



Research article

Effect of monosodium glutamate on serum sex hormones and uterine histology in female rats along with its molecular docking and *in-silico* toxicityMahfoudh A.M. Abdulghani^{a,b,**}, Salah Abdulrazak Alshehade^{c,d}, Sareh Kamran^d, Mohammed Abdullah Alshawsh^{d,*}^a Faculty of Medicine and Health Sciences, University of Science and Technology, Aden, Yemen^b Department of Pharmacology & Toxicology, Unaizah College of Pharmacy, Qassim University, Al Qassim 51911, Saudi Arabia^c Department of Pharmacology, School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 USM Penang, Malaysia^d Department of Pharmacology, Faculty of Medicine, Universiti Malaya, 50603 Kuala Lumpur, Malaysia

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ABSTRACT

Monosodium glutamate (MSG) is commonly used worldwide as a food flavour enhancer by the food industry. The current study investigated the *in vivo* toxic effects of MSG on the uterus in adult female Sprague Dawley rats and *in vitro* using MCF-7 and MDA-MB-231 cells, computational toxicity and molecular docking. The average levels of progesterone and oestrogen in the MSG-treated animals significantly altered. Besides, the average uterine lumen area (μm^2) was smaller than the control group. MSG showed high-affinity binding to acetylcholine receptors and disrupted the normal nerve signal with a predicted LD50 of 4500 mg/kg. MSG also demonstrated good binding affinity to human oestrogen receptors beta and some other proteins that have an oxidative stress role in the female reproductive organs. Therefore, a precaution should be taken when utilising this compound, especially for females under the risk factor of hormonal abnormality.

1. Introduction

Monosodium glutamate (MSG) is a controversial food additive that is widely used as a flavour enhancer in the food processing industry, restaurants, and institutional service. Chemically, MSG is a bright, and white powder similar to salt. It is manufactured by fermenting starch, sugar cane, or molasses followed by purifying and drying [1]. According to IHS Markit's Chemical Economics Handbook, the global consumption of MSG in 2019 was nearly 3.9 million metric tons (MMT) and its market size was USD 5143.6 million in 2021. Furthermore, by 2026, the MSG market is expected to register a 6.2% compound annual growth rate (CAGR) in terms of revenue, reaching a global market size of USD 6177.7 million [2].

Several studies provided evidence of the toxic effects of MSG in humans [3, 4] and experimental animals [3, 5], which raised the increasing interest in MSG consumption [3]. The reported side effects of MSG included but were not limited to dizziness, headaches, numbness, weakness, flushing, and sweating [4]. Moreover, more serious side

effects were reported, such as atopic dermatitis, asthma, urticaria, ventricular arrhythmia, and neuropathy [6]. In addition, there has been increasing interest in exploring the effect of MSG on the reproductive organs. Several studies have reported the toxic effects of MSG on male reproductive organs in animals by causing oligozoospermia, abnormal sperm morphology, and testicular haemorrhage [7, 8, 9, 10, 11].

Endocrine disruptors such as organic compounds, pesticides, phthalates, bisphenol A, and heavy metals may cause impaired reproductive systems, such as poor semen quality and quantity in males, and some gynaecological medical conditions in females, such as endometriosis, benign tumours and infections of reproductive organs [12, 13, 14, 15]. Uterine tissues of humans and rodents are similar in essential characteristics. Measurement of uterine toxicity could be done by assessing many parameters, including an alteration in uterine weight, hormonal levels and histological examination of lumen area, length of stroma and myometrium [16, 17, 18].

In the past decade, fear had increased due to the adverse effects and toxicity of MSG. In addition, there is limited literature about the

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hormonal and histological effects of MSG on the uterus. Therefore, this study was conducted to assess the *in vivo* toxic effects of MSG on the uterus in adult female Sprague Dawley rats and on oestrogen receptor-positive (MCF-7) and oestrogen receptor-negative (MDA-MB-231) breast cancer cells as well as to elucidate the possible mechanism of toxicity using computational toxicity and molecular docking.

2. Materials and methods

2.1. *In vivo* animal experiment

Twelve virgins sexually matured Sprague Dawley female rats (6 animals per group), 10–14 weeks old and weighing 237 ± 34 g were obtained from the Animal Research and Service Centre of Qassim University (QU), Qassim, Saudi Arabia. The animal experiment was done according to the National Research Council's Guide for the Care and Use of Laboratory Animals and the protocol of the animal experiment was approved by the committee of research ethics at Qassim University with ethics reference number "20-02-01". The animals were housed in a temperature-controlled room (25 ± 2 °C) with a 12 h light/dark cycle. Standard chow diet (1st Milling Company, KSA) and reverse osmosis (RO) water were provided *ad libitum*.

Only animals with a regular oestrous cycle (EC) were included in this study. Before the experiment started, two normal ECs were recorded according to the non-staining method described by the previous study [48]. Briefly, daily vaginal fluid obtained using a micropipette filled with 10 μ l normal saline and viewed under a light microscope at $\times 10$ magnification was performed to determine the phase of the oestrous cycle. The EC of rodents usually has four phases, namely proestrus, oestrus, metestrus and diestrus and lasts for 4–5 days [49]. Proestrus phase can be recognized by the predominance of epithelial cells, oestrus characterized by cornified cells, diestrus indicated by the predominance of leukocytes, and metestrus characterized by the presence of an equal number of epithelial, leukocytes, and cornified cells [50]. A total of 12 female SD rats were randomly and equally divided into two groups. The first group was assigned as a control group and administered 10 ml/kg of distilled water (DW), and the second group was administered daily with MSG (2 g/kg) via oral gavage for 14 days [42]. The selected dose of MSG (2 g/kg) was chosen based on the LD₅₀ of MSG in the rat model as reported previously [44] and a pilot study of MSG effects on ECs regularity which caused a marked increase in the diestrus phase. Vaginal lavage was performed on the control and treated animals. At the end of the experiment, each animal was anaesthetised with 75 mg/kg ketamine and 10 mg/kg xylazine. The blood samples were collected via cardiac puncture at the oestrus phase for serum progesterone and oestrogen analysis. The animals were euthanized by cervical dislocation, and the uterine tissues were isolated in the oestrus phase [29]. After recording the relative weight of each wet uterine, it was fixed in 10% buffered formalin for histological evaluation.

2.2. Histological examination of the uterus

Each Fixed uterine tissue was subjected to dehydration processing and embedded in paraffin wax. The prepared paraffin blocks were sent to a Specialized Medical lab for sectioning and stained with hematoxylin and eosin. The images of uterine sections were taken using a light microscope attached to a digital camera (Leica DM750). Microscopically, three major features were assessed using Image J software, namely, the lumen area, which is the inside space of the tubular structure of the uterus, the stromal uterus, which is a deep layer of the endometrium, and the myometrium layer, which is the structural wall of the uterus and composed primarily of smooth muscle. Six uterine sections from each animal were evaluated, and the mean relative value to the respective control was calculated.

2.3. Female sex hormone levels measurement

At the end of the experiment, each animal with the oestrus phase was anaesthetised. The blood sample was collected in a non-heparinised test tube. The serum was separated by centrifuging (5000 rpm for 15 min) and kept at -80 °C until analysis. The serum oestrogen and progesterone levels were measured using the chemiluminescence immunoassay (Beckman coulter Access II, USA). The relative value of hormone was calculated according to the following formula:

The relative value of hormone = measured value for treated animal/ average of control *100.

2.4. MTT cytotoxicity assay

To grow cells, media supplemented with 10 % fetal bovine serum (FBS) was used. MTT assay was conducted by seeding the oestrogen-positive MCF-7 and the oestrogen-negative MDA-MB-231 cells (5×10^3 cells/well) in 96 well plates. After overnight incubation in a CO₂ incubator at 37 °C, cells were treated with a 2-fold serial range of concentrations of MSG (100–0.78 μ g/ml) followed by incubation for 24, 48, and 72 h. After that, MTT (5 mg/ml) was added to each well and re-incubated for 4 h. Formazan crystals were dissolved in DMSO, and optical density was recorded using a microplate reader at 570 nm. The cell viability percentage was estimated in comparison to the control [51]. The assay was carried out in triplicates measurement and at least three independent experiments.

2.5. Exploring the computational toxicity of MSG

Computational chemistry was used to explore the pharmacokinetic properties of MSG. Then, to screen the toxic effects of the MSG computationally, acute toxicity was predicted using the ProTox-II tool based on many computational approaches, including molecular similarity, fragment propensities, and machine-learning [52]. Furthermore, the iSafeRat[®] tool was used to provide a reliable and accurate computational analysis to replace experimental OECD guideline studies [53]. Electrophilicity index (ω) was calculated (equation No. 1) using the molecular and quantum descriptors, which were calculated using PaDEL-Descriptor and Spartan (v.8), respectively [54, 55].

$$\omega = \frac{\mu^2}{2\eta} \approx \frac{-(I+A)/2)^2}{2(I-A)} \approx \frac{-(HOMO+LUMO)/2)^2}{2(-HOMO+LUMO)} \quad (1)$$

where (μ) is the chemical potential, (η) hardness, (I) ion energy, and (A) represents the electron affinity energies of the highest occupied orbital. HOMO and LUMO were calculated using the ω B97X-D model with a Basis set of 6–31G*. The ADF package (SCM, V. 2021.102) was used to calculate the local philicity (equation No. 2) at any atom (k) based on the Hirshfeld model of the Fukui function (f_k^α) [56].

$$\omega_k^\alpha = \omega \cdot f_k^\alpha \quad (2)$$

where α represents local philic quantities describing nucleophilic (ω_k^-), and electrophilic (ω_k^+) radical attacks. The ω_{max}^+ value was calculated on the carbon atom with maximum f_k^+ and the ω_{max}^- were calculated on nitrogen atom with maximum f_k^- .

Finally, the quantum dissimilarity (Eq. (3)) was calculated to check the effects of both the electron acceptor and donor on cellular toxicity [57].

$$\Delta\omega = (\omega_{max}^+ - \omega_{max}^-)^2 \quad (3)$$

2.6. Molecular docking

AutoDock Vina (v. 1.1.2) was used to blindly dock MSG with several selected targets [58]. Besides validating the findings was carried out

using commercial inhibitors. The 3D structure of the ligands was downloaded from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) and the targeted proteins were downloaded from the Protein data bank (<https://www.rcsb.org/>). The proteins were cleaned and then optimized by removing the solvent, besides adding hydrogen atoms and charges. Since the ligand is flexible and proteins are rigid, then semi-flexible docking analysis was used (3000 steps).

2.7. Statistical analysis

The *in vivo* data was represented as the mean relative value to the respective control \pm SEM ($n = 6$) and analysed using the Student's *t*-test. The *in vitro* data were analysed using two-way ANOVA and the results were expressed as mean \pm SD. GraphPad Prism 8.4 software was used to perform the analysis. *P*-values ≤ 0.05 were considered statistically significant.

3. Results

3.1. Effect of MSG on uterine tissue

The effect of oral administration of MSG (2 g/kg) on the uterus was evaluated in rats. In the MSG-treated group, the means of the relative lumen area ($77.2 \pm 12.48 \mu\text{m}^2$) and length of stroma ($85.5 \pm 14.23 \mu\text{m}$) were smaller than the control group ($100.3 \pm 16.01 \mu\text{m}^2$ and $100.2 \pm 10.63 \mu\text{m}$, respectively), while the mean of the relative length of the myometrium ($106.3 \pm 9.19 \mu\text{m}$) was slightly higher than control ($100.1 \pm 13.32 \mu\text{m}$) (Figure 1). The mean relative weight of the uterine weight in the MSG-treated group was less than the control group but the difference was not statistically significant (Figure 2). Histological findings of the uterus showed that The Lumen area and length of stroma were higher in the control group than in the treated group, while the length of myometrium was higher in the treated group. Figure 3 shows

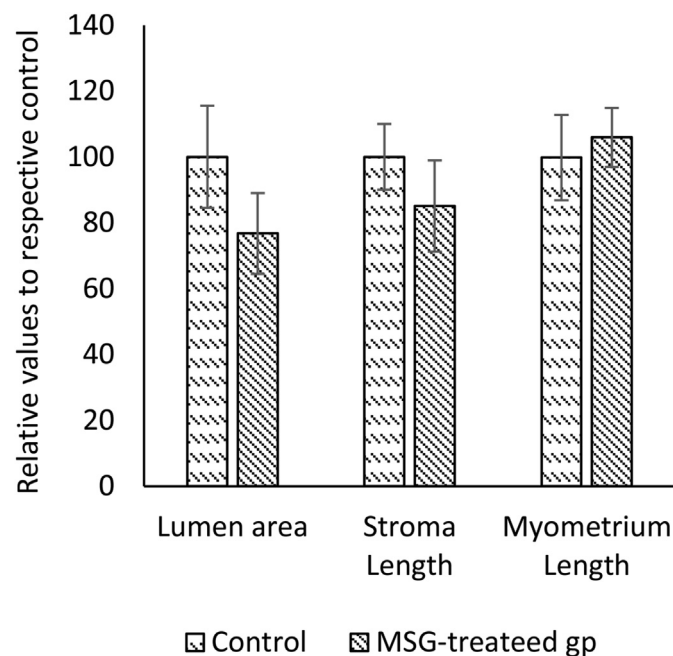


Figure 1. Effect of monosodium glutamate (MSG) oral administration on the morphological parameters of uterus during oestrus phase in female rats. Comparing the mean of lumen area (μm^2), stroma length (μm) and myometrium length (μm) in uterine tissues between control and treated groups. Image J software was used for measurement. Data were presented as mean \pm SEM, ($n = 6$ rats per group) and student's *t*-test was used to compare the significant difference between groups.

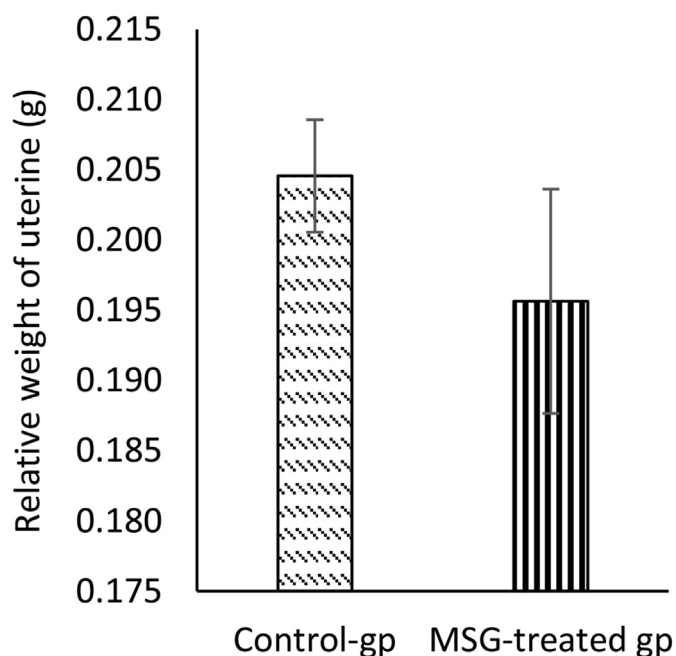


Figure 2. Effect of monosodium glutamate (MSG) oral administration on the relative weight of uterine in female rats. Comparing the mean of the relative weight of uterine tissues between control and treated groups. Data were presented as mean \pm SEM, ($n = 6$ rats per group). At the end of the experiment, the weight of uterine tissues was recorded using an electronic balance. The student's *t*-test was used to compare the significant difference between MSG-treated group and control.

representative photomicrographs of rat uterine sections obtained from MSG-treated rats and control.

3.2. Effects of MSG on serum sex hormones (oestrogen and progesterone), body weight and food consumption

The mean relative level of progesterone in the MSG-treated female rats ($144 \pm 11.8 \text{ ng/ml}$) was significantly higher ($p < 0.05$) compared to the control group ($100 \pm 6.05 \text{ ng/ml}$). On the contrary, the mean relative level of oestrogen in the MSG-treated animal group ($59.6 \pm 2.9 \text{ pg/ml}$) was significantly lower ($p < 0.05$) compared to the control group ($100 \pm 2.9 \text{ pg/ml}$) (Figure 4).

Oral feeding of female rats with MSG (2 g/kg) for 14 days showed a non-significantly increase in body weight of rats compared to the control group (Figure 5a). However, the mean food consumption (g/day/rat) showed no difference between the MSG-treated animal and the control group (Figure 5b).

3.3. Cytotoxic effect of MSG on oestrogen-positive and negative cells

The cytotoxic effect of MSG on MCF-7 and MDA-MB-231 cells was investigated using an MTT assay. MSG caused a mild cytotoxic effect ($<20\%$) on both cell lines after 24, 48, and 72 h, particularly at concentrations between 25 and $100 \mu\text{g/ml}$. MSG showed a higher cytotoxic effect on oestrogen-positive MCF-7 cells after 48 h compared to oestrogen negative MDA-MB-231 cells (Figure 6). However, the IC_{50} of MSG that causes 50% cell growth inhibition in both cell lines needs higher concentrations of MSG to be achieved.

3.4. Computational toxicity of MSG and molecular docking

The computational prediction of toxicity results showed relatively a low systemic toxic effect of the MSG (PubChem ID 23672308). According to the ProTox-II tool, the predicted toxicity class of MSG is V and the

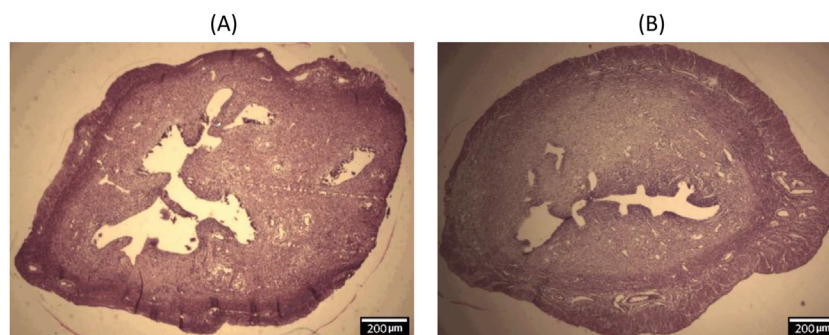


Figure 3. Photomicrographs of rat uterine sections. Lumen area (μm^2), and length of stroma (μm) were higher in the control group (A) than monosodium glutamate-treated group (B), while the length of myometrium was higher in the monosodium glutamate-treated group (H & E stain, magnification 10x).

predicted LD_{50} was 4500 mg/kg, which indicates a possibility to be harmful if swallowed ($2000 < \text{LD}_{50} \leq 5000$). In addition, the iSafeRat[®] tool predicted the mechanism of MSG toxicity to be due to high binding affinity to acetylcholine (ACh) receptors (muscarinic or nicotinic), which may involve in disrupting the normal nerve signal transmissions (class 5.2) and release of protons in the cytosol of cells for all species (class 6.2).

Furthermore, the MSG descriptor was calculated (Table 1). The ω is considered as an indicator of reactivity that allows a quantitative classification of the global electrophilic nature of a molecule. Then $\Delta\omega$ was assessed as the smaller the value indicates the stronger the electrophile-nucleophile interaction. Findings showed that MSG is weak as an electrophilic attacker on the biological macromolecules.

Additional details on the quantitative structure-activity relationship (QSAR) prediction for MSG are provided in Supplementary Table S1.

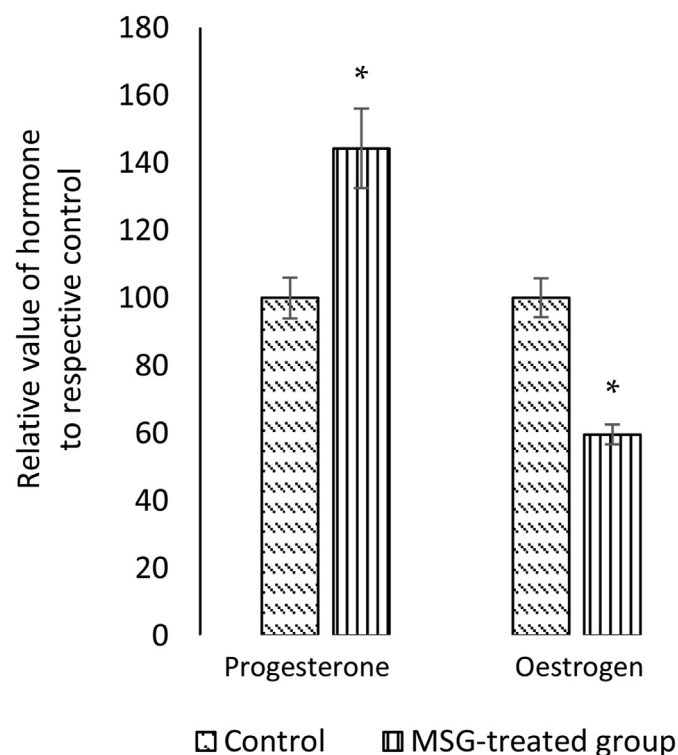


Figure 4. Effect of monosodium glutamate (MSG) oral administration on relative levels of female sex hormones during oestrus phase in female rats. Comparing the mean relative levels of serum oestrogen (pg/ml) and progesterone (ng/ml) between control and MSG-treated group. The chemiluminescent immunoassay (Beckman coulter Access II, USA) was used. Data were presented as mean \pm SEM, (n= 6 rats per group). Student's *t*-test was used to compare between MSG-treated group and control, and differences were considered significant at **p* < 0.05 compared to control group.

By searching the previous studies [19, 20, 21, 22, 23, 24], we found 33 proteins that have a role in the oxidative stress and hormonal regulation of female reproduction (Supplementary Table S2). By comparing the binding affinity and the number of interacting residues (Table 2) of the protein-ligand between MSG and the commercial inhibitors, the analysis showed that MSG has very weak and nonspecific interaction with the majority of the targets. However, a good binding affinity was noticed with some critical proteins, including cytochrome C oxidase subunit 2, proline-rich AKT1 substrate 1, prostaglandin synthase, and transforming protein RhoA. Interestingly MSG showed a good binding affinity with low inhibition constant with human oestrogen receptors beta.

4. Discussion

Oral administration of MSG (2 g/kg) for 14 days to SD female rats with regular ECs resulted in an alteration of serum progesterone and oestrogen levels without significant changes in uterine morphology. For the *in vitro* study, a slight cytotoxic effect was observed on MCF-7 and MDA-MB-231 cells after treatment with serial concentrations of MSG over three-time points, however, the cytotoxic was more pronounced in oestrogen-positive MCF-7 cells after 48 h of treatment. Neuronal toxicity and oxidative stress were the predicted mechanisms of toxicity.

Treating the female rats with MSG for two weeks altered the serum levels of progesterone and oestrogen, which are comparable to the findings of previous studies that reported abnormality in the ovarian hormone with increased serum progesterone levels [25, 26]. However, the observed oestrogen levels in this study are inconsistent with a study that reported a significant 2-fold increase in serum oestrogen levels compared to control when Wistar rats were treated with 100 mg/kg MSG for 60 days [27]. The effects of MSG on oestradiol (oestrogen) levels could be due to the long exposure time, which could be attributed to the activation of aromatase, which catalyses the conversion of testosterone to β -oestradiol and the aromatization of ring A of β -oestradiol, resulting in increased oestradiol synthesis. In addition, treating rats with MSG showed some uterine morphological changes including the lumen area and lengths of stroma and myometrium compared to the control.

A previous histological assessment of the rats' ovaries administrated MSG showed cellular hypertrophy, degenerative and atrophic changes [28]. Moreover, a study by AL-Shemary (2017) showed that administrating MSG for one month significantly decreases the weight of ovaries, and some changes in the histology of ovaries were observed, including congested blood vessels of the medulla, and increased atretic follicles. Furthermore, examining the uterus showed necrosis in the perimetrium and the presence of lacunae in the wall structure [29]. In our study, neither the change of the body weight nor uterine weight was significantly different when compared to the control group. This observation was not in line with Koffuor's finding [30], who reported a significant increase in uterine weight when compared to the control. Unlike the observation of Adamo & Ratner [31], who reported an insignificant changes in the relative uterine weight compared to the control.

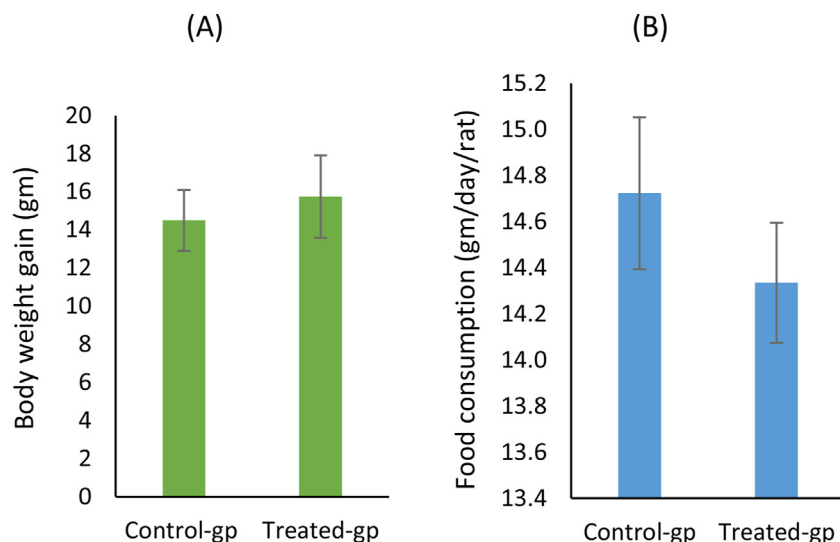


Figure 5. Effect of monosodium glutamate (MSG) oral administration on body weight and food consumption in female rats (A) Comparing the mean body weight gain (gm) and (B) food consumption (gm/day/rat) in the control and treated groups.

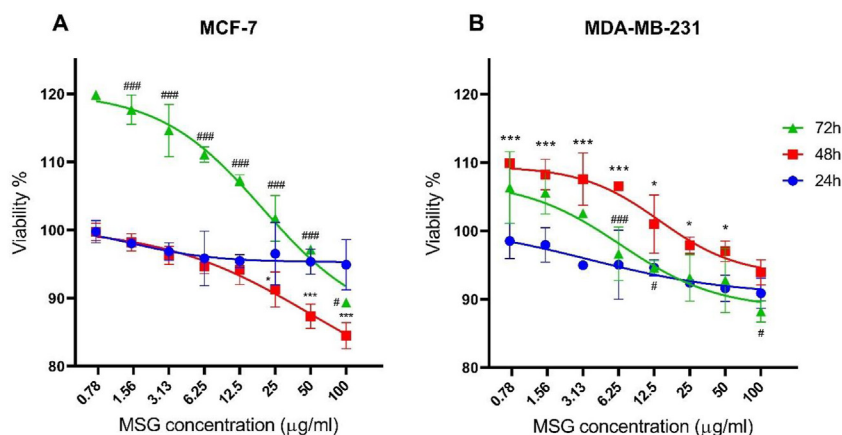


Figure 6. The cytotoxic effect of monosodium glutamate (MSG) on the cell viability of the oestrogen-positive MCF-7 (A) and the oestrogen-negative MDA-MB-231 (B) cells at 24, 48 and 72 h. The data were presented as mean \pm SD, $n = 3$. * $p \leq 0.05$; and *** $p \leq 0.001$ indicate a significant difference between 24 h and 48 h, while # $p \leq 0.05$; and ### $p \leq 0.001$ indicate a significant difference between 48 h and 72 h.

The previous studies showed that prolonged intake of food containing MSG or glutamate salt in humans and animals results in altering the metabolism processes and oxidation balance in different organs such as the liver, pancreas, and kidney, or systems such as the nervous system

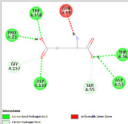
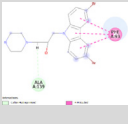
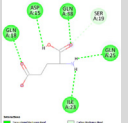
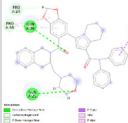

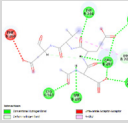
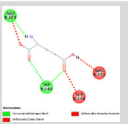

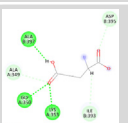
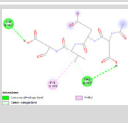




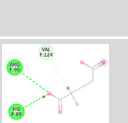
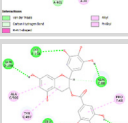
and endocrine system [32, 33, 34]. The systemic alteration of the metabolism and oxidation processes by MSG may affect the function of males' and females' reproductive systems. Previous research showed impairment of the male reproductive function after consuming MSG, including a reduction in sperm count and morphology, and testicular haemorrhage, which was connected to male infertility [8]. Histologically, higher doses of MSG in rats showed deleterious effects on the morphology of fallopian tubes, ovary and uterus, which also connected to infertility [26, 35, 36]. These harmful effects of MSG on the reproductive system could be attributed to the indirect alteration of the regulatory axis of the hypothalamic-pituitary-gonads [11]. Several interesting aspects need to be explored further by long-term study.

Physiologically, several changes to the uterine lining (endometrium) occur during the uterine cycle, it grows to a thick, blood vessel-rich tissue lining, representing an optimal environment for the implantation of a blastocyst upon its arrival in the uterus. The uterine cycle is controlled by a series of changes in hormone levels, primarily oestrogen and progesterone. Thus any changes in their levels directly affect the fertility function [37]. A previous study by Mondal et al. found that administering MSG to rats impaired ovarian function and decreases the duration of proestrus, oestrus and metestrus phases in each EC, which could be due to the significant increase in the secretion of luteinizing hormone (LH)

Table 1. The quantum descriptor of monosodium glutamate (MSG).

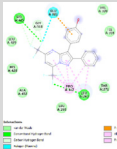
PubChem CID	23672308
Formula	C ₅ H ₉ NO ₄ Na
Enthalpy (au)	-551.3
Entropy (J/mol-K)	443
Gibbs Energy (au)	-551.4
Cv (J/mol-K)	163.54
LUMO (ev)	2.13
HOMO (ev)	-8.89
ω (eV)	0.52
ω -max	0.09
ω + max	0.04
$\Delta\omega$	0.002
LogP	5.64

Table 2. Molecular Docking results of monosodium glutamate (MSG) and the commercial inhibitors with the oxidative stress and hormonal-related proteins.

Protein Name/Symbol	PDB ID ^a	MSG				Commercial inhibitor					
		2D diagram of interaction	No of interacting residues	Affinity ^b	Ki ^c	Inhibitor Name	PubChem ID	2D diagram of interaction	No of interacting residues	Affinity ^b	Ki ^c
Apoptosis regulator BAX (BAX)	6EB6		8	-4.6	415.9	BAI1	2729027		2	-6.4	20.21
Bcl-2-related protein A1 (BCL2A1)	5WHI		6	-4.8	315.3	S55746	71654876		5	-8.6	0.51
Caspase 3 (CASP3)	1NME		2	-4.5	471.6	Ac-DEVD-CHO	644345		8	-6.1	36.57
Caspase 7 (CASP7)	4FDL		4	-4.5	468.7	Ac-DEVD-CHO	644345		8	-6.2	28.51
Caspase-8 (CASP8)	1QTN		6	-4.7	340.8	Ac-DEVD-CHO	644345		3	-9.4	0.13
Caspase-9 (CASP9)	1NW9		13	-4.8	312.9	Ac-DEVD-CHO	644345		13	-8.6	0.52
Cytochrome C oxidase subunit 2 (MT-CO2)	5Z62		14	-5.6	75.6	Indomethacin	3715		18	-8.2	0.93
Glutamate dehydrogenase 1 (GLUD1)	1L1F		3	-4.8	311.1	(-)-Epigallocatechin Gallate	65064		9	-9.6	0.09


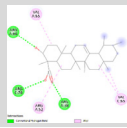

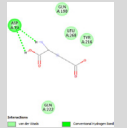
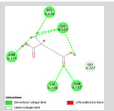
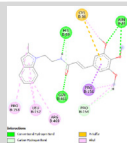





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Table 2 (continued)

Protein Name/Symbol	PDB ID ^a	MSG				Commercial inhibitor					
		2D diagram of interaction	No of interacting residues	Affinity ^b	Ki ^c	Inhibitor Name	PubChem ID	2D diagram of interaction	No of interacting residues	Affinity ^b	Ki ^c
Glutaminase (GLS)	3CZD		12	-4.8	324.9	BPTES	3372016		20	-7.7	2.11
Human estrogens receptors alpha (ESR1)	1ERE		2	-4.8	323.1	AZD9496	11201035		6	-7.4	3.53
Human oestrogen receptor beta (ESR2)	1U3Q		15	-5.2	151.0	PHTPP	11201035		12	-6.7	11.64
Insulin like growth factor binding protein 1 (IGF1R)	2DSQ		6	-4.1	1012.7	NVP-AEW541	11476171		8	-7.5	2.97
Macrophage migration inhibitory factor (MIF)	1CA7		12	-5.0	200.7	ISO-1	4633677		12	-7.8	1.88
Oxytocin receptor (OXTR)	6TPK		11	-4.7	354.3	L-371,257	6918320		25	-9.9	0.05
Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform (PIK3CA)	2AR5		7	-5.0	202.8	BAY1082439	135905473		8	-6.9	9.38
Progesterone receptors (PRGR)	1A28		12	-4.7	384.2	Mifepristone	55245		15	-6.7	13.02
Proline-rich AKT1 substrate 1 (AKT1S1)	6NPZ		15	-5.4	106.0	3CAI	152961		13	-6.3	25.34

(continued on next page)

Table 2 (continued)

Protein Name/Symbol	PDB ID ^a	MSG				Commercial inhibitor					
		2D diagram of interaction	No of interacting residues	Affinity ^b	Ki ^c	Inhibitor Name	PubChem ID	2D diagram of interaction	No of interacting residues	Affinity ^b	Ki ^c
Prostaglandin E synthase (PTGES)	3DWW		12	-5.6	82.3	beta-Boswellic acid	168928		6	-6.8	10.95
Prostaglandin F synthase (PTGFR)	2F38		4	-5.4	105.4	N- (4-chlorobenzoyl)-melatonin	86086004		5	-9.3	0.16
Prostaglandin G/H synthase 2 (PTGS2)	5IKR		6	-5.4	116.8	TG4-155	5886965		9	-8.5	0.59
Transforming protein RhoA (RhoA)	1S1C		7	-5.9	46.2	Rhosin hydrochloride	4304262		9	-9.1	0.21
Type-1 angiotensin II receptor (AGTR1)	4YAY		3	-4.8	325.8	Valsartan	60846		8	-8.3	0.81
Tyrosinase (TYR)	5M8N		5	-5.1	172.4	Isobutylamido thiazolyl resorcinol	71543007		6	-7.4	3.91

^a Proteins data bank (<https://www.rcsb.org/>).

^b Binding Affinity (kcal/mol).

^c Inhibitor constant (μmol).

and follicle-stimulating hormone (FSH) from the pituitary gland and oestradiol from the proliferating ovarian follicles [38]. Moreover, it was suggested that MSG probably promotes the maturation of the ovarian follicles from the primary follicles due to the suppression of progesterone production during the dioestrus phase in EC as revealed by the regression of corpus luteum in comparison to control ovarian tissues sections [38]. In addition, MSG may impair the female reproductive organs through increased oxidative stress [39] and decreased antioxidant enzymes like glutathione reductase, and glutathione peroxidase in ovarian tissues [26].

Furthermore, MSG may cause some metabolic abnormality [40]. It has been reported that excessive intake of MSG is connected to an increase in the incidence of type two diabetes mellitus [33], and increase body weight and fat mass in rodents models [32, 41, 42].

The cytotoxic effect of MSG was tested on MCF-7 and MDA-MB-231 cells. The two cell lines were selected to cover different phenotypic and genotypic variabilities of oestrogen receptors. Cell viability results showed that the MSG compound has a mild cytotoxic effect on both cell lines, especially at concentrations between 25 and 100 µg/ml. However, MCF-7 positive oestrogen receptor cells revealed slightly less cell proliferation after 48 h compared to MDA-MB-231 treated cells, which indicates that oestrogen-positive cells are more sensitive to the cytotoxic effects of MSG. Although the triple-negative MDA-MB-231 cell line is more resistant to treatment, another study reported that MDA-MB-231 showed more susceptibility to drug toxicity in comparison to MCF-7 [43]. Our result revealed that MDA-MB-231 cell proliferation dropped after 72 h treatment with MSG. Therefore, long-term MSG cytotoxicity could be further explored in future studies using higher concentrations. Moreover, according to the ProTox-II tool, the predicted lethal dose was determined as 4500 mg/kg. To apply this dose in an *in vitro* cytotoxicity model, investigations using a higher MSG concentration on both cell lines may result in a more cytotoxic effect.

The computational chemistry tools were used to explore the toxicity of MSG. Overall, MSG showed a possibility of being harmful if swallowed (OECD class 5), and neurological toxicity was predicted by affecting ACh receptors, which consist of the nature of glutamate as a neurotransmitter. Therefore, excessive intake of MSG in the long term may cause several neurological adverse effects. Further neurotoxicity associated with MSG has also been linked to the over-activation of glutamate receptors, which in turn are implicated in brain function and pathology [44]. We found that the predicted mechanism of toxicity of MSG could be through disrupting the normal nerve signal transmissions and the release of protons in the cytosol of cells. Intriguingly, it was reported that the glutamate receptors, including GluR 2/3, metabotropic glutamate receptor 2/3, kainate 2, and N-methyl D-aspartate receptor 1 (NMDAR 1) were localized in the different structures of the female reproductive system, including ovaries, uterine cervix, and endometrium [45], suggesting that the neuroexcitatory targets the female reproductive function.

Furthermore, we assessed the toxicity using the quantum descriptors, based on the principle that cytotoxicity could occur by an electrophilic and nucleophilic attack and forming covalent bonds with an electron-rich nucleophilic target, e.g., amino acids, enzymes, or DNA. Findings showed that MSG was able to make an electrophilic attack against nucleophilic cellular macromolecules. However, MSG safety could be attributed to the contribution of other physicochemical properties, e.g., lipophilicity and ionisation state, which could affect the electrophile nature. Besides, the antioxidant mechanisms include glutathione-electrophile conjugation [46]. However, the computational study is more focusing on the acute action, not on the chronic exposure to MSG, which limits our results.

Furthermore, to explore the interaction of MSG with the uterus function we performed molecular docking with many uterus-related proteins. In general, there was weak interaction of MSG compared to the commercial inhibitors. However, this does not exclude the indirect inhibition, thus *in vitro* validation is needed. Surprisingly, a good binding affinity of MSG with human oestrogen receptor beta was found. These findings matched with a previous study that explored the relationship of

oestrogen receptors α and β with GluR in the female rodent brain, which results in modulating intracellular G-protein signalling cascades to stimulus neuronal physiology, and ultimately behaviour [47]. Furthermore, docking results indicate good binding with different isomers of prostaglandin, indicating involving of MSG with the inflammation pathway, which could be a lighting point to explore the cytotoxicity based on the long term.

5. Conclusions

The alteration in serum progesterone and oestrogen levels caused by oral administration of 2 g/kg MSG for 14 days may result in an abnormality in the functions of reproductive organs in young female rats. Neuronal toxicity can be one of the mechanisms of MSG influencing progesterone and oestrogen levels. Involving of MSG with the inflammatory pathway and binding human oestrogen beta receptors play roles in uterine functions. Therefore, a precaution should be taken when utilising MSG, especially for females associated with a high risk of hormonal disturbance. Further studies should be conducted to evaluate the long-term safety of MSG on corpus luteum function.

Declarations

Author contribution statement

Mahfoudh A. M. Abdulghani: Analyzed and interpreted the data; Conceived and designed the experiments; Wrote the paper.

Salah Abdulrazak Alshehade; Sareh Kamran: Analyzed and interpreted the data.

Mohammed Abdullah Alshawsh: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

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