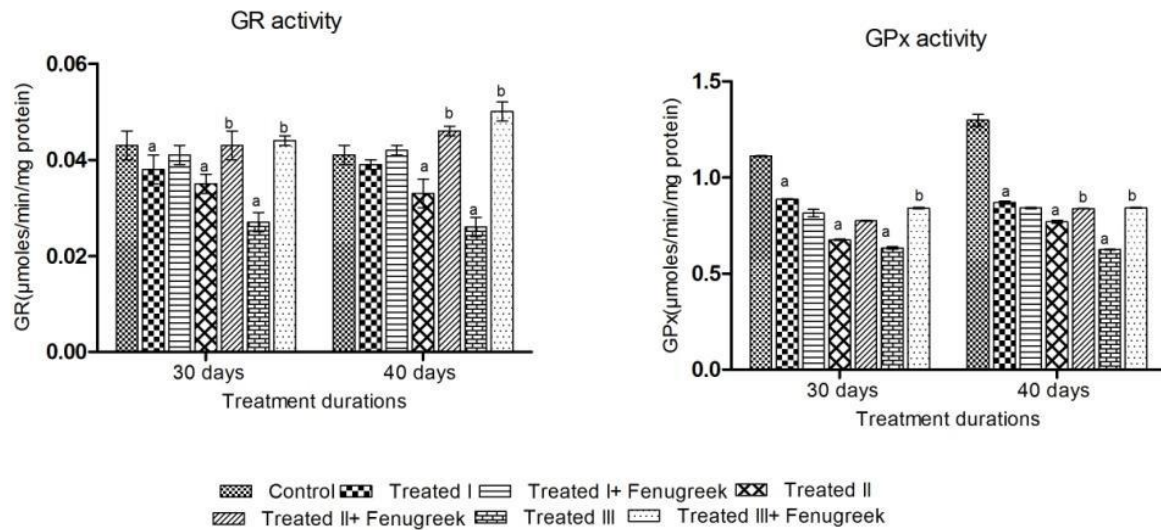


Figure 2. Bar diagram shows the activities of glutathione reductase and glutathione peroxidase in uterine tissue homogenate of control; MSG exposed and MSG in combination with fenugreek seed extract groups of rats for thirty-day and forty-day treatment periods. Data expressed as Mean \pm SEM, seven observations, ^a p<0.05 vs. control and ^b p<0.05 vs. MSG.

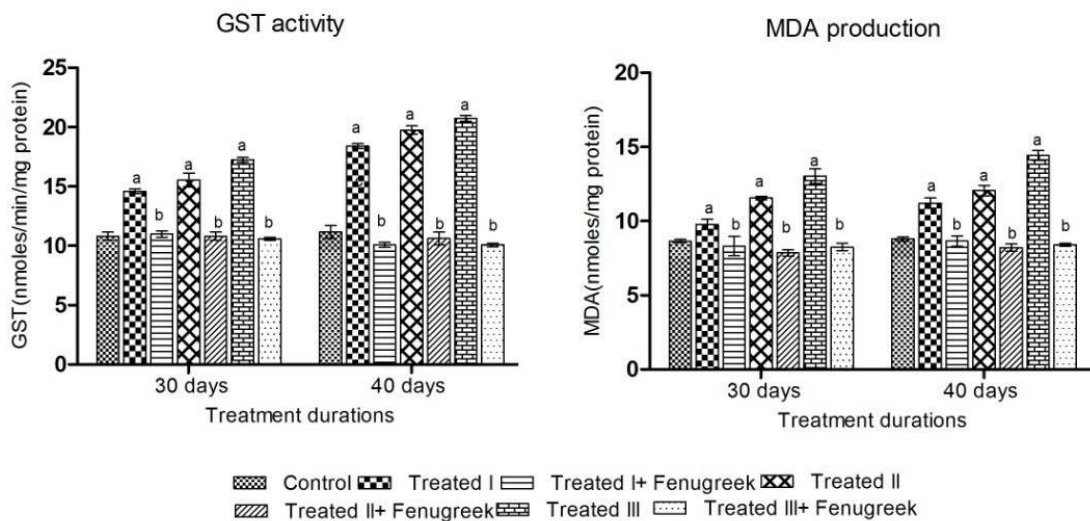


Figure 3. Bar diagram shows the activity of glutathione-s- transferaese and amount of malonaldehyde production in uterine tissue homogenate of control; MSG exposed and MSG in combination with fenugreek seed extract groups of rats for thirty-day and forty-day treatment periods. Data expressed as Mean \pm SEM, seven observations, ^a p<0.05 vs. control and ^b p<0.05 vs. MSG.

Moreover, the diameter of the endometrium was also reduced in the exposed uterus. Significant degenerative changes in the perimetrium of the uterus were also observed. Whereas, the application of MSG in combination with fenugreek seed extract, no remarkable morphological changes were observed in the uterine wall (Figure 4).

Discussion

The study aimed to evaluate the efficacy of fenugreek seed extract in mitigating the MSG-induced increase in mean body weight and oxidative stress-induced structural degenerations of uterine tissues. In this study, the mean body weight (Table 1) of MSG-exposed groups of rats was significantly increased compared to the control group of rats.

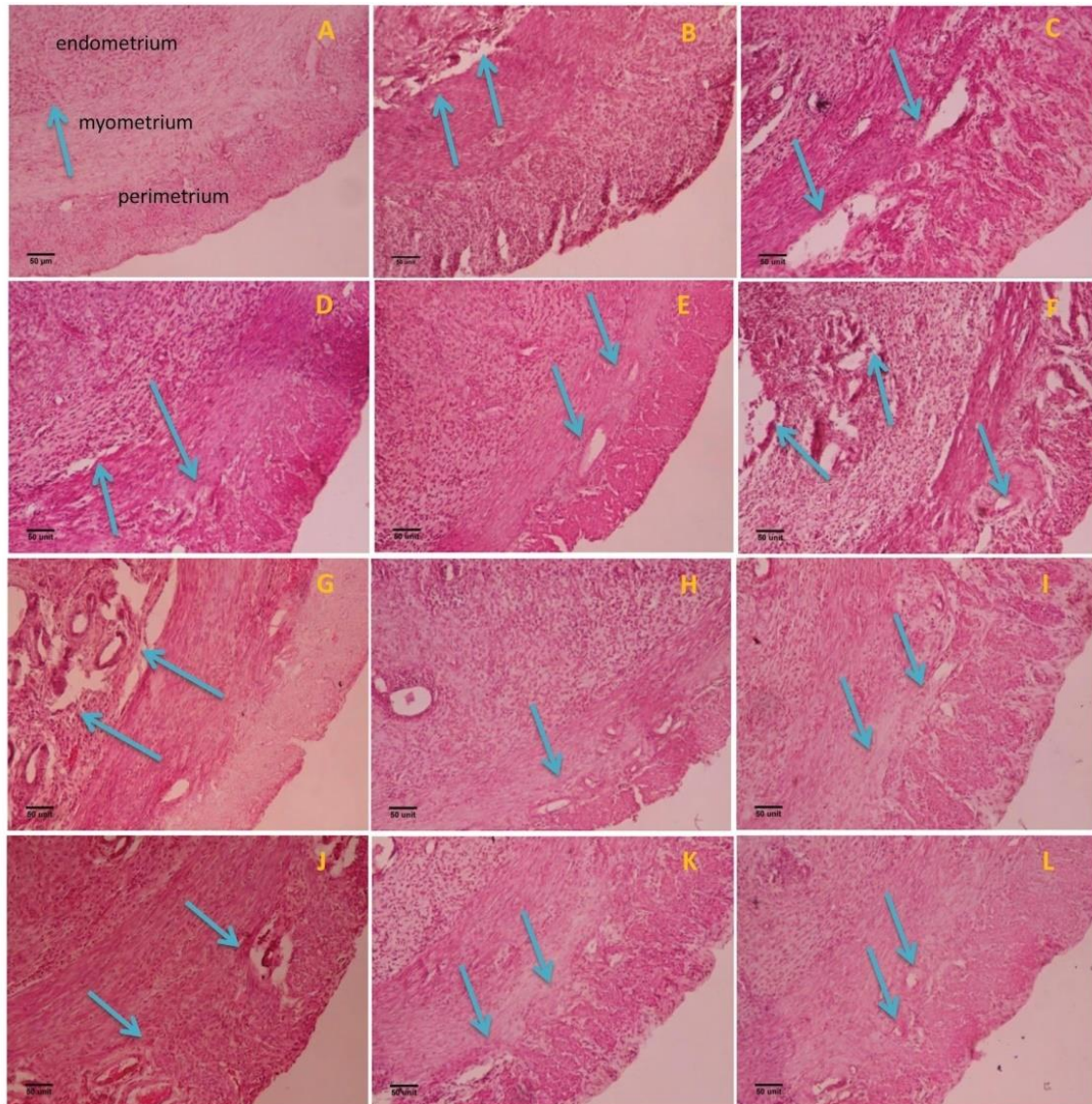


Figure 2. Transverse Section of wall structure of uterus (H and E staining) of control; MSG exposed and MSG in combination with fenugreek seed extract groups of rats for thirty-day and forty-day treatment periods (100X magnification). A: control, B:Treated II, C:Treated III (thirty-day), D:Treated I, E:Treated II, F:Treated III (forty-day), G:Treated I+ fenugreek seed extract, H:Treated II+ fenugreek seed extract, I:Treated III+ fenugreek seed extract (thirty-day), J:Treated I+ fenugreek seed extract, K:Treated II+ fenugreek seed extract, L:Treated III+ fenugreek seed extract (forty-day). (↑) indicate the site of morphological changes. Images were obtained by digital SLR Olympus Camera (E-620) fitted with an Olympus light microscope (CH20i).

Further, no significant changes in body weight of rats exposed to fenugreek seed extract in combination with MSG were observed compared to the control groups of rats (Table 1). The results suggest that MSG may stimulate the glutamate system situated in hunger-satiety centers of hypothalamus set-point to the hunger centres due to the overstimulation of the hunger centre and suppression of the satiety center. Thus, food intake was enhanced due to the increased of appetite for MSG contaminated foods that cause an increase in body weight due to increase adipose tissue in the body. So, it may be concluded that MSG probably increases the mean body weight by impairing the set-point homeostatic mechanism. The fenugreek seed extract is crucial in inhibiting the hunger center and stimulating the satiety center. From our experimental study, no significant degenerative changes were observed in uterine tissues, and there were changes in mean body weight in MSG and fenugreek seed extract (exposed combined) groups in comparison with control. In this study, MSG produced significant cytoarchitectural changes in the tissues of the uterus. From the results, it can be hypothesized that MSG might probably induce degenerative changes in the uterine tissues by inducing oxidative stress in uterus.

GPx have not decreased. The activity of GST and the production of MDA have not increased significantly in groups of rats exposed to MSG and fenugreek in combination compared to control (Figure 1, 2 and 3). The results suggest that fenugreek might counteract the MSG-induced decrease the antioxidant enzymes activities and increase MDA production. In order to ascertain the mitigative role of fenugreek seed extract in MSG-induced necrosis of uterine tissues, probably due to oxidative stress, the histopathology of uterine tissues has been studied in groups of rats exposed to MSG and fenugreek in combination for both the treatment periods. It has been observed that no significant histo-pathological alterations were observed in groups of rats exposed to MSG and fenugreek seed extract in combination compared to the histopathological changes in the uterus of rats exposed to MSG only (Figure 4). This result indicates that fenugreek might exert protective action by counteracting the oxidative stress-induced necrosis of uterine tissue. In conclusion, fenugreek seed extract might probably exert mitigative action in MSG-induced suppression of the uterus function by promoting the antioxidant defense in the uterine tissues (Figure 5).

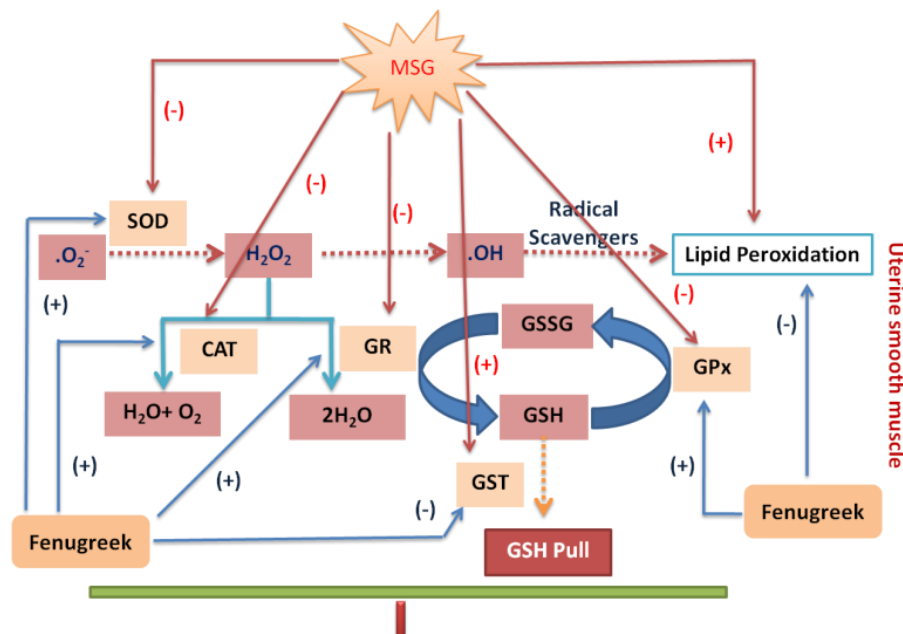


Figure 3. Diagram illustrating the possible mechanism by which fenugreek seed extract protects rat uterine tissues from MSG-induced free radical generations. (+) denotes increased or stimulating secretion, and (-) denotes decreased or altered secretion.

Significant decreases in the activities of SOD, CAT, GR, GPx and an increase in the activity of GST and MDA production in MSG exposed groups of rats have been observed when compared to control. Significantly, the activities of antioxidant enzymes like SOD, CAT, GR,

Conclusion

Based on the above research findings, it can be concluded that fenugreek seed extract mitigates MSG-induced uterine dysfunction in rats through an enhanced antioxidant defense mechanism in the uterine tissues.

Conflict of Interest

No conflicts of interest are present.

Acknowledgement

The author Dr. Mukti Mondal, expresses gratitude to the Department of Science and Technology, Ministry of Science and Technology of the Government of India, for providing financial support for full-time doctoral (Ph.D.) programme under INSPIRE Fellowship scheme and the Department of Physiology, Kalyani University for lending the research facilities to acquire the degree of doctor of philosophy.

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How to cite this Article:

Mukti Mondal, Kaushik Sarkar and Goutam Paul (2024). Fenugreek Seed Extract Mitigates MSG-Induced Uterine Dysfunction in Rats through Enhanced Antioxidative Defense Mechanisms. *International Journal of Experimental Research and Review*, *37*(Spl.), 139-148.

DOI : <https://doi.org/10.52756/ijerr.2024.v37spl.012>



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