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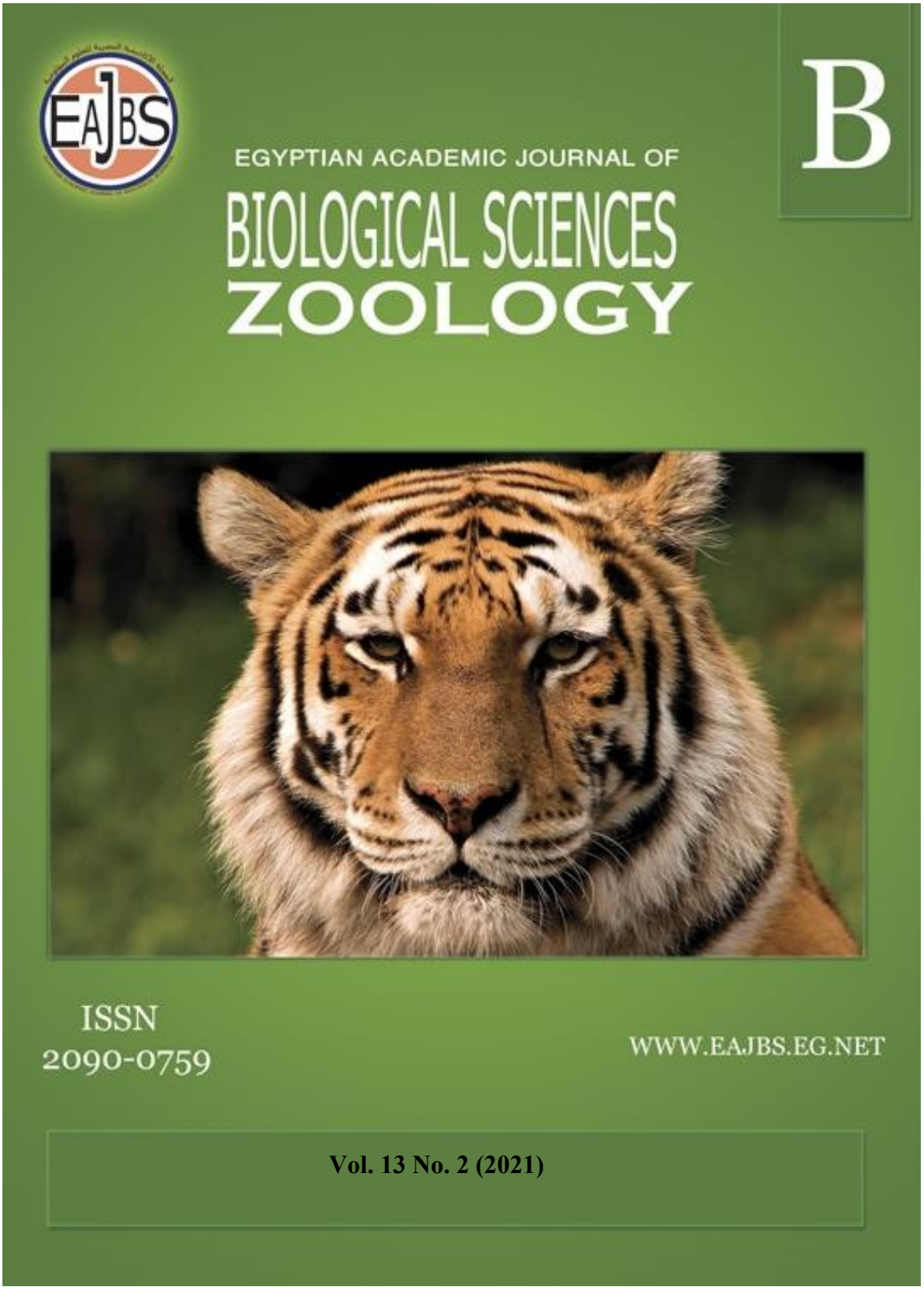

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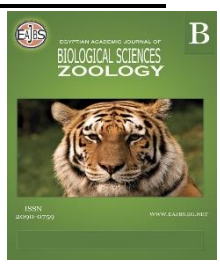

Possible Ameliorative Role of Ethanolic Ginger Extract (*Zingiber officinale*) against Testicular Toxicity Induced By Monosodium Glutamate In Albino Rats

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Monosodium glutamate (MSG), testicular alterations, food additive, Oxidative stress, Zingiber officinale.

ABSTRACT

The paper aims to investigate the protective effect of ethanolic ginger extract against Monosodium glutamate (MSG) induced reproductive disorders in male rats. Forty-eight male albino rats were separated into four experimental groups: normal control, ginger extract, MSG only, and MSG plus ginger extract groups. All experimental regimens were administrated orally for four weeks. Dissection was done after two and four weeks from the starting of the experiment. Body and testis weight, sperm abnormalities, biochemical investigations were recorded. Additionally, molecular, histopathological, and immunohistochemical techniques in testis tissue were performed. The present data shows a significant increase in body weight, sperm abnormalities, and serum total cholesterol, with a significant decline in testis weight, serum Follicle-stimulating hormone (FSH), Luteinizing hormone (LH), and testosterone levels after MSG administration. There was an upregulation in the expression of Bax protein whereas downregulation in the expression of B-cell lymphoma-2 (BCL-2) protein in the MSG group when compared with the normal control. Furthermore, testicular alterations were distinguished in the group treated with MSG only, along with enhanced caspase-3 and declined BCl-2 positive cells in testis tissue. While administration with ethanolic ginger extract showed an ameliorative effect contrasted with testicular alterations produced by Monosodium glutamate.

INTRODUCTION

Male infertility affects about fifty percent of sterile couples (Agarwal *et al.*, 2015). Oxidative stress is a recognized contributing factor in the sterility of males (Martin-Hidalgo *et al.*, 2019). Moreover, oxidative stress is a cellular incident that happens at the molecular level due to the disparity among free radicals and antioxidants which caused oxidative destruction of all cell components (Azab *et al.*, 2017)

Some of the associated factors for male reproductive dysfunction involve drug treatment, chemotherapy, poisons, and environmental reasons that can have a dangerous influence on spermatogenesis (Harchegani *et al.*, 2019). MSG is formed from H_2O,

sodium, and glutamate, and it's a chief food taste enhancer, which improves to overstate the inherent flavor of foods (Kayode *et al.,* 2020). Moreover, MSG is a subset of glutamate which is an important but non-vital amino acid that has an important role in human metabolism (Bera *et al.,* 2017). It may be used in packaged foods involving beef, milk, tuna, and vegetables. Furthermore, MSG consumption is associated with numerous effects on the brain, obesity, sex organs, and metabolism(Kazmi *et al.,* 2017). It has a harmful effect on the testis by producing a significant oligozoospermia as it raises sperm abnormalities in male rats (Krynytska *et al.,* 2019). The excessive usage of MSG may have adverse effects all over the body due to the formation of free radicals and increased oxidative stress(Hamza & Al-Baqami, 2019). Likewise, the formation of free radicals causes many hazards to the testis tissue and several cells in the body such as cells of the hypothalamic-pituitary-gonadal axis as well as Leydig and Sertoli cells(Mahidin *et al.,* 2018; Martin-Hialgo *et al.,* 2019). Recently, medicinal plants attracted attention for their antioxidant properties. Numerous researches stated that the defending effect of antioxidants arises from scavenging free radicals and retrieving the functions of body organs and reproductive functions (Seif *et al.,* 2017; Madboli & Seif, 2021). Ginger (*Zingiber officinale*) belongs to Zingiberaceae family, which is commonly utilized as a nutritional enhancement in beverages, and also in the treatment of many illnesses (Morakinyo *et al.,* 2010; Sharifi-Rad *et al.,* 2017). Many studies revealed that ginger has various activities such as immune-modulatory, antioxidant and anti-apoptosis (Hosseinzadeh *et al.,* 2017). Zingiber has a constructive influence on improved sperm numbers, motility, testosterone levels, and reproductive activities in rats (Odo *et al.,* 2020; Seif *et al.,* 2021). Consequently, this study has been conducted out to assess the defending effects of the ethanolic extract of ginger against the testicular alterations produced by MSG administration on male albino rats.

MATERIALS AND METHODS

Chemicals*:*

Mono-sodium glutamate (C5H9NO·Na) of 98% purity was acquired from Al-Dawlya chemicals company, Egypt. All substances and chemicals used were of analytical quality. Firstly, a working solution of MSG was prepared with dissolving 125 g of MSG powder in one liter of distilled H_2O, in the present study the selective dose was 2 mg/g/body weight, it was adjusted for animals as their weight.

Preparation Of The Ethanolic Ginger Extract:

Fresh roots of ginger were purchased from a local market in Cairo, Egypt. About 20 grams of ginger roots were washed carefully under tap water, then sliced into tiny pieces, allowed to dry on the stove, and crushed into fine powder by using a blender. In 80% ethanol for 48 h, the powder was subsequently soaked. Ginger extract was then filtrated, the obtained liquid was heated in the oven at 40 °C to evaporate the alcohol, concentrated and stored at 4 °C until usage (Akbari *et al.,* 2017).

Experimental animals*:*

From the animal house of the National Research Centre, Dokki, Giza, Egypt, 48 adult male albino rats of the genus (*Rattus rattus*) weighing about (180-200) g were purchased. Rats were maintained under the same conditions (23 ± 2 °C and 55 ± 5% humidity with a 12 h light:12 h dark cycle), also they were maintained to a standard diet and water available *ad libitum*. The experimental method and animals care were achieved under the experiments directed agreed by the Board of Animal Ethics in the National Research Centre (NRC), Dokki, Cairo, Egypt.

Study Protocol:

Rats were left for one week as an acclimatization period before treatment. The animals were separated into 4 experimental groups (12 animal/group). The first group served as the normal control group which was given distilled water. The second group was given Ginger Extract (GE) only at 50 mg/kg b.wt according to (Bordbar *et al.*, 2013), while the third group received MSG only at 2 mg/g b.wt according to (Igwebuike *et al.*, 2011). Finally, the 4th group was received GE at 50 mg/kg b.wt, and MSG at 2 mg/g b.wt. All experimental regimens were given orally to rats for 4 consecutive weeks daily, animals were dissected after two and four weeks from the beginning of the experiment.

Samples Collection:

After two and four weeks, the weight of each animal was documented, then all rats were autopsied through diethyl ether (Sigma, USA) inhalation anesthesia, after which animals were carefully dissected. Epididymis was removed for sperm abnormalities and testes weights were recorded. Blood was collected by cardiac acupuncture in centrifuge tubes left to clot and centrifuged at 3000 rpm for twenty minutes. then serum was collected and kept at −20°C till usage. All animals tests were collected for molecular and histochemical studies.

Sperm Head Abnormality Test:

Sperm-shape abnormalities were made according to the method described by Wyrobek(1978). In detail, caudal epididymis was sited in a Petri-dish containing one ml of NaCl (0.9 %). The epididymis was cut off into small pieces to allow the sperms to get out. Then, three drops of this solution were used to prepare the smears. The slides were left to dry in air overnight, then fixed in absolute methanol for 10 minutes and stained by eosin Y. A thousand sperm per rat were observed to establish the morphological abnormalities by oil immersion, according to Ekaluo et al.(2009). The percentages of sperm head abnormality were determined.

Total Cholesterol And Hormonal Estimation In Serum Samples:

Total cholesterol in serum samples was assessed calorimetrically by spectrophotometer at 580 nm according to the method described by (Naito & Kaplan, 1984). Moreover, levels of testosterone, FSH, and LH were done in serum samples by using (ELISA**)** kits purchased from Bio-Diagnostic Co. Dokki, Giza, as illustrated by (Morley et al., 2002; Rose, 1998; Tietz & Ash, 1995) respectively.

Western Blotting Technique:

Recognition of Bcl-2 and Bax protein expressions was carried out by western blot technique utilizing V3 Western Workflow Complete System (Bio-Rad Hercules, California). Briefly, proteins were obtained from testes tissue homogenates expending ice-cold radioimmunoprecipitation assay (RIPA) buffer enhanced by phosphatase and protease inhibitors (50 mmol/L sodium vanadate (Na_3VaO_4). 0.5 mM phenylmethylsulphonyl fluoride, 2 mg/mL aprotinin, and 0.5 mg/mL leupeptin), then centrifugated at 12,000 rpm for twenty minutes. Protein concentration for each sample was verified by Bradford assay. Equivalent volumes of protein 20-30 μg per sample were electrophoresed on the 10%SDS-PAGE gel as described by (Laemmli, 1970). After that, the gel was transferred onto polyvinylidene difluoride membranes (Pierce, Rockford, IL, USA) with a Bio-Rad Trans-Blot system. After transfer, the membrane was blocked for 1 h at room temperature with 5% (w/v) skimmed milk powder in Tris-buffered saline-Tween (TBS-T: 0.05 M Tris, 0.138 M NaCl, 0.0027 M KCl, 0.05–0.1% Tween (Sigma). Following blocking, the membrane was washed by TBS-T for 5 min, then allowed to incubate overnight at pH 7.6 at 4° C (with calm shaking) with primary rabbit polyclonal Bcl-2 antibody (dilution 1:500, PAS-27094), primary rabbit polyclonal Bax antibody

(dilution 1:2000, PAS-11378), and rabbit β-actin polyclonal antibody (dilution 1:1000, PAS 585271) supplied by (Thermo-scientific, Rockford, Illinois, USA). The next day, the membrane was washed 3 times with TBS-T (5min each time) and then incubated at 37ºC for 1 h in secondary goat anti-rabbit (HRP) IgG antibody (dilution 1:2000 cat#65-6120). The membrane was again exposed for a repeated wash three times with TBS-T. The intensity of bands was evaluated by ChemiDocTM imaging system with Image LabTM software version 5.1 (Bio-Rad Laboratories Inc., Hercules, CA, USA). The results were stated as random units after standardization by expression of the β-actin protein. Western blot analysis was conducted according to the method reported by (Radwan & Karam, 2020).

Histological and Immunohistochemical Investigation*:*

For histological studies: testes tissue specimens were fixed in formalin then inserted in blocks of paraffin. By utilizing rotatory microtome sections of 5 μm thickness was achieved and stained with Hematoxylin &Eosin. Fixation and staining were carried out as the method described by (Bancroft & Gamble, 2008).

Another set of sections was stained immunohistochemically using the method described by (Kiernan, 2007) for localization of BCL2; (B-cell lymphoma 2) and Caspase-3. Anti- BCL2 and CASP3 antibodies respectively were used to stain the cells in an Avidin–biotin-complex (ABC) immune-peroxidase procedure. The sections were incubated in 5% hydrogen peroxide (H_2O_2) (in methanol) solution for ten min to block endogenous peroxidase action and then incubated with primary Anti- BCL2 and CASP3 rabbit monoclonal antibody respectively (at a dilution of 1:50 in 1% bovine serum albumin solution) for one hour at room temperature. to eliminate unbound primary antibody slides were rinsed with phosphate-buffered saline at pH 7.4, then allowed to incubate with diaminobenzidine (DAB) chromogenic solution for five min at 25°C. After that, the sections were then counterstained with hematoxylin stain for fifteen-sec methods. Finally, slides were observed by microscope (Olympus BX51, Tokyo, Japan) and images were taken by a digital camera (canon power shot A 620).

Statistical Analysis:

The collected data were expressed as mean ± SEM and p-value ≤ 0.05 was thought statistically significant. The significance of differences between means of the control and all treated rats (*). One-way analysis of variance and Duncan's test were made for several assessments between the experimental groups. This was performed by using SPSS version 19.0 software (SPSS Inc., Chicago, IL)

RESULTS

Phenolic And Flavonoid Content:

The total flavonoid and phenolic compositions of the ethanolic ginger extract were 0.17 mg /g and 5.41 mg/g dried extract. The results in Table (1) revealed that animals treated with MSG (2 mg/g body weight) even for two or four weeks showed a significant increase in both body weight and head sperm abnormalities, with a significant decline ($p \leq 0.05$) was recorded in testis weight as compared to control group. While co-administration with ethanolic ginger extract causes a significant decline in body weight and head sperm abnormalities, while a significant increase in testis weight was noted as compared with animals received MSG only.

Biochemical Investigation:

The data given in Table (2) illustrated that animals in the MSG group revealed a highly significant ($p \leq 0.05$) reduction in serum levels of FSH, LH, testosterone, and total cholesterol activity after two and four weeks of exposure, as compared to animals in the

normal control group. In contrast, the group treated with both MSG and ethanolic ginger extract presented a significant ($p \leq 0.05$) rise in the levels of FSH, LH, testosterone, and total cholesterol concentration as compared to the MSG treated group on both 2nd and 4th weeks.

Table 1: Effects of Monosodium glutamate (MSG) and ethanolic ginger extract on body weight, testis weight and sperm abnormalities

Animal group	Body weight (g)		Testis weight (g)		Head sperm abnormalities (%)	
	2 weeks	4 weeks	2 weeks	4 weeks	2 weeks	4 weeks
Control	201.13±1.17	208.00±0.73	1.45±0.02	1.45±0.01	7.00±0.37	6.00±0.37
Ethanolic Ginger extract	200.33±1.40	195.83±2.18	1.56±0.01	1.55±0.01	5.83±0.60	5.67±0.80
MSG	232.17±1.66[a]	261.83±1.78[a]	1.24±0.02[a]	1.22±0.03[a]	13.50±0.81[a]	16.67±0.99[a]
MSG + Ethanolic Ginger extract	214.33±0.96[b]	220.83±1.37[b]	1.44±0.01[b]	1.43±0.02[b]	8.67±0.67[b]	7.50±0.76[b]

Values are expressed as mean ± SEM., n = 12

[a]: Significant change at $P \leq 0.05$ in comparison with control group.

[b]: Significant change at $P \leq 0.05$ in comparison with MSG group.

Table 2: Effects of Monosodium glutamate (MSG) and ethanolic ginger extract on serum level of Follicle stimulating hormone (FSH), Luteinizing hormone (LH), testosterone and total cholesterol.

Animal group	FSH (mIU/ml)		LH (mIU/ml)		Testosterone(ng/ml)		Total Cholesterol(mg/dL)	
	2 weeks	4 weeks	2 weeks	4 weeks	2 weeks	4 weeks	2 weeks	4 weeks
Control	5.81 ± 0.30	5.40± 0.26	47.03 ± 1.02	47.30 ± 0.29	2.43 ± 0.13	2.40 ± 0.17	220.18± 2.61	220.50 ± 1.20
Ethanolic Ginger extract	5.82 ± 0.25	5.92 ± 0.22	50.23 ± 0.12	51.10 ± 0.25	2.27± 0.14	2.35 ±0.13	205.62 ± 0.87	209.18 ± 0.50
MSG	3.62 ± 0.01[a]	2.92 ± 0.15[a]	29.90 ± 0.84[a]	20.27 ± 1.08[a]	0.83 ± 0.08[a]	0.54 ± 0.04[a]	118.02 ± 0.66[a]	109.65 ± 0.23[a]
MSG+ Ethanolic Ginger extract	4.07 ± 0.03[b]	5.30 ± 0.13[b]	44.87 ± 0.22[b]	46.55 ± 0.19[b]	1.18 ± 0.10[b]	2.05 ± 0.04[b]	163.37 ± 1.18[b]	178.18 ± 0.69[b]

Values are expressed as mean ± SEM., n = 12

[a]: Significant change at $P \leq 0.05$ in comparison with control group.

[b]: Significant change at $P \leq 0.05$ in comparison with MSG group

Expressions of Bax and BCL2 Proteins:

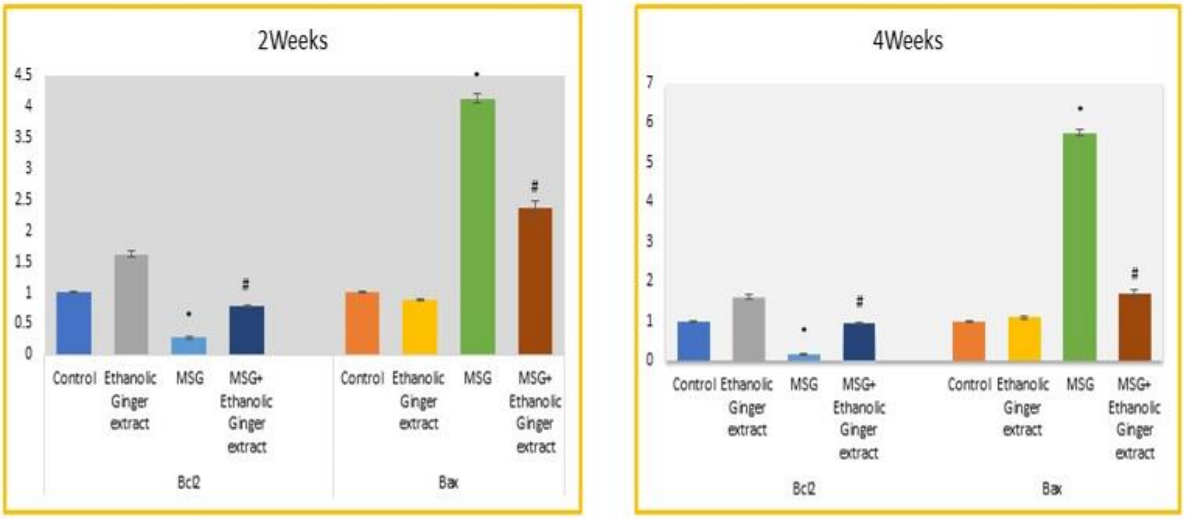
The expression of Bax protein in the MSG group at a dose of 2mg/g body weight even for 2 or 4 weeks was significantly higher than that in the normal control group ($p \leq 0.05$). In contrast, the group of animals that received MSG and ethanolic ginger extract at a dose (50 mg/kg body weight) showed a reduction in the level of Bax expression on both durations as compared with the MSG group. Administration of 2mg/g body weight of MSG for 2or 4 weeks led to a significant reduction in the level of expression of Bcl-2 protein than that in the normal control group, while animals which received the ethanol extract of ginger in combination with MSG showed an increased in the expression of the level of Bcl-2 protein ($p \leq 0.05$) compared to MSG group (Fig.1).

Histological and Immunohistochemical Examination of Testicular Tissue*:*

The testicular tissue of the MSG treated group displayed different alterations among damaged seminiferous tubules showing detachment of spermatogenic cells from the basal lamina, presence of vacuoles in spermatogonia and in between tubules. There were no sperms in the lumen of tubules that was occupied by acidophilic hyaline material and degenerated cells. The seminiferous tubules appeared to have irregular outlines basement membranes, empty interstitial connective tissue where the spaces between the tubules were occupied by acidophilic hyaline materials. Moreover, the testis tissue which was treated with MSG and ethanolic ginger extract showed improvement in seminiferous tubules with the presence of some vacuolated acidophilic material and partial separation as shown in Figure (2).

Immunohistochemical results: Testes sections showed a decline in the

expression of Bcl-2 protein in the rats treated with MSG only (Fig. 3) as compared with those of positive expression of the normal control and ethanolic ginger extract group. On contrary, Caspase-3 protein expression was increased in rats treated with MSG when compared with the control group. While the combination between MSG and ethanolic ginger extract showed less number of caspase-3-positive cells. An increase in BCL +ve cells is revealed in Figure (3).



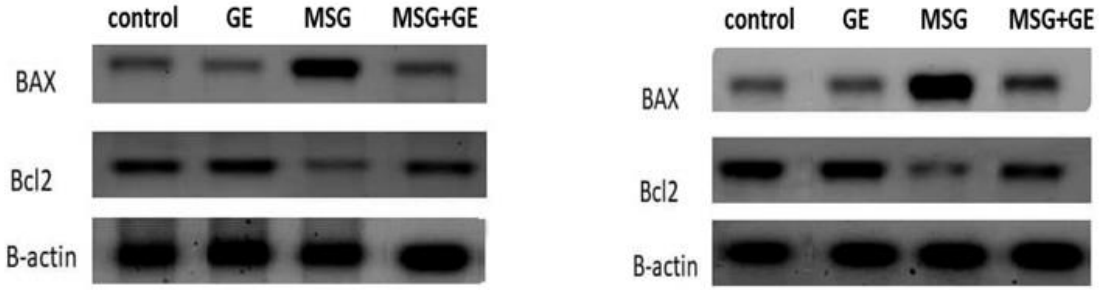


Fig.1: Western blot analysis showing the effect of ginger ethanol extract on protein expression of B-cell lymphoma-2 (Bcl-2) and BAX in testis tissues of albino rats exposed to Monosodium glutamate (MSG) for two and four weeks. Each value represents the mean ± SEM. * Significantly different from control group at $P \leq 0$ .05, # Significantly different from MSG group at $P \leq 0$ .05.

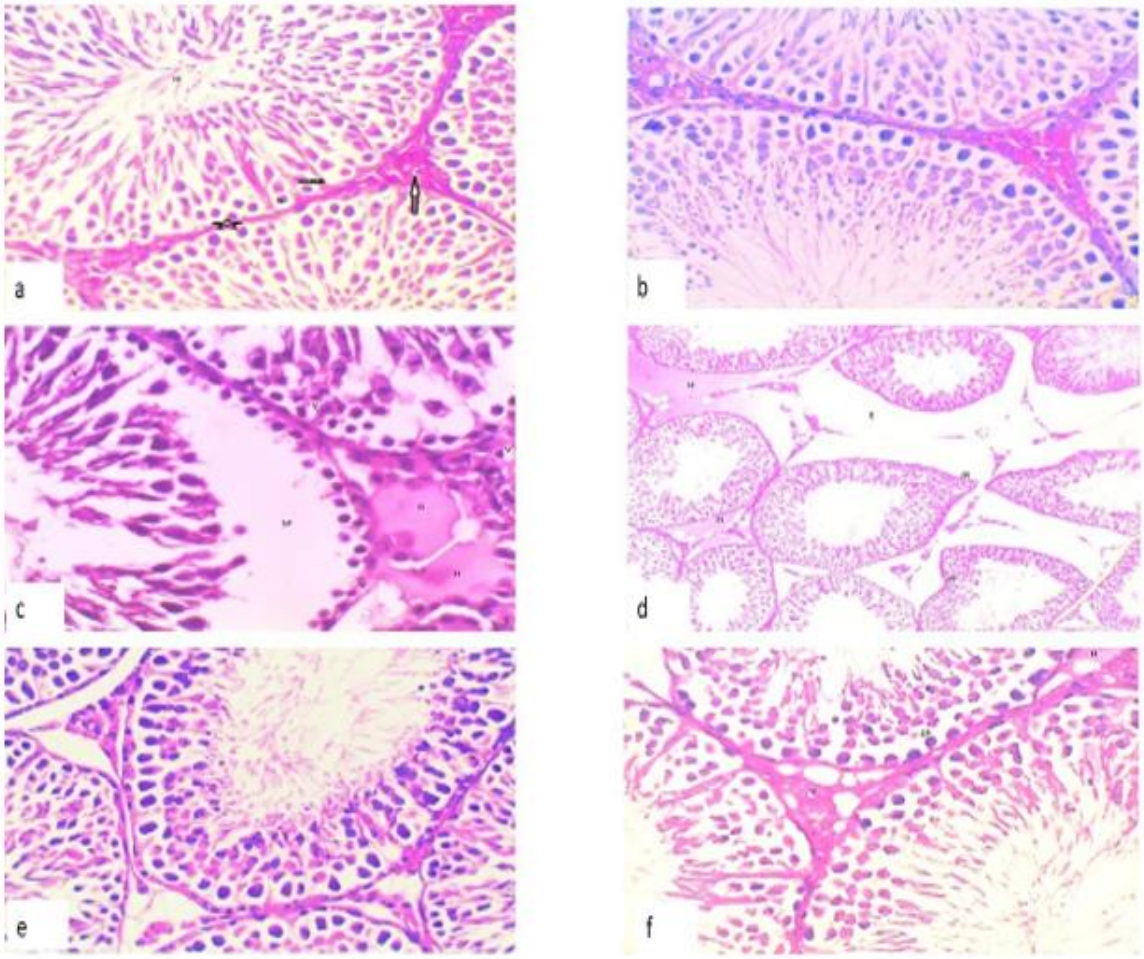


Fig.2: A photomicrograph of a rat testis of control group(a) showing the basement membrane(star),spermatogonia, primary spermatocytes and sperms in the lumen, Leydig cells (white arrow), Sertoli cells (black arrow) (H&E. X400). photomicrograph of a rat testis administrated ginger (b) showing similar structure to control. (H&E. X400) photomicrograph of a rat testis treated with Monosodium glutamate (MSG) (c) showing the basement membrane of some tubules showed separation of spermatogonia (SP) and presence of vacuoles(V) (H&E. X400). (d) showing the seminiferous tubules appeared to have irregular outlines (IR) basement membranes, empty (E) of interstitial connective tissue, the spaces between the tubules were occupied by acidophilic (H) hyaline materials. (H&E. X200). photomicrograph of a rat testis treated with MSG and ginger (e) showing enhancement in seminiferous tubules (H&E. X400) while appeared in some seminiferous tubules (f)presence of vacuolated acidophilic material and partial separation (H&E. X400).

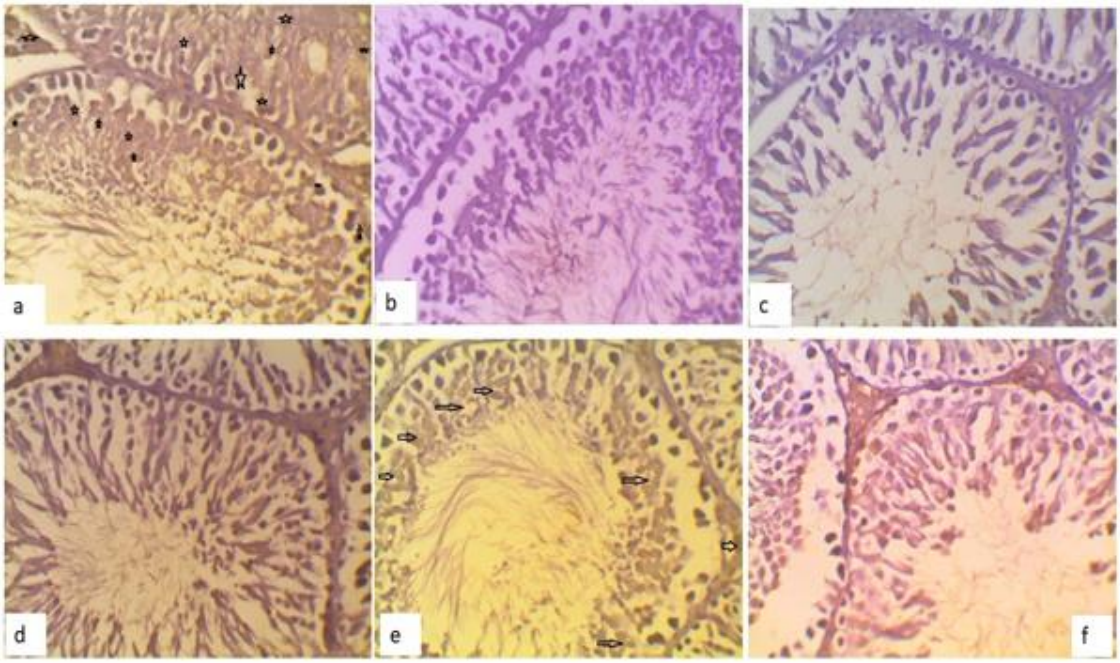


Fig. 3: Immunoexpression of B-cell lymphoma-2 (BCL2) (a-c) and caspase-3 (d-f) in testes of rats((immunoperoxidase,X400) :(a) control group showed high positive staining of BCL2 protein indicating the presence of BCL2 protein, (b) Monosodium glutamate (MSG) group showing -ve reaction of immunostaining BCL2 , (c) MSG and G showing weak diffuse reaction of immunostaining BCL2 , (d) control group showed negative reaction staining of Caspase-3 , (e) MSG group showing high diffuse of immunostaining Caspase-3 , (f) MSG and G showing weak diffuse reaction of immunostaining Caspase-3

DISCUSSION

Exposure to numerous environmental contaminants is able to produce a number of harmful health effects for animals and humans, involving toxicity in the reproductive system. One of the most widely used flavor enhancers all over the world is MSG (Seif *et al.,* 2021). Harmful effects of monosodium glutamate on male sterility were recorded through different ways of administrations, one of which is the oral exposure to MSG which was proved to destroy the nerve cells of the hypothalamus in mice and rats(El-Masry & Elsayed, 2019). This exposure also indicates a disturbance in the hypothalamic-pituitary-testis regulatory axis that is responsible for the steroidogenesis of Leydig cells (Al-Shahari & El-Kott, 2019). Additionally, MSG is responsible for the generation of oxidative stress by supporting the formation of lipid peroxidase and reactive oxygen species, which have adverse effects on the permeability of the plasma membrane (Hanipah *et al.,* 2018)**.**In the current research, the ability of MSG to cause testicular toxicity in male rats was investigated. Likewise, the possibility of ethanolic ginger extract to ameliorate the harmful effects of MSG on rats was assessed.

The current study showed a significant rise in body weight with a decrease in testis weight in the MSG-treated group. This was in accordance with many studies which established that a significant rise in body weight was observed with MSG administration (Alalwani, 2014; Shukry *et al.,* 2020)**.** The body weight rising might be related to the ability of MSG to enhance foods' palatability by having a favorable effect on the center of appetite (Egbuonu *et al.,* 2009; Alalwani, 2014) and increasing the chemosensory perception(Abd-Ella & Mohammed, 2016).

Sperm production can be disturbed by several causes, involving vitamin defect by free radical generation in the testis which is able to decrease sperm concentrations and lead to male sterility (Morinobu *et al.*, 2002). The data in this study revealed a significant rise in sperm abnormalities in the animals administrating MSG either for two or four weeks. These results are in harmony with Nayanatara *et al.* (2008) who reported that treatment with MSG is associated with a decrease in sperm count and an increase in the percentage of abnormal sperm. Likewise, Igwebuike *et al.* (2011) stated that a decline in caudal epididymal sperm count was found in the MSG-treated rats. Additionally, treatment with MSG leads to a decrease in testes and epididymis weights, sperm count and germ cell height, with a rise in sperm abnormalities (Ekaluo *et al.*, 2009)**.** Moreover, Rahmawati *et al.* (2020) showed that administration with MSG at the dose of 140 mg/b.wt/day for 21 consecutive days caused a reduction in sperm quality, Leydig and Sertoli cell counts.

MSG has neurotoxic effects which lead to a disturbance in the functions of the hypothalamic-pituitary-gonadal axis. Consequently, after monosodium intake, the testis is immediately affected by glutamate receptors and be able to disintegrate through the hypothalamus-pituitary gonadal axis(Alalwani, 2014). This leads to alterations in the levels of testosterone, follicle-stimulating hormone, and luteinizing hormone, following several alterations of the spermatogenesis process. In the current research, MSG administration led to significantly decreased serum levels of reproductive hormones. Gad *et al.* (2020) revealed that administration of rats with monosodium glutamate for six weeks produced a significant decline in serum of testosterone, LH and FSH hormones concentrations lead to a decline in gonadotropin-releasing hormone (GnRH) excretion by the hypothalamus. This was followed by a reduction in levels of FSH and LH hormones. Decreased FSH level will be followed by declined spermatogenesis, while reduction of LH will be followed by reduced testosterone level produced by Leydig cells in testis tissue (Dong & Robbins, 2015). In addition, the decrease in the level of FSH causes Sertoli cells to fail to produce androgen binding protein (ABP), this protein was necessary for transporting testosterone to seminiferous tubules for the maturation of spermatozoa.

Any androgen hormones disorder will consequently have adverse effects on the reproductive tissues (Hamza & Al-Harbi, 2014). The production of the Testosterone hormone includes two major steps: translocation of cholesterol to mitochondria as a precursor substrate and establishment of the pregnenolone by steroidogenic enzymes (Wang *et al.*, 2017). The present results demonstrated that MSG treated group showed a significant reduction in serum total cholesterol level in comparison to the normal control group (Ochiogu et al., 2015; Okoye *et al.*, 2016).

Western blot results showed that MSG administration to animals induced significant up-regulation of Bax and downregulation of Bcl-2 protein expressions in testis tissue during the whole examination period. Many reports supported these findings**,** Pavlovic *et al.*, (2009) confirmed that MSG administration in animals significantly caused a reduction in cell viability with significant down-regulation of Bcl-2 protein, whereas no difference was shown in the expression of Bax protein in rat thymocytes. Moreover, Shukry *et al.*, (2020) showed that MSG administration revealed significant rises in caspase-3, P53, and apoptotic protein (Bax), accompanied by a decline in anti-apoptotic protein (Bcl-2).

Numerous testicular histological alterations in the MSG-treated group were revealed in this study. This is in accordance with (El Wakf *et al.*, 2009). Furthermore, few numbers of spermatogenic cells with the absence of sperms were shown in the MSG group (AL-Sharkawy *et al.*, 2017; Sarhan, 2018). This was related to testosterone

inhibition which produced blocking of the spermatogenesis process. Current work showed pyknosis of cell nuclei that could reveal loss of functional proficiency of the cells as mentioned by Ortiz *et al*.(2006).

Moreover, the acidophilic hyaline material might be the consequence of lymphatic exudates from a degenerative lymphatic vessel or due to enhanced vascular permeability of crowded blood vessels(Salama *et al.,* 2003). It is perfectly recognized that B-cell lymphoma 2 (Bcl-2) protein is expressed in the inner mitochondrial membrane (Roset *et al.,* 2007). The partners of the Bcl-2 protein family are determined to manage the release of apoptosis-activating factors that the ratio of Bcl-2 to Caspase-3 controls cell survival or cell mortality as described by (Oltval *et al.,* 1993). Currently, Immunohistochemical examinations showed that Caspase-3 protein expression was found to be improved even though the expression of Bcl-2 protein was depressed in the experimental group treated with MSG only. Anbarkeh *et al.* (2019) showed that oral administration of MSG can lead to increase apoptotic changes in the germinal epithelium of the testicular tissue. Also, Abd-Ella & Mohammed (2016) revealed that Caspase-3 protein expression was found to be significantly increased whereas Bcl-2 protein expression was significantly depressed in the rats treated by MSG in testicular tissues through immunohistochemical studies, through oxidative stress mechanisms (Gad *et al.,* 2020; Jubaidi *et al.,* 2019).

The occurrence of excess glutamate because of the intake of monosodium affects the acute initiation of the glutamate receptors. Several reports have demonstrated that the presence of a glutamate system involving metabotropic (mGlu) and ionotropic glutamate receptors and transporters in various tissues (Pavlović *et al.,* 2007). Stimulation of mGlu5 receptors has the probability to generate intracellular Calcium ions (Ca^{2+}) waves inside the cells, which stimulates several of the reactions that perform a basic function in differentiation, permanence, and cell growth (Alalwani, 2014). Alternatively, calcium-dependent enzymes, such as proteases and endonucleases (caspases) become active and provide preliminaries for apoptosis when the level of Ca^{2+} in the cell rises or excessive amounts of calcium penetrate the organelles such as the endoplasmic reticulum, nucleus, and mitochondria (Rajaei *et al.,* 2005).

When damage occurs in testicular cells, it is restored by using enzymatic and non-enzymatic antioxidants that are currently in the cells, even though the repair is not wholly completed. Antioxidants from external supply can play an essential role in scavenging ROS and the survivability of the cell. Administration of natural antioxidants, especially from plant sources, can improve the adverse effects of ROS(Sharma & Singh, 2012). Concerning the protecting effects of Ginger, the present results showed that ethanolic ginger extract enhanced the testicular damage brought by MSG. This result was in harmony with Morakinyo *et al.* (2008) who revealed that *Zingiber officinale* extract avoids pro-fertility estates in male rats which could be due to its strong antioxidant belongings and androgenic actions.

Also, (Khaki *et al.,* 2009) showed ginger administration caused a significant enhancement in sperm percentage, viability, motility, and serum total testosterone. This indicated that ginger might be hopeful in enhancing sperm's healthy parameters. Additionally, Tende *et al.* (2013) confirmed that the aqueous extract of ginger might possess an androgenic activity. Recently, Afzali & Ghalehkandi (2018) suggested that ginger improved spermatozoa characteristics and semen hormone levels.

In the present study rats that were administrated with ethanolic ginger extract and MSG improved the reduction in serum total cholesterol and sex hormones levels in male rats, these results are in harmony with (Obisike *et al.,* 2020). Ginger has been previously reported to protect the male reproductive system from oxidative injury and

biochemical alterations produced by sodium arsenite (Seif *et al.,* 2021). On the other hand, ethanolic extract caused a significant up-regulation of Bcl2 and downregulation of Bax proteins expressions in testis tissue during the whole examination period. This was in harmony with (Zahedi *et al.,* 2012) who revealed that ginger exhibited DNA protection and strong antioxidant properties of ginger.

Additionally, Asl *et al.* (2013) suggested that ginger might reduce the apoptotic effects of 3-4, methylenedioxymethamphetamine (MDMA) in male rats hippocampus. Mukherjee *et al.,* (2015) indicated the extract of ginger avoids the advancement of phosphamidon (PHO) produced apoptotic signaling way which is introduced by ROS. Photomicrographs of testes tissue of rats that received MSG and ethanolic ginger extract showed regular and well-organized tubules with normal interstitial connective tissue and Leydig cells. This was in agreement with a study by El Wakeel *et al.*(2020) who reported that ginger improved and prevented the histological changes caused by MSG in testes of rats that were administered a combination of MSG and ginger. Ginger defeated reproductive toxicity and stimulated spermatogenesis through the enhancement of testosterone level, due to its antioxidant properties (Siddaraju & Dharmesh, 2007; Zahedi *et al.,* 2012). Ginger rhizome is contained several worthy antioxidant composites and volatile oils (An *et al.,* 2016). Likewise, our results have shown that the ethanolic extract of ginger contains great concentrations of polyphenols and flavonoids. Additionally, ginger extract has high antioxidant activities, which keep the equilibrium between ROS and antioxidant resistance enzymes in testis cells (Banihani, 2019) Consequently, it may lead to the enhancement of the biosynthesis of testosterone hormone, and improvement of the male reproductive functions in rats. Based on our result (Ozola *et al.,* 2019) reported that ginger includes numerous antioxidants involving vitamin C, flavonoids, and phenol elements, as well as (Seif *et al.,* 2021) indicated that ginger ethanol extract contains great concentrations of tannins and alkaloids and includes high levels of phenolic and flavonoid elements which displayed significant regained testis histopathological alterations.

In conclusion. Ginger has a protective and improving role against the effects of MSG on testicular tissue of rats and this was due to the great extent and reduced oxidative stress on testis tissue.

Authors' contributions :

The authors participated equally in all parts of the research.

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