
**E-ISSN:** 2706-9575
**P-ISSN:** 2706-9567
IJARM 2024; 6(2): 12-24
Received: 12-01-2024
Accepted: 17-02-2024

**Nehal M Gabr**
Physiology Department, Faculty of Medicine, Tanta University, Tanta, Egypt

**Abeer Abed Abo Zeid**
Physiology Department, Faculty of Medicine, Tanta University, Tanta, Egypt

**Nahid M Tahoon**
Physiology Department, Faculty of Medicine, Tanta University, Tanta, Egypt

**Manar Elnady**
Pathology Department, Faculty of Medicine, Tanta University, Tanta, Egypt

**Maessa Mohamed Elnhas**
Physiology Department, Faculty of Medicine, Tanta University, Tanta, Egypt

**Corresponding Author:**
**Nehal M Gabr**
Physiology Department, Faculty of Medicine, Tanta University, Tanta, Egypt

# Study the effect of vitamin D on monosodium glutamate induced testicular and ovarian toxicity in pubertal male and female albino rats

**Nehal M Gabr, Abeer Abed Abo Zeid, Nahid M Tahoon, Manar Elnady and Maessa Mohamed Elnhas**

**DOI:** https://doi.org/10.22271/27069567.2024.v6.i2a.548

**Abstract**
**Background and Aim:** Monosodium glutamate (MSG) a commonly used food additive, has been reported to have adverse effects on reproductive organs. Vitamin D, known for its role in its various physiological functions, including reproductive health, was hypothesized to mitigate the toxic effects of MSG on reproductive organs. The aim of this work was to evaluate the toxic effect of MSG on testicular and ovarian tissue in pubertal male and female albino rats and the possible protective effect of vitamin D.
**Material and Methods:** A total of 80 pubertal albino local strain rats were divided into eight groups: four groups for males and four groups for females (10 rats) in each group control, vitamin D, MSG, MSG + vitamin D. The rats were administered MSG orally at a dose of 60 mg/kg/day dissolved in 0.5 ml distilled water for 30 consecutive days. Vitamin D was administered orally at a dose of 200 IU/kg /day for the same duration. 0.5 ml distilled water was administered to control groups.
**Results:** MSG administration caused degeneration, necrosis, disruption of normal architecture, multiple apoptotic cells shown by immunohistochemical examination of testicular caspase-3, DNA damage, and decreased TAC in testicular and ovarian tissue. Changes in male and female rats included increased body weight, decreased kisspeptin, serum GnRH, FSH, LH, testosterone, estradiol, and elevated TNFα levels. Vitamin D and MSG co-administration significantly reduced histopathological and hormonal alterations.
**Conclusions:** Vitamin D supplementation may have a role against MSG-induced ovarian and testicular toxicity in pubertal male and female albino rats.

**Keywords:** Vitamin D, MSG, kisspeptin, testicular toxicity, ovarian toxicity, pubertal rats

## Introduction
Reproductive dysfunction is often distinguished by the malfunctioning of reproductive tissues, resulting in the disruption of the expected synergistic rhythm that is expected to facilitate the advancement of sexual activities and the conception of offspring [1]. Infertility is a serious medical issue among modern nations. The reproductive systems of both males and females are very susceptible to many detrimental environmental conditions [2].
Various variables have been linked to the development and advancement of reproductive dysfunction, such as inadequate nutrition, adverse effects of medications, pathological conditions, consumption of hazardous substances, industrial pollutants, and dietary additives. One dietary additive that has been identified is monosodium glutamate (MSG) [3, 4, 5].
MSG is a frequently used flavor enhancer or food additive that finds extensive application in various culinary products, including both fast-food establishments and handmade meals [6].
The use of this substance has seen a notable expansion over the years, and it is now ubiquitously present in a wide range of ingredients and processed food products available in various markets and grocery stores. MSG imparts a distinct fragrance to processed foods, sometimes referred to as umami [7].
Multiple investigations have shown that MSG toxicity is linked to physiological complications such as hypertension, obesity, gastrointestinal tract issues, and impairment of brain, neurological system, reproductive, and endocrine system function [8].
Vitamin D3 is conventionally recognized for its role in preserving and controlling calcium and phosphorus balance.

In addition to its overall influence on mineral metabolism, vitamin D3 serves as a crucial endocrine hormone that has a substantial influence on the regulation of reproductive processes in both males and females [9].

The correlation between vitamin D and reproduction has gained recognition as a result of the existence of vitamin D receptors (VDR) in the reproductive organs of both males and females, as well as in the brain, namely in neurons and surrounding glial cells [10]. Furthermore, the eating of MSG has been identified as a potential risk factor for obesity, which might have an impact on fertility. Therefore, the administration of Vitamin D has been shown to inhibit weight growth in rats with obesity generated by MSG [11].

The objective of this study was to assess the potential harmful impact of MSG on ovarian and testicular tissue in pubertal male and female albino rats, as well as to investigate the potential protective effects of vitamin D.

## Materials and Methods

### Drugs

Vitamin D3 (cholecalciferol) in a dose 200 IU/kg /day VD (DEVİT-3® Deva, Turkey) via oral gavage at 9Am daily for 30 days [12], MSG in a dose of 60 mg /kg/day dissolved in 0.5 ml distilled water given by oral gavage at 9 Am daily for 30 days [13]. Vitamin D3, at the same dose of group II, along with MSG at the same dose of group III given by oral gavage at 9 Am daily for 30 days.

### Animals

This experimental work was performed on 80 pubertal albino rats (Forty pubertal male albino rats and forty pubertal female albino rats of local strain) with average age of approximately 6 or 7 weeks, weighing between 150-180 grams. The animals were picked in a random manner from the animal houses located inside the college of science at Tanta University. The animals are housed in separate animal enclosures, with carefully regulated environmental conditions. They are exposed to a 12/12-hour light/dark cycle and are maintained at ambient temperature (23 ± 2 °C). They have unrestricted access to standard rat food and drinking water. The experiment was carried out according to the committee's guidelines for Research and Ethical Issues of Tanta University, Tanta, Egypt. (approval code: 32397/06/18).

### Experimental design

The animals were acclimatized for one week, and then randomly divided into four groups for males and four groups for females: A) Male and female group: divided into 4 groups (10 rats) in each group: Group l: Control group: received 0.5 ml saline by oral gavage at 9 Am daily for 30 days, Group II: vitamin D3 group: treated with vitamin D3 (cholecalciferol) in a dose 200 IU/kg /day VD (DEVİT-3® Deva, Turkey) via oral gavage at 9Am daily for 30 days [12], Group III: MSG group: treated with MSG in a dose of 60 mg /kg/day dissolved in 0.5 ml distilled water given by oral gavage at 9 Am daily for 30 days [14]. and Group IV: MSG + Vitamin D3 group: treated with vitamin D3, at the same dose of group II, along with MSG at the same dose of group III given by oral gavage at 9 Am daily for 30 days.

### Sample collection and analysis

Following body weight measurements, the rats were administered anesthesia using diethyl ether. Subsequently, the animals underwent cervical decapitation to induce scarification. Blood samples were collected during the estrous phase in the female groups, and subsequently subjected to centrifugation at 3000 rpm for a duration of 10 minutes. The serum samples were kept at -80 °C for analysis of the following parameters: Serum Kisspeptin level [15], serum gonadotropin releasing hormones [16], serum FSH [17], serum LH [18], serum free testosterone level in male groups [19], serum free estradiol level in female groups [20] and serum Tumour necrosis factor alpha (TNFα) [21].

### Tissue sampling

After decapitation of rats, testes from male groups and ovaries from female groups were dissected carefully to avoid mechanical trauma, weighed, washed three times with ice cold saline to remove extraneous materials. The testes and ovaries were divided into three parts, two parts were wrapped in aluminum foil and stored at –80 °C till used for preparation of tissue homogenates and DNA extracts. The first Part of testes and ovaries were homogenized in phosphate buffer (pH 7.4) by Potter homogenizer. The homogenate was centrifuged at 4000 rpm for 15 min at 4 °C. The homogenates were used for estimation of the following parameters [total oxidant capacity in testicular and ovarian tissues [22], and the second part of testes and ovaries were used for detection of ttesticular and ovarian Caspase-3 activity [23] and DNA damage in testicular and ovarian tissues [24].

### Vaginal smear for female group:

Vagina of each female rat was first cleaned and swabbed daily from first day of the experiment via tip of cotton applicator swab soaked in saline solution for 5 days then immediately rolled on the glass slide in a thin layer (smear) and allowed to air dry. Dry fixed slide was stained with metachromatic stain (H&E). This method is used for rats' estrous cycle detection [25].

Following body weight measurements, the rats were administered anesthesia using diethyl ether. Subsequently, the animals underwent cervical decapitation to induce scarification. Blood samples were collected during the estrous phase in the female groups, and subsequently subjected to centrifugation at 3000 rpm for a duration of 10 minutes. For the examination of the following parameters, the serum samples were stored at a temperature of -80 °C. Serum Kisspeptin concentration [26], serum gonadotropin releasing hormones (GnRH) [27], serum FSH [17], Serum LH [28], serum free testosterone level in male groups [19], serum free estradiol level in female groups [20] and serum TNFα [21].

### Histopathological examination

For histological investigation, the third section of each rat's testes and ovaries were embedded in paraffin and preserved in 10% neutral buffered formalin (pH 7.2). Light microscopy was used to analyze paraffin-embedded tissues that had been sectioned into 5µm thick slices using microtone and stained with haematoxylin-eosin (H&E) stain. Following all necessary safety and infection control procedures, the slaughtered animals were carefully packaged in a designated container.

### Measurement of serum Tumor Necrosis Factor alpha (TNF- α) by ELISA [21]

The test operates as follows: an enzyme well is coated with a rat TNF-α monoclonal antibody, left to incubate. Next,

biotin-labeled TNF-α antibodies are added, and the immune complex is combined with Streptavidin-HRP. The uncombined enzyme is removed by incubation and washing. After adding Chromogen Solutions A and B, the liquid becomes blue; acid causes the color to turn yellow. The level of the rat drug TNF-α in the sample was strongly linked with the color chromium. Dilute to the standard concentration Introduce samples: Only chromogen solutions A and B, as well as stop solutions, are permitted in the blank well. Put 50 µl of standard and 50 µl of streptavidin-HRP into the standard wells (since the standard already contains mixed biotin antibody). Before adding the sample (40 µl), combine the TNF-α-antibody (10 µl) and Streptavidin-HRP (50 µl). After gently shaking, close the sealing memberance and incubate at 37 ℃ for 60 minutes. Thicken the 30×washing concentrate by mixing it with D 30 times. Reserved a spot. Gently peel back the membrane, pour out the contents, and then shake off any excess water. Pour 50 µl of chromogen solution A into each well, followed by 50 µl of chromogen solution B. Combine gently and let incubate at 37 ℃, shaded, for 10 minutes. When the blue color turns to yellow, it's time to halt the reaction. Add 50 µl of halt Solution to each well. As a last step, measure the optical density (OD) at 450 nm within 15 minutes after applying the stop solution, using the blank well as a reference point. To determine the concentration of a sample, first determine its optical density (OD) using the appropriate standard concentration and OD values. Then, use the sample's OD values to plug into the linear regression equation for the standard curve.

**Determination of serum Kisspeptin level by ELISA [15]**

Test principle: Introduce Kisspeptin 1 (KISS1) into a monoclonal antibody Enzyme well that has been previously coated with Rat KISS1 monoclonal antibody. Incubate the mixture. Next, introduce KISS1 antibodies labeled with biotin and combine them with Streptavidin-HRP to create an immune complex. Finally, incubate and wash the mixture again to eliminate any remaining enzyme. Subsequently, the introduction of Chromogen Solution A and B induces a transformation in the liquid's color, resulting in a blue hue. Upon exposure to acid, the color ultimately transitions to yellow. There was a positive correlation observed between the chroma of color and the concentration of the Rat Substance KISS1 in the sample. The test kit provides a single original standard reagent, which will be diluted in accordance with the provided instructions. Administer specimens: Samples and KISS1-antibody labeled with biotin, Streptavidin-HRP, Chromogen solution A and B, and stop solutions are prohibited from being added to the blank well. In the standard wells, 50¼l of standard and 50 µl of Streptavidin-HRP should be added. It is not essential to add the antibody since the standard already contains a combined biotin antibody. Introduce a 40¼l sample, followed by the addition of 10¼l of (KISS1)-antibody and 50¼l of Streptavidin-HRP. Next, securely close the sealing membrane and gently agitate it, then incubate for 60 minutes at a temperature of 37 ℃. Dilute the 30 washing concentration by a factor of 30 with distilled water as a backup. Carefully remove the membrane and proceed to drain the liquid, ensuring to shake off any leftover water. Introduce 50¼l of chromogen solution A, followed by 50¼l of chromogen solution B, into each well. Lightly combined and incubated for 10 minutes at a temperature of 37℃. To halt the reaction, introduce a volume of 50¼l of Stop Solution into each well, resulting in an instantaneous transition from blue to yellow. Set the blank well to zero and measure the optical density (OD) at 450 nm wavelengths within 15 minutes after adding the stop solution. Based on the concentration standards and their associated optical density (OD) values, the standard curve linear regression equation can be computed. Subsequently, the OD values of the sample may be used in the regression equation to determine the concentration of the corresponding sample.

**Determination of serum gonadotropin releasing hormones by ELISA [16]**

Test principle: To prepare an immune complex, first coat an enzyme well with a monoclonal antibody that binds to rat gonadotropin-releasing hormone (GnRH). Then, add biotin-labeled GnRH antibodies and mix them with Streptavidin-HRP. Finally, incubate the mixture and wash it again to remove any uncombined enzyme. When you add Chromogen Solutions A and B, the liquid becomes blue; when you add acid, it turns yellow. There was a positive correlation between the sample's content of rat Substance gonadotropin-releasing hormone and its color chroma. Instructions for standard dilution are included with this test kit, which includes one original standard reagent. Inject samples: Blank well: only Chromogen solution A and B, and stop solutions are allowed. Standard wells: add standard 50 µl, Streptavidin-HRP 50 µl. Add sample 40 µl, and then add both GnRH antibody 10 µl and Streptavidin-HRP 50 µl. Then seal the sealing membrane was gently shaken and incubated at a temperature of 37 ℃ for a duration of 60 minutes. Dilute the 30-washing concentration by a factor of 30 with distilled water as a backup.

**Determination of serum FSH level by ELISA [17]**

Test principle: The biotin labeled FSH and unlabeled FSH are subjected to a competitive inhibition process with the pre-coated antibody that specifically targets FSH. Following the incubation period, the conjugate that is not bonded is rinsed off. Following this, Streptavidin that has been conjugated to Horseradish Peroxidase (HRP) is introduced into each well of the microplate and subjected to incubation. The quantity of HRP conjugate that is bound has a negative correlation with the concentration of FSH present in the sample. Following the introduction of the substrate solution, the observed color intensity exhibits a negative correlation with the concentration of follicle-stimulating hormone (FSH) present in the sample. The serum sample was subjected to refrigeration at a temperature range of 2-8 °C for a duration of up to 5 days. The samples were cryopreserved at temperatures of -20 °C or below for extended durations, up to 30 days. A temperature of 22-28 °C was maintained for all reagents. Subsequently, set aside at ambient temperature (22-28 °C) for a duration of one hour. The substances present in each well were extracted, and then, the wells were rinsed with a 300 µl solution of diluted wash. The washing method was iterated twice by ensuring proper drainage of the wash. Place the sample in a dark environment at ambient temperature (22-28° C) for a duration of 15 minutes. The absorbance (E) was measured at a wavelength of 450 nm from a blank sample.

**Determination of serum LH level by ELISA [28]**

Test principle: A pre-coating of antibody is applied to the microtiter plate. Subsequently, standards or samples are introduced into the suitable wells of the microtiter plate together with an HRP-conjugated LH and an LH-specific antibody preparation, followed by incubation. Substrate solutions are then introduced into each well. The enzyme-substrate reaction is concluded by the introduction of a solution containing sulfuric acid, and the resulting change in color is quantified using spectrophotometry at a wavelength of 450 nm ± 2 nm. To determine the concentration of LH in the samples, the optical density (O.D.) of the samples is compared to the standard curve. The serum was obtained by using heparin as an anticoagulant and subjected to centrifugation during a 30-minute timeframe, following the previously stated procedure. The samples were kept at a temperature of -20 °C.

**Determination of serum free testosterone level by ELISA [19]**

The sample did not undergo any specific preparation. The serum samples were kept at a temperature range of 2-8o C for a maximum duration of 24 hours.

Test principle: The testosterone enzyme-linked immunosorbent assay (ELISA) technique relies on the competitive binding between testosterone in the test sample and testosterone-horseradish peroxidase (HRP) conjugate, with a consistent quantity of rabbit anti-testosterone. During the incubation process, wells coated with goat anti-rabbit Immunoglobulin G (IgG) are subjected to incubation with 10µl of testosterone standards controls patient samples, 100µl of testosterone-HRP conjugate reagent, and 50µl of rabbit anti-testosterone reagent. The incubation is carried out at a temperature of 37oC for a duration of 90 minutes. Throughout the incubation period, a predetermined quantity of testosterone tagged with HRP engages in competition with the naturally occurring testosterone present in the standard, sample, or quality control serum. This competition occurs for a certain number of binding sites held by the particular testosterone antibody. As the concentration of testosterone in the material increases, there is a gradual reduction in the quantity of immunologically bound testosterone peroxidase conjugates in the well. The sample did not undergo any specific preparation. The serum samples were kept at a temperature range of 2-8o C for a maximum duration of 24 hours. The holder was used to secure the required quantity of coated wells.

**Determination of Serum estradiol level by ELISA [20]**

Test principle: The wells were then supplemented with standards, test samples, and HRP conjugated detection antibody. The mixture was then incubated, and any unbound conjugates were subsequently washed away using wash buffer. The HRP enzymatic reaction was seen using 3,3',5,5'-Tetramethylbenzidine (TMB) substrates (A & B). The reaction of TMB was catalyzed by HRP, resulting in the formation of a blue product that then transitioned to yellow with the addition of an acidic stop solution. The yellow density is directly proportional to the quantity of Estradiol present in the sample that is captured on the plate. The concentration of Estradiol may be determined by measuring the optical density (O.D) absorbance at a wavelength of 450 nm using a microplate reader.

Perform a 15-30 minute equilibration of the kit components at ambient temperature. The standard, test sample, and control (zero) wells were established on the pre-coated plate, and their respective locations were then recorded. Introduction of 50µl of diluted standards into the standard wells is recommended. To the control (zero) well, introduce 50µl of Standard diluent buffer. It is advised to refrain from introducing the sample and HRP conjugated antibody into the first control well.

**Determination of Total Antioxidant Capacity by Colorimetric Method [22]**

Test principle: Antioxidant capacity is assessed by the interaction between antioxidants present in the sample and a predetermined quantity of exogenously supplied hydrogen peroxide (H2O2). The presence of antioxidants in the sample results in the removal of a certain quantity of the given hydrogen peroxide. The calorimetric determination of residual H2O2 is achieved by an enzymatic process that entails the conversion of 3, 5, dichloro-2-hydroxyl benzensulphonate into a product with a distinct color.

**Determination of Testicular and Ovarian Caspase-3 activity by Immunohistochemical detection [29]**

**Immunohistochemical staining:** The procedures involved in this study include the inhibition of endogenous peroxidase, the use of microwave antigen retrieval, the prevention of nonspecific staining, the application of primary antibody, the application of streptavidin enzyme label, the production of a working color reagent, the development of color, and the evaluation of immunohistochemical staining data.

**The steps were performed according to Yeh *et al*. [30] the steps can be summarized as follows**

1. **Deparafinization and rehydration of sections:** Segments The formation fixed paraffin embedded blocks on positive charged sides (superfrost plus-biogenix) were subjected to cutting at a depth of 3-5 microns. The samples underwent dewaxing in a hot xylene bath and were then incubated at ambient temperature for a duration of 5 minutes. This process was iterated twice. After draining the excess liquid, the slides were immersed in a new 100% ethyl alcohol solution for a duration of 3 minutes at ambient temperature. Additionally, this procedure was iterated twice. After draining the excess fluids, the slides were immersed in a new solution containing 95% ethyl alcohol for a duration of 3 minutes at room temperature. This process was done again. The slides underwent a one-minute rinsing process using distilled water. To eliminate any surplus liquid, a firm tap was made on the edge of each slide using filter paper.
2. **Blocking endogenous peroxidase:** The sections were submerged in a solution containing 3% hydrogen peroxide in methanol for a duration of 30 minutes in order to inhibit the activity of endogenous peroxidase at ambient temperature inside a humid environment. The surplus reagent is removed by tapping. Next, the slides were rinsed with PBS for a duration of 5 minutes. The slides were subjected to a drying process around the tissue slice, while ensuring that the section itself was not dried.

3. **Microwave antigen retrieval:** The sections were immersed in a 10 ml mol/L citrate buffer (pH 6.0) for a duration of 10 minutes at a temperature of 100 °C using a microwave. The slides were then cooled to ambient temperature, washed with distilled water, and subsequently transferred to phosphate-buffered saline (PBS).
4. **Blocking nonspecific staining:** To ensure that the tissue slices were well coated, two or three drops of normal goat serum, a nonspecific blocking reagent, were applied to each slide. After a 10-minute incubation period at room temperature, the slides were transferred to the humidity chamber. We tapped off the excess reagent.
5. **Exposure to primary antibody:** For caspase-3 immunostaining two to three drops of caspase-3, mouse monoclonal antibody was placed on each slide. In the humidity chamber, the monoclonal antibody was diluted 1:50 and left to sit at room temperature. After removing any surplus reagent, the slides were rinsed for a duration of 5 minutes. After that, the tissue pieces were dried separately from the slides. Prior to exposure to secondary biotinylaed antibody, two or three drops of the antibody were applied to each slide to fully cover the tissue sections. For half an hour, the slides were left at room temperature in the humidity chamber. We tapped out any excess reagent and then rinsed the slides for 5 minutes in PBS. After that, we dried them.
6. **Exposure to streptavidin enzyme label:** Two to three drops of streptavidin enzyme label were placed on each slide. The slies were incubated for 30 minutes at room temperature in the humidity chamber. Excess reagent was tapped off and the slides were washed for 5 minutes in PBS.
7. **Interpretation of immunohistochemical staining results:** Caspase-3: The immunostaining results of caspase -3 were scored semi quantitatively according to [31] each section was carefully examined for the presence of nuclear immunostaining under high power of light microscope. Detection of DNA damage in testicular and ovarian tissues by electrophoresis [32]: Sample: Tissue homogenate. Analysis of DNA Fragmentation Using Agarose Gel Electrophoresis [32].

**Detection of DNA damage in testicular and ovarian tissues by electrophoresis [24]. DNA extraction by EZ-10 Column Genomic DNA Minipreps kit, Animal**

Test principle: The selective adsorption of DNA on a silica-based membrane embedded in an EZ-10 Spin Column is contingent upon the advantageous properties of silica-based DNA purification technology. During the wash processes, the column facilitates the removal of various components and contaminants. Subsequently, the genomic DNA is extracted from the column and may be easily used in various subsequent procedures, such as polymerase chain reaction (PCR) or gel electrophoresis. Prior to usage, it is recommended to add 1 ml of sterile water to the tube containing Proteinas K in order to homogenize the tissue sample. Maintain the solution at a temperature of -200 °C. Prior to use, combine 48 ml of 100% ethanol with 12 ml of Wash Solution, with a ratio of 4:1 between the volume of ethanol and the volume of Wash Solution.

To get animal tissue, it is recommended to cut a maximum of 30 mg of tissue and place it in a 1.5 ml centrofugal tube. Combine 300 µl of Animal Cell Lysis Solution (ACL Solution) with 1.5 ml of Proteinase K in a centrifuge tube. Allow the tissue to incubate at a temperature of 55 °C until it is fully lysed, typically taking 1-3 hours. Intermittent vortexing. Reduce the temperature to the ambient room temperature. Perform a vortexing motion for 20 seconds and then centrifuge at 12,000 rpm for 5 minutes. Transfer 300µl of the liquid portion to a fresh Eppendorf tube, then introduce 300µl of AB solution. Blend by periodically flipping the tube and let it sit for 2 minutes. Proceed to transfer the whole of the solution into an EZ-10 Spin Column. Rotate the mixture at a speed of 4,000 revolutions per minute for 2 minutes and dispose of the liquid. Introduce 500¬l of Wash Solution and subject to centrifugation at 10,000 rpm for a duration of 2 minutes. Please repeat step 8. Eliminate the flow-through. Rotate the centrifuge at a speed of 10,000 revolutions per minute for an extra minute to eliminate any remaining quantity of Wash Solution. Fill the central region of the membrane in the column with 30µl of Elution Buffer. Cultivate at ambient temperature for a duration of 2 to 3 minutes. The DNA was eluted from the column by centrifuging at 10,000 rpm for a duration of 1 minute. To evaluate the purity of genomic DNA, an analytical 0.7% agarose gel was used.

**Analysis of DNA Fragmentation Using Agarose Gel Electrophoresis [24]**

Test principle: Using agarose gel electrophoresis, this technique offers a qualitative way of evaluating cell death. Genomic DNA cleaves into oligonucleosomal pieces with multiples of 180-200 bp; this is a hallmark of cell death. One way to help characterize an apoptotic event is to see these pieces. Tris-acetate EDETA (TAE) buffer preparation:1 liter of 5x TAE buffer was prepared (24.2 gtris base, 5.71 ml acetic acid and 10 ml 0.5M EDETA completed to 1 liter of distilled water). TAE buffer was diluted 1:5 by adding distilled water to it to form a 1xTAE solution. The PH of this buffer was about 8.5. Agarose gel preparation: 1.8% agarose was prepared by weighing 0.9 gm agarose powder and dissolve it in up to 50 ml 1x TAE. The solution was boiled, stained by 3µl of ethidium bromide, poured into the assembled electrophoresis plate and allowed to solidify. 20µl of eluted DNA were prepared in 4µl loading dye (6x DNA loading defragments) (60% glycerol, 60 mM EDETA, 10mM Tris-HCl,0.03% xylene cyanol, 0.03%bromophenol blue).

**Electrophoresis**

Subsequently, the samples were settled in the wells. After 75 minutes, the gel was subjected to 88 volts. As a standard for DNA fragmentation size, a VC 100bp plus DNA ladder (ready to use) (100-3000 bp) at a concentration of 0.5µg/µl was used. The visualization and photography of DNA bands were accomplished using an ultraviolet transilluminator.

**Statistical analysis**

The statistical study was conducted using SPSS v26, developed by IBM Inc. in Chicago, IL, USA. The quantitative variables were presented using the mean and standard deviation (SD), and thereafter compared across the three groups by an analysis of variance (ANOVA) with a post hoc test (Tukey). The Chi-square test was used to report and assess the frequency and percentage (%) of

qualitative variables. A result was considered statistically significant if the two-tailed P value was less than 0.05.

## Results

Daily administration of vitamin D3 alone orally for 30 days induced insignificant changes (p= 0.999) in the body weight, kisspeptin level, gonadotropin releasing hormone, FSH level, LH level, TNFα level and total antioxidant capacity level in normal control compared to vitamin D3 group in normal control compared to vitamin D3 group in male and female group. While administration of MSG alone (60 mg/kg/day) orally for 30 days revealed a significant increase ($p$<0.001) in the body weight and TNFα level in MSG group compared with the previous values of normal control group in male and female group. While animals received MSG along with vitamin D3, a significant decrease ($p$<0.001) was detected in the body weight, TNFα level and total antioxidant capacity in MSG + Vit. D3 group compared with the later values of MSG group, although still significantly higher than the normal control group value in male and female group. Administration of MSG alone (60 mg/kg/day) orally for 30 days revealed a significant decrease ($p$<0.001) in the Kisspeptin level, gonadotropin releasing hormone, FSH level, LH level in MSG group compared with the previous values of normal control group in male and female group. While animals received MSG along with vitamin D3, a significant increase ($p$<0.001) was detected in the Kisspeptin level, gonadotropin releasing hormone, FSH level, LH level and total antioxidant capacity in MSG + Vit. D3 group compared with the later values of MSG group, although still significantly lower than the normal control group value in male and female Table 1.

**Table 1:** Effect of MSG and/or Vit. D3 on body weight, kisspeptin level, gonadotropin releasing hormone, FSH, LH level, TNFα, total antioxidant capacity among studied groups

| | Male | | | | Female | | | | P |
|---|---|---|---|---|---|---|---|---|---|
| **Body Weight (g)** | **Control** | **Vit. D3** | **MSG** | **MSG + Vit. D3** | **Control** | **Vit. D3** | **MSG** | **MSG + Vit. D3** | |
| | 164.8±10.19 | 170±11.15 | 209±9.42 | 190±6.87 | 169.3±8.27 | 174.1±9.19 | 209.7±9.55 | 192.1±6.71 | <0.001* |
| Kisspeptin (pg/ml) | 173.2±4.44 | 173.7±2.98 | 130.7±1.89 | 168.6±5.56 | 182.3±4.24 | 183.2±3.88 | 132.5±2.46 | 169.6±4.67 | <0.001* |
| Gonadotropin releasing hormone (pg/ml) | 22.26±1.20 | 22.83±1.38 | 11.37±0.68 | 18.59±0.75 | 19.65±0.40 | 19.61±0.37 | 9.68±0.30 | 18.14±0.42 | <0.001* |
| FSH (mlU/ml) | 4.07±0.167 | 4.20±0.180 | 3.47±0.194 | 3.98±0.139 | 0.042±0.013 | 0.048±0.010 | 0.007±0.001 | 0.037±0.011 | <0.001* |
| LH (mlU/ml) | 7.02±0.18 | 7.01±0.14 | 5.01±0.56 | 6.87±0.14 | 0.27±0.037 | 0.28±0.038 | 0.15±0.027 | 0.28±0.042 | <0.001* |
| TNFα(ng/L) | 8.62±1.05 | 8.36±0.86 | 32.88±4.97 | 9.42±1.06 | 8.42±1.11 | 8.48±0.95 | 33.03±4.98 | 9.44±0.99 | <0.001* |
| Total antioxidant capacity (mM/g tissue) | 40.7±1.23 | 40.64±1.05 | 29.87±3.50 | 53.79±2.76 | 37.07±2.08 | 37.72±1.71 | 31.18±1.44 | 61.45±1.49 | <0.001* |
| Post hoc test | a=0.999, b=<0.001*, c=<0.001*, d=<0.001* | | | | | | | | |

Daily administration of vitamin D3 alone (200 IU/kg) orally for 30 days induced insignificant changes (p = 0.999) in free testosterone level in male and free estradiol level in female in normal control compared with vitamin D3 group in normal control group compared to vitamin D3 group. While administration of MSG alone (60 mg/kg/day) orally for 30 days revealed a significant decrease ($p$<0.001) in free testosterone level in male and free estradiol level in female in MSG group compared to the previous values of normal control group. While animals received MSG along with vitamin D3, a significant increase ($p$<0.001) was detected in the free testosterone level in male and free estradiol level in female, MSG + Vit. D3 group compared with the later values of MSG group, although still significantly lower than the normal control group value Table 2.

**Table 2:** Effect of MSG and/or Vit. D3 on free testosterone level among male groups and free estradiol level among female groups

| | Male | | | | P |
|---|---|---|---|---|---|
| | **Control** | **Vit. D3** | **MSG** | **MSG + Vit. D3** | |
| Free testosterone level(ng/ml) | 3.46±1.07 | 3.51±1.085 | 2.4±0.79 | 3.34±1.045 | <0.001* |
| | a=0.999, b=<0.001*, c=<0.001*, d=<0.001* | | | | |
| | Female | | | | |
| Free estradiol level(pg/ml) | 90.48±1.08 | 90.89±1.03 | 81.39±0.80 | 90.05±0.79 | <0.001* |
| | a=0.999, b=<0.001*, c=<0.001*, d=<0.001* | | | | |

**As regarding to Immunohistochemical results of caspase-3 in male and female groups**

Group l: (Control group) and Group II: (Vit. D3 group): Immunohistochemical staining of testicular and ovarian sections from animals in both groups showed no apoptotic cells. The cytoplasm of the seminiferous epithelium and interstitial Leydig cells in males, as well as the follicular lining and stromal cells in females, exhibited negative expression of caspase-3 (score -1). ale (Figure 1, A,D).

Group III: (MSG group): In MSG group, the cytoplasm and nuclei of the seminiferous epithelium exhibited a positive expression of caspase-3 (score-3) in over 10% of the cells. The Leydig cells exhibited robust expression of caspase 3. (Figure 1, B). While in female in atretic follicles. Stromal cells also showed strong caspase-3 expression (Figure 1, E).

Group IV: (MSG + Vitamin D3 group): In MSG + Vit. D3, <10% of cells positive for caspase-3 (score-2) expression was detected in the cytoplasm of the seminiferous epithelium and in Leydig cells in male (Figure 1, C) and in mature Graffian follicles and in stromal cells in female (Figure 1, F).

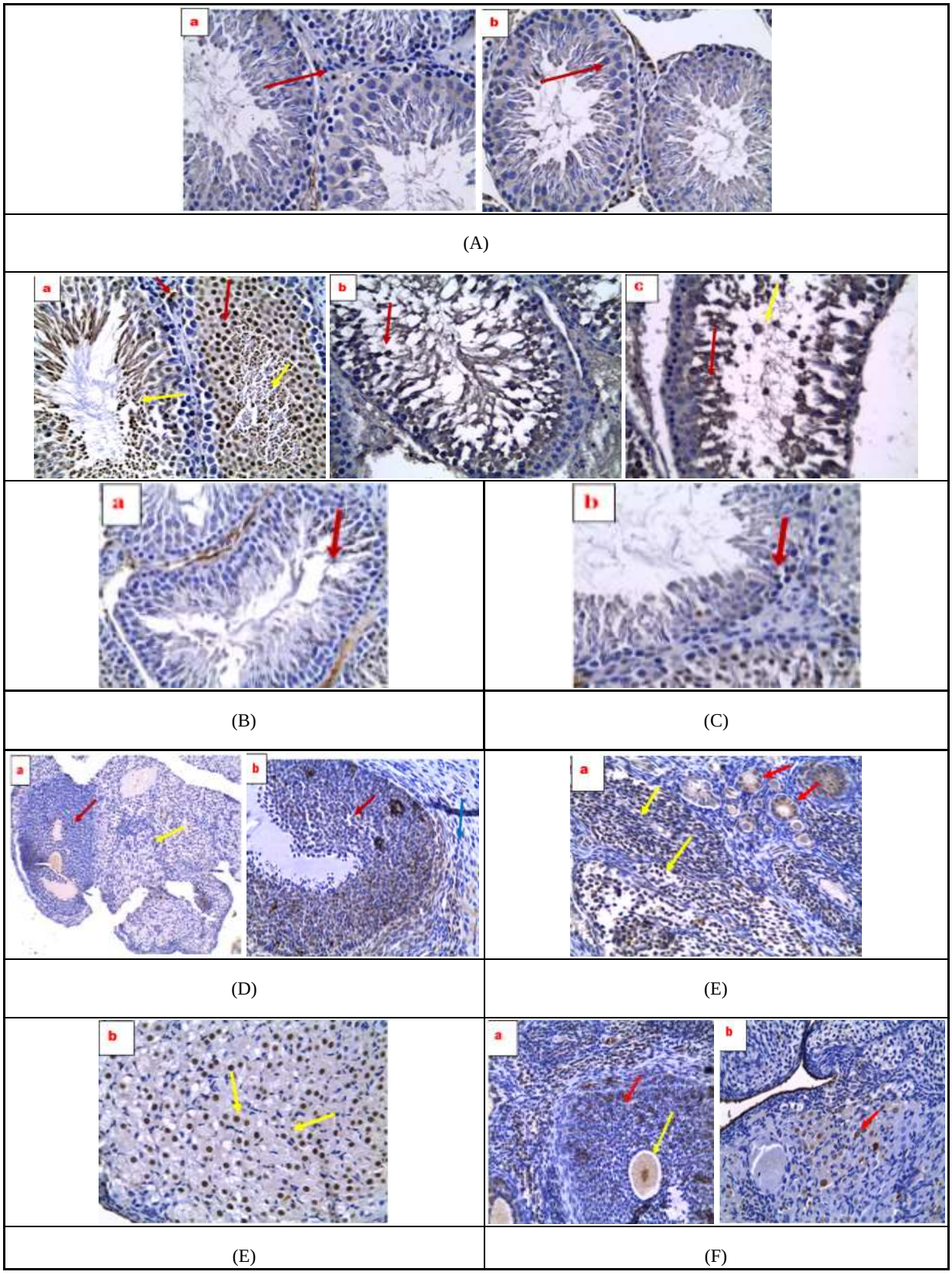



**Fig 1:** In male group , (A) Testicular section (a) from control group, (b) Testicular section from Vit. D3 group showing no apoptotic cells (score -1) negative for caspase -3 immunostaining expression in cytoplasm of the seminiferous epithelium and in the interstitial Leydig cells (red arrows) (Immunoperoxidase ×400), (B) Testicular section from MSG group showing >10% of cells positive for caspase-3 (score-3) expression in the cytoplasm and nuclei of the seminiferous epithelium and the interstitial Leydig cells showing strong caspase-3 expression (red arrows) Furthermore, apoptotic bodies filling lumens of seminiferous tubules (yellow arrow) lumens of seminiferous tubules (Immunoperoxidase ×400), (C) Testicular section from MSG + Vit. D3 group showing <10% of cells positive for caspase-3 (score-2) expression in the cytoplasm of the epithelium (red arrows) and in the interstitial Leydig cells (red arrows) (Immunoperoxidase ×400). In female group, (D) Ovarian section (a) from control group (Immunoperoxidase ×100) (b) from Vit. D3 group (Immunoperoxidase ×200) showing negative caspase -3 immunostaining expression in follicular lining (red arrows) and stromal cells showing no apoptotic cells (yellow ), Ovarian section (E) MSG group was positive to caspase 3 immunostaining in the atretic follicles (red arrows)and stromal cells (yellow arrow)showing many apoptotic cells (Immunoperoxidase ×200), (F) MSG + Vit. D3 group was weak positive for caspase -3 immunostaining, showing negative expression in mature Graffian follicle (a) (yellow arrow) and focal positivity in few stromal cells (a and b) (red arrow) (Immunoperoxidase x400)

**Testicular histopathology in male group**

**Group l: Control group:** All animals within this cohort exhibited typical testicular architecture, including the basement membrane of seminiferous tubules, basal spermatogonia, various stages of spermatogenesis, and mature sperm inside the lumen of seminiferous tubules. Additionally, the number of Sertoli cells was found to be within normal parameters. (Figure 2, A, a). Group II: vitamin D3 group: All animals in this group showed preserved normal architecture as the control group. (Figure 2, A, b). Group III: MSG group: The 10 animals examined in this study had irregular seminiferous tubules characterized by large lumens, which were bordered by a limited number of layers of spermatogenic cells. Additionally, the tubes contained a small number of sperm, which were separated by hyalinized interstitial tissue and congested blood arteries. Among the 10 animals that were examined, it was observed that seven of them had apoptotic cells inside the hyalinized interstitial tissue and seminiferous tubules. (Figure 2, B, a, b, c). Group IV: MSG + Vitamin D3 group: All animals of this group showed normal seminiferous tubules and Spermatogenesis with few apoptosis. (Figure 2, C)

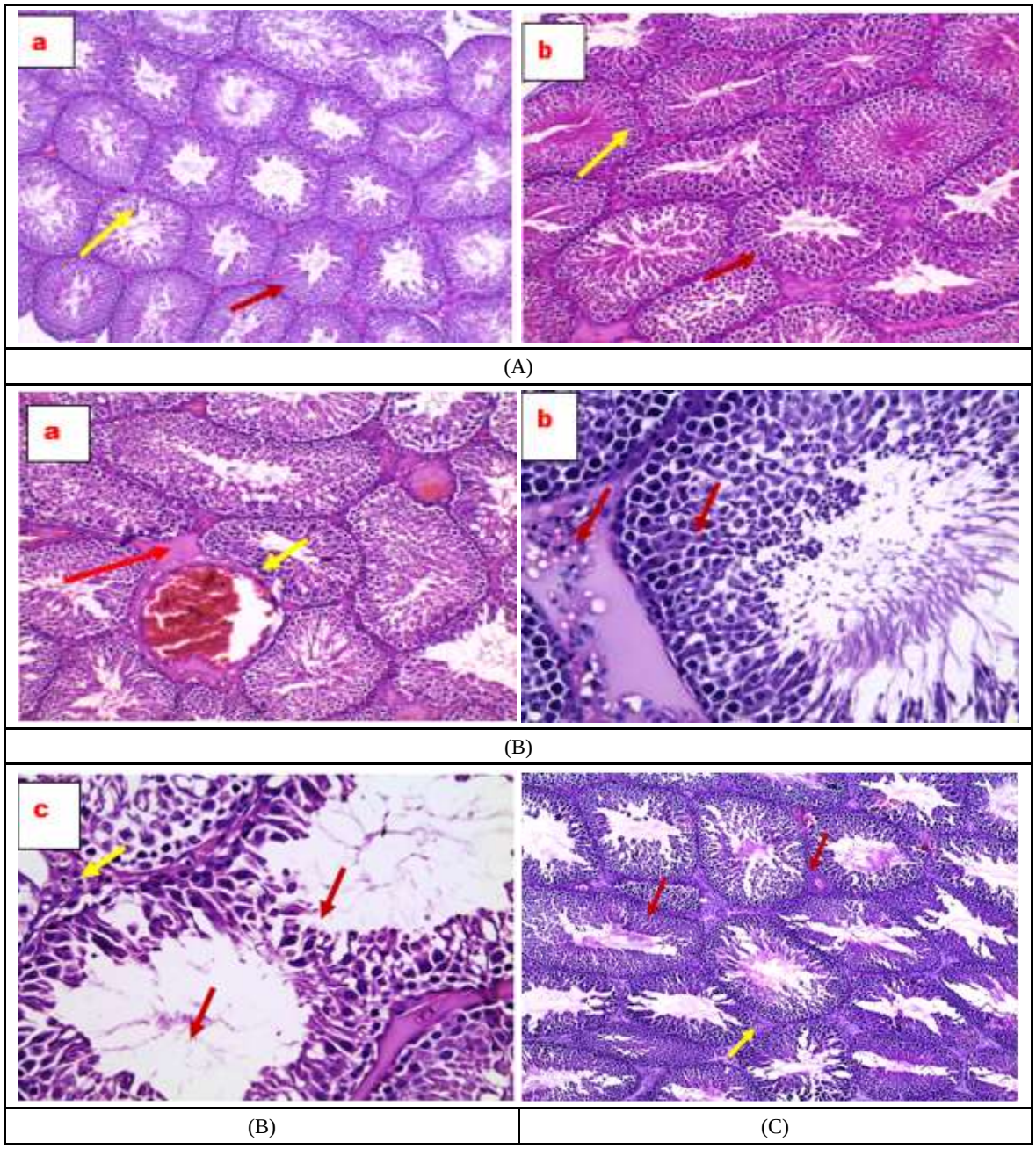



**Fig 2:** In male group, (A) Testicular section (a) control group ((H&E x100), (b) Vit. D3 group (H&E x200) showing normal testicular architecture with regular seminiferous tubules (yellow arrow) lined by multiple layers of spermatogenic cells (red arrow) in different stages of maturation up to mature sperms, (B) MSG group showed (a) hyalinized interstitial tissue (red arrow) with congested blood vessels (yellow arrow) (H&E x200), (b)apoptotic cells in hyalinized interstitial tissue and in seminiferous tubules(red arrow) (H&E x200) ,(c) seminiferous tubules with wide lumens lined by few layers of spermatogenic cells and sperms (red arrow) with leukocytic infiltration(yellow arrow) (H&E x400), (C) MSG + Vit. D3 group showing retained architecture with mild irregularity in seminiferous tubules Spermatogenesis retained to normal with multiple layers of spermatogenic cells lining the tubules with few apoptosis (red arrow). Absence of hyalinization, congestion, and apoptosis in the interstitial tissue (yellow arrow) (H&E x200)

**Ovarian histopathology in female group**

Group l: Control group: All animals in this group showed normal ovarian architecture. Mature Graffian follicle lined by granulosa and theca cells and filled by liquor folliculi. (Figure 3, A, a). Group II: vitamin D3 group: All animals in this group showed preserved normal architecture as the control group. (Figure 3, A,b). Group III: MSG group: The studied ten animals in this group showed distorted Graffian follicle with wide lumen and thin atrophic lining with vacuolated cytoplasm and pyknotic nuclei with congested blood vessels in ovarian medulla. Out of the studied ten animals, six animals showed apoptotic changes in ovarian stromal cells with vacuolated cytoplasm and pyknotic nuclei. (Figure 3, B, a, b, c). Group IV: MSG + Vitamin D3 group: All animals of this group showed ovarian Graffian follicle with retained maturation lined by multiple layers of granulosa and theca cells .Stromal cells show some cytoplasmic vacuolation but with rounded nuclei(Figure 3,C).

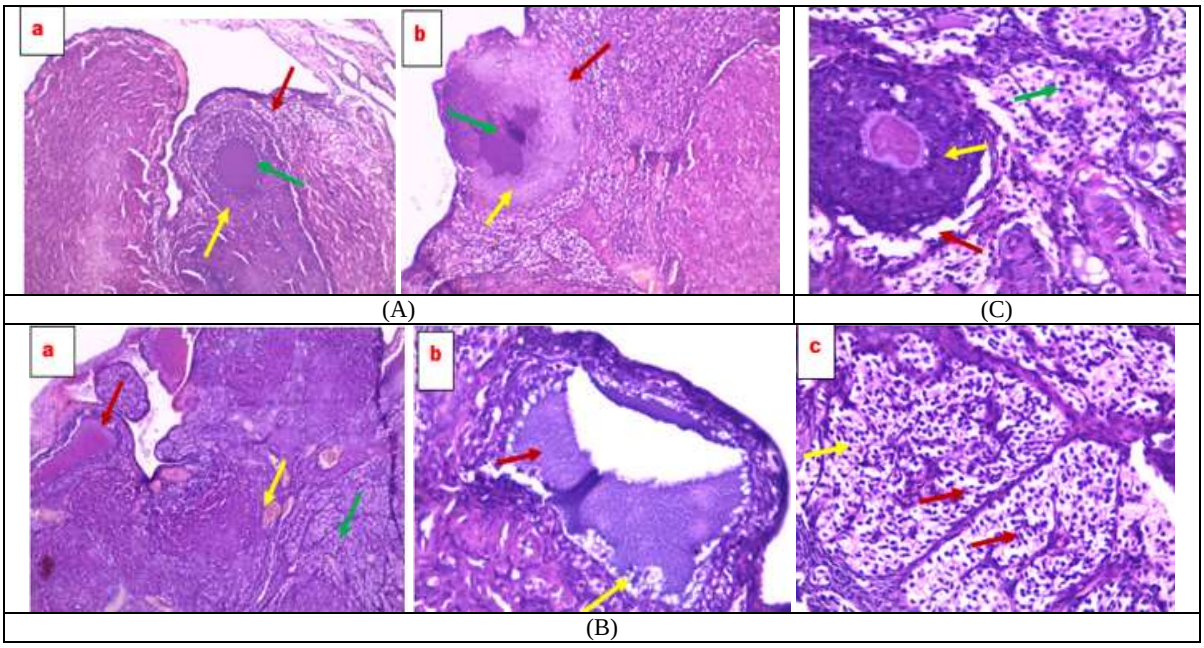
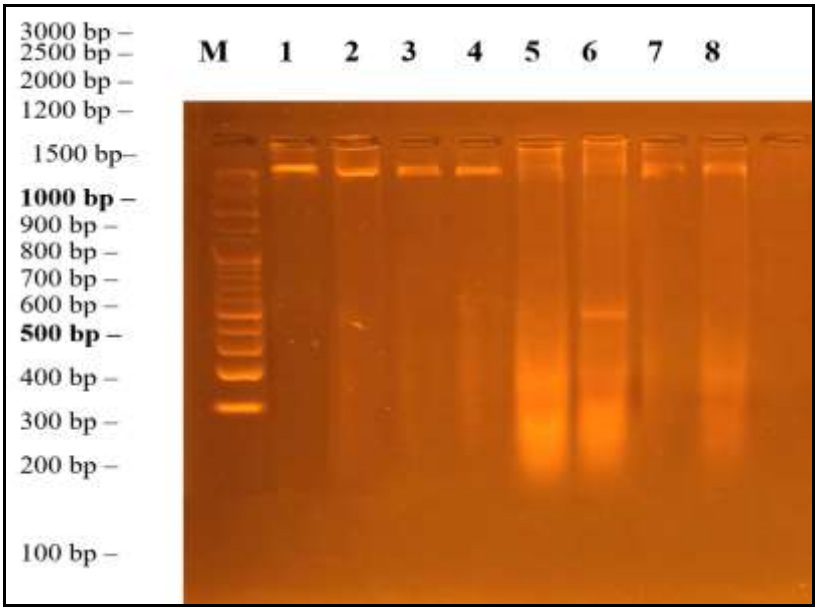



**Fig 3:** (A) Ovarian section from (a) control group, (b) Vit. D3 group showing normal ovarian architecture. MatureGraffian follicle is seen (red arrow) lined by granulosa and theca cells (yellow arrow) and filled by liquor folliculi (green arrow) (H&E x200),(B) MSG group showing Graffian follicle with wide lumen and thin atrophic lining (red arrow) (a) Ovarian medulla showed congested blood vessels (yellow arrow) and vacuolated stromal cells (green arrow) (H&E x100) .(b) The lining cells are atrophic with vacuolated cytoplasm and pyknotic nuclei (yellow arrow) (H&E x400). (c) stromal cells with vacuolated cytoplasm and pyknotic nuclei (red arrows) with leukocytic infiltration yellow arrow) (H&E x400), (C) MSG + Vit. D3 group showing ovarian Graffian follicle with retained maturation red arrow lined by multiple layers of granulosa and theca cells (yellow arrow) Stromal cells show some cytoplasmic vacuolation but with rounded nuclei (green arrow) (H&E x400).

### 4.4. DNA damage




**Picture 4:** Agarose gel electrophoresis for DNA extracted from testicular and ovarian tissue of control, VitD3, MSG and MSG+VitD3 groups.

**Lane (M):** represents (100-3000 base pair DNA ladder
**Lane (1):** represents DNA extracted from testicular tissue of control group and shows no apoptotic fragmentation.
**Lane (2):** represents DNA extracted from ovarian tissue of control group and shows no apoptotic fragmentation.
**Lane (3):** represents DNA extracted from testicular tissue of VitD3 group and shows no apoptotic fragmentation.
**Lane (4):** represents DNA extracted from an ovarian of VitD3 group and shows no apoptotic fragmentation.
**Lane (5):** represents DNA extracted from testicular tissue of MSG group and shows apoptotic DNA fragmentation at approximately 400 bp.
**Lane (6):** represents DNA extracted from ovarian tissue of MSG group and shows apoptotic DNA fragmentation at approximately 377 bp.
**Lane (7):** represents DNA extracted from testicular tissue of VitD3 + MSG group shows a reduction of DNA apoptotic fragmentation at approximately 370 bp in comparison to MSG group and normal band start to appear.
**Lane (8):** represents DNA extracted from ovarian tissue of VitD3 + MSG group shows a reduction of DNA apoptotic fragmentation at approximately 317 bp in comparison to MSG group and normal band start to appear.

## Discussion

There is evidence suggesting that individuals who are overweight or obese, regardless of gender, are susceptible to experiencing reproductive dysfunction. Additionally, certain blood metabolites or hormones may serve as indicators of the extent of this dysfunction. Moreover, there is a strong correlation between being overweight or obese and polycystic ovarian syndrome, which is the primary factor contributing to anovulatory infertility in women [33].

Regarding the effect of MSG on reproductive hormones, the results of the present study showed that MSG administration for 30 days could induce reproductive dysfunction, as indicated by the significant decrease in (Kisspeptin, GnRH, FSH, LH and testosterone in male and estradiol in female, the group treated with MSG in comparison to the control group. The extended use of MSG and glutamate salt in conjunction with food leads to mostly metabolic and oxidative imbalances in several physiological systems, such as the neurological system, endocrine system, liver, pancreas, and kidneys, both in humans and animals [34].

The effect of kisspeptin on the release of testosterone in rodents appeared to be mediated via GnRH/LH. Moreover, an increase in KiSS-1 expression was observed at puberty in both male and female monkeys [35].

Kisspeptin exerts its effects by acting upstream of GnRH, in response to paracrine stimulatory and inhibitory signals from neurokinin B and dynorphin (referred to as KNDy, which stands for kisspeptin / neurokinin B [NKB]/dynorphin). Direct signals are sent to GnRH neurons in order to regulate the pulsatile release of GnRH. When kisspeptin is provided to people in various isoforms, methods, and dosages, it effectively enhances the production of LH and increases the frequency of LH pulses [36]. Overall, many studies indicate a reduction in hypothalamic NPY levels in rats injected with MSG [37]. In contrast, Torrezan *et al*. [38] It has been found that there was a considerable rise in the expression levels of NPY in both the hypothalamus and the pituitary gland of rats treated with MSG.

Unlike previous studies, A recent research found that there was an elevation in the production of LH and FSH by the anterior pituitary gland. This was followed by an increase in the secretion of FSHRH and LHRH by the paraventricular and supraoptic hypothalamic nuclei. These changes had a detrimental effect on the reproductive system [39]. Leisegang *et al*. [40] supposed that There exists a positive correlation between elevated levels of inflammatory cytokines and adverse effects on male reproductive function. These effects manifest as disruptions in the hypothalamic-pituitary-testes axis and steroidogenesis cascades, leading to hypogonadotropic hypogonadism. Additionally, spermatogenesis is impaired, resulting in subclinical prostatitis and prostate hyperplasia.

Based on the findings of the present investigation, a correlation can be established between the inflammatory state generated by MSG and a notable elevation in TNF-α levels seen in the MSG group when compared to the control group. The presence of a chronic inflammatory state was further substantiated by histological findings, which revealed a significant infiltration of lymphocytes. The observed inflammatory condition may be ascribed to the simultaneous presence of obesity and dyslipidemia, which aligns with prior research indicating that obese individuals and animals exhibit a persistent low-level inflammatory state defined by elevated levels of TNF-κ in their plasma [41].

In the current investigation, it was shown that MSG exhibited an oxidative stress impact on the testicular and ovarian tissue. This was substantiated by the significant reduction in testicular and ovarian TAC within the MSG group. These findings are consistent with previous research [42, 43].

The production of oxidative stress is the underlying process responsible for the damage generated by MSG, irrespective of the specific organ or cell type involved. This process involves an elevation in the amounts of reactive oxygen species (ROS) within the cellular environment, resulting in detrimental effects on cell proteins, lipids, polysaccharides, and nucleic acids. Consequently, several cellular functions become impaired [44].

MSG triggers apoptosis via LPO pathways in the arcuate nucleus, hypothalamus, and other tissues around the ventricle. Furthermore, the occurrence of oxidative stress induces apoptosis in rat thymocytes via the downregulation of Bcl-2 expression. Nevertheless, this phenomenon is associated with a rise in intracellular calcium levels, which initiates a series of enzymatic reactions and consequent death. Furthermore, it leads to the activation of calcium-dependent protease, calpain, and apoptosis-inducing factor (AIF) [45].

The findings of this study revealed the presence of numerous apoptotic figures using immunohistochemistry analysis of caspase-3 in the MSG group, in comparison to the control group, after a 30-day dose of MSG. According to Kianifard *et al*. [46], excessive consumption of MSG leads to an excessive production of reactive oxygen species (ROS) as a result of the inactivation and depletion of antioxidants. Consequently, these oxidants contribute to additional tissue damage and are believed to play a crucial role as a trigger molecule for apoptosis, resulting in cellular damage and the activation of additional caspases.

Vitamin D is classified as a member of the steroid hormone family and is recognized for its involvement in the maintenance of calcium and phosphate balance, as well as its contribution to bone health. Additionally, this substance

exhibits a diverse range of cytoprotective characteristics, including anti-fibrotic, anti-oxidative, and anti-inflammatory capabilities [47].

Furthermore, vitamin D assumes a pivotal function in the regulation of reproductive hormonal processes. Multiple studies have shown a correlation between vitamin D and the synthesis and secretion of reproductive hormones into the bloodstream [48]. There has been growing interest in vitamin D's potential involvement in obesity. There is a lot of curiosity in vitamin D's potential function in changing energy balance to a negative condition [49].

Leydig cells are the primary cells responsible to produce testosterone in the testis of mammals. The production of testosterone starts with the transformation of cholesterol into pregnenolone by the action of the mitochondrial enzyme CYP11A1 (cytochrome P450 Cholesterol side-chain cleavage) [50]. In a study conducted by Zhu *et al*. [51], it was shown that the expression of the CYP11A1 enzyme gene in human adult primary testicular cells was elevated by VD/VDR.

A substantial prospective observational study has identified a potential correlation between vitamin D deficiency and anovulatory infertility as a symptom of polycystic ovary syndrome (PCOS). Vitamin D deficiency has been linked to the most common manifestations of PCOS, including insulin resistance, adiposity indices, systemic proinflammatory indices, and ovulatory dysfunction. Vitamin D administration has the potential to enhance reproductive function in women diagnosed with polycystic ovary syndrome (PCOS) by facilitating the restoration of regular menstrual periods [52]. Additionally, Cowan *et al*. [53] added that Vitamin D deficiency may exacerbate symptoms of polycystic ovarian syndrome (PCOS), associated with ovulatory and menstrual irregularities and lower pregnancy success.

Our study showed a significant decrease in the serum level of TNF-α in the MSG + vitamin D group which may prove the anti-inflammatory effect of vitamin D administration.

Our results about antioxidant effect of Vit D on testis and ovary agree with other studies on different organs, Vit D was reported to be attributed to the prevention of some chronic diseases as type 2 diabetes through increasing the antioxidant defense system including GSH, GPx and SOD and suppressing the expression of NADPH oxidase [54]. Edris *et al*. [55] reported that diminished expressions of Caspase3 in the MSG + vitamin D groups.

Histopathological examination and DNA damage of the testicular and ovarian tissue that received vitamin D supplementation confirmed our biochemical improvement as there was retained testicular architecture with mild irregularity in seminiferous tubules.

Recommendations of this study included that reduced consumption of foods containing MSG which can be fortified with minute quantities of Vit D to overcome its adverse effects. Also, recommended use of vitamin D3 in obese patients with reproductive disorders as Vit D modulates reproductive processes in women and men as part of therapeutic protocols.

## Conclusions

Vitamin D supplementation may have a role against MSG-induced ovarian and testicular toxicity in pubertal male and female albino rats.

**Financial support and sponsorship:** Nil

**Conflict of Interest:** Nil

## References

1. Kayode OT, Rotimi DE, Kayode AA, Olaolu TD, Adeyemi OS. Monosodium glutamate (MSG)-induced male reproductive dysfunction: a mini review. Toxics. 2020;8:7-11.
2. Carré J, Gatimel N, Moreau J, Parinaud J, Léandri R. Does air pollution play a role in infertility?: a systematic review. Environmental Health. 2017;16:82-99.
3. Gillois K, Lévêque M, Théodorou V, Robert H, Mercier-Bonin M. Mucus: An underestimated gut target for environmental pollutants and food additives. *Microorganisms*. 2018;6:53-59.
4. Niaz K, Zaplatic E, Spoor J. Extensive use of monosodium glutamate: A threat to public health? *Excli Journal*. 2018;17:273-278.
5. Roychoudhury S, Saha MR, Saha MM. Environmental toxicants and male reproductive toxicity: oxidation-reduction potential as a new marker of oxidative stress in infertile men. Environmental Toxicology. 2019;175:99-115.
6. Sugimoto M, Murakami K, Fujitani S, Matsumoto H, Sasaki S. Dietary free glutamate comes from a variety of food products in the United States. Nutrition Research. 2019;67:67-77.
7. Deepa Indoria KVK, Chittorgarh, MPUAT, Udaipur. Health hazards of food additives. International Journal of Current Microbiology and Applied Sciences. 2020;87:1-8.
8. Chakraborty SP. Patho-physiological and toxicological aspects of monosodium glutamate. Toxicology Mechanisms and Methods. 2019;29:389-396.
9. Jeremy M, Gurusubramanian G, Roy VK. Vitamin D3 regulates apoptosis and proliferation in the testis of D-galactose-induced aged rat model. Scientific Reports. 2019;9:8-15.
10. Nandi A, Sinha N, Ong E, Sonmez H, Poretsky L. Is there a role for vitamin D in human reproduction? Hormone Molecular Biology and Clinical Investigation. 2016;25:15-28.
11. Banerjee A, Mukherjee S, Maji BK. Worldwide flavor enhancer monosodium glutamate combined with high lipid diet provokes metabolic alterations and systemic anomalies: An overview. Toxicology Reports. 2021;8:938-61.
12. Kaplan S, Türk A, Aydın H, Erten M, Kırıcı P. Vitamin D improves oxidative stress and histopathological damage in rat ovaries caused by hyperthyroidism. Journal of Obstetrics and Gynaecology Research. 2021;47:3551-3560.
13. Hamza RZ, Al-Harbi MS. Monosodium glutamate induced testicular toxicity and the possible ameliorative role of vitamin E or selenium in male rats. Toxicology Reports. 2014;15:1037-1045.
14. Hamza RZ, Al-Harbi MS. Monosodium glutamate induced testicular toxicity and the possible ameliorative role of vitamin E or selenium in male rats. Toxicology Reports. 2014;1:1037-1045.
15. Gaytan F, Gaytan M, Castellano JM, Romero M, Roa J, Aparicio B, *et al*. KiSS-1 in the mammalian ovary:

distribution of kisspeptin in human and marmoset and alterations in KiSS-1 mRNA levels in a rat model of ovulatory dysfunction. American Journal of Physiology-Endocrinology and Metabolism. 2009;296:E520-E31.
16. Iwabe T, Harada T, Sakamoto Y, Iba Y, Horie S, Mitsunari M, *et al*. Gonadotropin-releasing hormone agonist treatment reduced serum interleukin-6 concentrations in patients with ovarian endometriomas. Fertility and Sterility. 2003;80:300-304.
17. Gay V, Midgley Jr A, Niswender G, editors. Patterns of gonadotrophin secretion associated with ovulation. Federation Proceedings; c1970.
18. Haavisto A-M, Pettersson K, Bergendahl M, Perheentupa A, Roser J, Huhtaniemi I. A supersensitive immunofluorometric assay for rat luteinizing hormone. Endocrinology. 1993;132:1687-91.
19. Morley JE, Patrick P, Perry Hr. Evaluation of assays available to measure free testosterone. Metabolism Clinical and Experimental. 2002;51:554-9.
20. Shaikh AA. Estrone and estradiol levels in the ovarian venous blood from rats during the estrous cycle and pregnancy. Biology of Reproduction. 1971;5:297-307.
21. Wang Y-S, Chi K-H, Chu R-M. Cytokine profiles of canine monocyte-derived dendritic cells as a function of lipopolysaccharide- or tumor necrosis factor-alpha-induced maturation. Veterinary Immunology and Immunopathology. 2007;118:186-98.
22. Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for the measurement of antioxidant activity in human fluids. Journal of Clinical Pathology. 2001;54:356-61.
23. Kim S-K, Yoon Y-D, Park Y-S, Seo JT, Kim J-H. Involvement of the Fas-Fas ligand system and active caspase-3 in abnormal apoptosis in human testes with maturation arrest and Sertoli cell-only syndrome. Fertility and Sterility. 2007;87:547-53.
24. Kasibhatla S, Amarante-Mendes GP, Finucane D, Brunner T, Bossy-Wetzel E, Green DR. Acridine orange/ethidium bromide (AO/EB) staining to detect apoptosis. Cold Spring Harbor Protocols. 2006;2006:4493.
25. Cora MC, Kooistra L, Travlos G. Vaginal cytology of the laboratory rat and mouse: Review and criteria for the staging of the estrous cycle using stained vaginal smears. Toxicologic Pathology. 2015;43:776-93.
26. Gaytan F, Gaytan M, Castellano JM, Romero M, Roa J, Aparicio B, *et al*. KiSS-1 in the mammalian ovary: distribution of kisspeptin in human and marmoset and alterations in KiSS-1 mRNA levels in a rat model of ovulatory dysfunction. American Journal of Physiology-Endocrinology and Metabolism. 2009;296:520-31.
27. Iwabe T, Harada T, Sakamoto Y, Iba Y, Horie S, Mitsunari M, *et al*. Gonadotropin-releasing hormone agonist treatment reduced serum interleukin-6 concentrations in patients with ovarian endometriomas. Fertility and Sterility. 2003;80:300-4.
28. Haavisto A-M, Pettersson K, Bergendahl M, Perheentupa A, Roser J, Huhtaniemi I. A supersensitive immunofluorometric assay for rat luteinizing hormone. *Endocrinology*. 1993;132:1687-91.
29. Kim SK, Yoon YD, Park YS, Seo JT, Kim JH. Involvement of the Fas-Fas ligand system and active caspase-3 in abnormal apoptosis in human testes with maturation arrest and Sertoli cell-only syndrome. Fertility and Sterility. 2007;87:547-553.
30. Yeh KL, Hoffmann M, Hebling J, Nelson KA. Generation of 10μJ ultrashort terahertz pulses by optical rectification. Applied Physics Letters. 2007;90:8-20.
31. Wong AR, Clements A, Raymond B, Crepin VF, Frankel G. The interplay between the Escherichia coli Rho guanine nucleotide exchange factor effectors and the mammalian RhoGEF inhibitor EspH. mBio. 2012;3:9-20.
32. Kasibhatla S, Amarante-Mendes GP, Finucane D, Brunner T, Bossy-Wetzel E, Green DR. Acridine orange/ethidium bromide (AO/EB) staining to detect apoptosis. Cold Spring Harbor Protocols. 2006;2006:4493-4496.
33. Georgieva E, Stoyanova S, Turgai Mehmedov T, Tomov S, Velcheva I, Yancheva V. Main factors causing male infertility: A review. Ecology. 2023;15:8-15.
34. Zanfirescu A, Ungurianu A, Tsatsakis AM, Nițulescu GM, Kouretas D, Veskoukis A, *et al*. A review of the alleged health hazards of monosodium glutamate. Comprehensive Reviews in Food Science and Food Safety. 2019;18:111-134.
35. Xie Q, Kang Y, Zhang C, Xie Y, Wang C, Liu J, *et al*. The role of kisspeptin in the control of the hypothalamic-pituitary-gonadal axis and reproduction. Frontiers in Endocrinology. 2022;13:6-15.
36. Prashar V, Arora T, Singh R, Sharma A, Parkash J. Hypothalamic Kisspeptin neurons: integral elements of the GnRH system. Reproductive Sciences. 2023;30:802-822.
37. Rodrigues KC, Bortolatto CF, da Motta KP, de Oliveira RL, Paltian JJ, Krüger R, *et al*. The neurotherapeutic role of a selenium-functionalized quinoline in hypothalamic obese rats. Psychopharmacology. 2021;238:1937-1951.
38. Torrezan R, Malta A, de Souza Rodrigues WdN, Dos Santos AAA, Miranda RA, Moura EG, *et al*. Monosodium l-glutamate-induced obesity onset is associated with disruption of central control of the hypothalamic-pituitary-adrenal axis and autonomic nervous system. Journal of Neuroendocrinology. 2019;31:5-15.
39. Haddad M, Esmail R, Khazali H. Reporting the effects of exposure to monosodium glutamate on the regulatory peptides of the hypothalamic-pituitary-gonadal axis. International Journal of Fertility and Sterility. 2021;15:246-55.
40. Leisegang K, Henkel R, Agarwal A. Obesity and metabolic syndrome associated with systemic inflammation and the impact on the male reproductive system. American Journal of Reproductive Immunology. 2019;82:13-17.
41. Mandlik DS, Mandlik SK, Patel S. Protective effect of sarsasapogenin in TNBS induced ulcerative colitis in rats associated with downregulation of pro-inflammatory mediators and oxidative stress. Immunopharmacology and Immunotoxicology. 2021;43:571-583.
42. Hajihasani MM, Soheili V, Zirak MR, Sahebkar A, Shakeri A. Natural products as safeguards against

monosodium glutamate-induced toxicity. Iranian Journal of Basic Medical Sciences. 2020;23:416-430.
43. Rostami A, Vakili S, Koohpeyma F, Jahromi BN, Aghajari ZA, Mahmoudikohani F, *et al*. Ellagic acid effects on testis, sex hormones, oxidative stress, and apoptosis in the relative sterility rat model following busulfan administration. BMC Complementary Medicine and Therapies. 2022;22:1-14.
44. Liao R, Wood TR, Nance E. Superoxide dismutase reduces monosodium glutamate-induced injury in an organotypic whole hemisphere brain slice model of excitotoxicity. Journal of Biological Engineering. 2020;14:1-12.
45. Al-Nasser MN, Mellor IR, Carter WG. Is L-Glutamate toxic to neurons and thereby contributes to neuronal loss and neurodegeneration? A systematic review. Brain Sciences. 2022;12:577-590.
46. Kianifard D, Shoar SMM, Karkan MF, Aly A. Effects of monosodium glutamate on testicular structural and functional alterations induced by quinine therapy in rat: An experimental study. International Journal of Reproductive BioMedicine. 2021;19:20-29.
47. Voltan G, Cannito M, Ferrarese M, Ceccato F, Camozzi V. Vitamin D: an overview of gene regulation, ranging from metabolism to genomic effects. Genes. 2023;14:9-20.
48. Brennan E, Butler AE, Nandakumar M, Drage DS, Sathyapalan T, Atkin SL, *et al*. Association between organochlorine pesticides and Vitamin D in female subjects. Biomedicines. 2023;18:2-15.
49. Esposito F, Sansone L, Srivastava A, Cusano AM, Campopiano S, Giordano M, *et al*. Label-free detection of vitamin D by optical biosensing based on long period fiber grating. Actuators B: Chemical. 2021;347:5-15.
50. Ge R-S, Li X, Wang Y. Leydig cell and spermatogenesis. Molecular Mechanisms in Spermatogenesis. 2021;423:111-129.
51. Zhu Y, Yin Q, Wei D, Yang Z, Du Y, Ma Y. Autophagy in male reproduction. Systems Biology in Reproductive Medicine. 2019;65:265-272.
52. Morgante G, Darino I, Spanò A, Luisi S, Luddi A, Piomboni P, *et al*. PCOS physiopathology and vitamin D deficiency: biological insights and perspectives for treatment. Journal of Clinical Medicine. 2022;11:10-15.
53. Cowan S, Lim S, Alycia C, Pirotta S, Thomson R, Gibson-Helm M, *et al*. Lifestyle management in polycystic ovary syndrome–beyond diet and physical activity. BMC Endocrine Disorders. 2023;23:14-22.
54. Kim DH, Meza CA, Clarke H, Kim JS, Hickner RC. Vitamin D and endothelial function. Nutrients. 2020;12:8-18.
55. Edris K, Najafi Tazekand G, Farrocki F. Protective effects of vitamin D on testicular tissue, oxidative stress and apoptotic genes expression in rats treated with simvastatin. Journal of Advanced Biomedical Science Research. 2023;11:4088-4099.

**How to Cite This Article**
Gabr NM, Zeid AAA, Tahoon NM, Elnady M, Elnhas MM. Study the effect of vitamin D on monosodium glutamate induced testicular and ovarian toxicity in pubertal male and female albino rats. International Journal of Advanced Research in Medicine. 2024;6(2):12-24.