PROTECTIVE EFFECT OF VITAMIN C AGAINST MONOSODIUM GLUTAMATE (MSG) INDUCED APOPTOSIS IN OVARIES OF ADULT FEMALE RATS

BY

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ABSTRACT

The reproductive system is very sensitive to different harmful environmental factors. A great danger is hidden in an increased use of different food additives like monosodium glutamate (MSG). Earlier studies have shown that higher doses of MSG induce oxidative damage which can result in apoptosis. Accordingly, this work was carried out to study the possible protective effect of vitamin C on monosodium glutamate induced ovarian toxicity in adult female albino rats. Sixty adult female albino rats were included in this study which were divided into four main groups. Group I served as control group (10 rats). Group II (10 rats) received 100mg/kg/day vitamin C. Group III (20 rats) was further subdivided as subgroup I (10 rats) that was treated with 100mg/kg/day MSG and subgroup II (10 rats) which was treated with 4gm/kg/day MSG. Group IV (20 rats) was further subdivided into two subgroups and received both MSG and vitamin C in their previous doses. All animals were treated orally by gastric tube for two months. Body weights for all studied groups were measured. Histopathological examination using H&E stain and caspase-3 immunostaining was carried out. The present work revealed that there was a significant increase in the body weight of MSG-treated animals (group III) compared to the other groups (I,II,IV). This increase was dose dependent and was more significant in 4gm/kg than in 100mg/kg MSG-treated animals. H&E stained sections revealed many atretic follicles and corpora lutea with few primary and secondary follicles in 100mg/kg MSG-treated group, while many atretic follicles and corpora lutea only without any developmental stages of the follicles were observed in 4gm/kg MSG-treated group. There was also marked increase in caspase-3 expression in 4gm/kg MSG treated group compared to other groups. Moreover, there was significant improvement in all studied parameters on administration of vitamin C concomitant with MSG which was more observed with low MSG dose (100mg/kg). The present work has demonstrated that oral administration of monosodium glutamate resulted in increased body weight of adult female rats and overt pathological lesions in their ovaries. These effects were dose dependent. Oral administration of vitamin C along with MSG provided a significant improvement in the pathological changes in ovaries of rats administrated MSG at low dose (100mg/kg) in comparison with high dose (4gm/kg) MSG treated group. Thus, vitamin C holds a promise as an agent that can potentially reduce MSG induced toxic effects in ovaries.

Key words: Vitamin C, monosodium glutamate, apoptosis, ovaries.
INTRODUCTION

Female infertility is a very real medical problem in developed and developing countries. The female reproductive system is very sensitive to different harmful environmental factors. Increased use of chemicals like food additives can seriously harm female fertility. One of these important additives is monosodium glutamate (MSG) (Bojanic et al., 2009).

Monosodium Glutamate (MSG) is a sodium salt of nonessential amino acid glutamic acid (Kondoh and Torii, 2008). Glutamate was reported to have many functions in the human body as a metabolic intermediate and protein constituent (Zai et al., 2009). It occurs naturally in virtually all foods, including meat, fish, poultry, breast milk and vegetables, with vegetables tending to contain proportionally higher levels of free glutamate (as MSG). Today, MSG is widely used as a popular flavoring agent in canned foods, processed meat, soups and similar products (Pavlovic et al., 2006).

In the early part of the twentieth century, MSG was extracted from seaweed and other plant sources; today it is produced in many countries around the world through a natural fermentation process using molasses from sugar cane or sugar beets, as well as starch and corn sugar (Walker and Lupien, 2000).

In 1988, Joint FAO/WHO expert committee on food additives has recognized MSG as generally safe for human consumption (Garattini, 1974). However, the safety of MSG usage has generated such controversial argument. MSG has been alleged to cause many ills. It was suggested to trigger symptoms, which were referred to collectively as “Chinese Restaurant Syndrome” consisting of numbness at the back of the neck and arms, weakness and palpitations (Geha et al., 2000).

However, high doses produced neuroendocrine abnormalities (Moreno et al., 2005), neurodegeneration, neurotoxicity (Chaparro-Huerta et al., 2002) and oxidative damage with excess generation of ROS in different organs (Farombi and Onyema, 2006 and Pavlovic et al., 2007). Excess generation of ROS in cells is known to damage DNA, lipids, and proteins resulting in several biological effects, ranging from alterations in signal transduction, gene expression, mutagenesis, and apoptosis (Rollet-Labelle et al., 1998 and Kannak and Jain, 2000).

It was found that MSG altered the activity and sensitivity of rat hypothalmo-pituitary-adrenocortical axis and thus changed several endocrine functions in neonatally treated rats (Seo et al., 2010).

Vitamin C has been established biochemically as an antioxidant which mops
The animals were divided into four main groups:

**Group I:** (control group): included 10 rats that received 0.2ml of distilled water (the diluting vehicle for MSG and vitamin C).

**Group II:** (Vitamin C treated animals): included 10 rats that received vitamin C in a dose of 100 mg/kg/day, prepared as 20mg/0.2ml solution in distilled water (Obochi et al., 2009).

**Group III:** (MSG treated animals): included 20 rats that further subdivided into two subgroups (ten rats each).

**Subgroup (i):** received MSG in a dose of 100mg/kg/day, prepared as 20mg/0.2ml solution in distilled water (Obochi et al., 2009). The average human daily intake of MSG is estimated to be 0.3-1.0 gm in industrialized countries (Geha et al., 2000). Dose of 100mg/kg day MSG used for rats in this group was equal to the average intake in human. It could be calculated by the use of the formula of dose translation based on body surface area (Reagan-Shaw et al., 2008).

**Subgroup (ii):** received MSG in a dose of 4 gm/kg/day, prepared as 800mg/4ml solution in distilled water (Monno et al., 1995). This is the subchronic MSG dose, and has been widely used in studying up free radicals produced in the body and shows the ability to scavenge superoxide, hydrogen peroxide, and hydroxyl radicals (Young and Woodside, 2001).

In view of the recorded hazardous effects of MSG, the present study was designed to evaluate histopathological and immunohistochemical changes in the ovaries of MSG treated female rats and the potential protective role of vitamin C.

**MATERIAL & METHODS**

**Chemicals:**

Monosodium glutamate was obtained in the form of L- Glutamic acid Sodium Salt 500 gm (Eastrin Fine Chemicals. LTD).

Vitamin C was obtained in the form of L- ascorbic acid 100 gm (Universal Fine Chemicals PVT. LTD India A.R. grade).

Animal management: Sixty healthy adult female albino rats with an average weight ranged from 150 to 200g were used in this study. They were housed in clean properly ventilated cages under the same environmental conditions with free access to food and water throughout the whole period of the experiment. All procedures on animals followed guideline for work on experimental animals approved by Ethical Committee of Faculty of Medicine in Tanta.
MSG-induced toxic reactions in experimental animals (Diniz et al., 2004; Farombi and Onyema, 2006). Moreover, this dose is 1/4 LD50 in rats as LD50 in rats and mice is 15,000-18,000 mg/kg body weight (Walker and Lupien, 2000).

**Group IV:** (Protective group): included 20 rats that were further subdivided into two subgroups (ten rats each):

Each subgroup received vitamin C concomitantly with MSG (at the same dose and duration of their correspondents).

The drugs and their vehicle were given orally by gastric tube once daily for two months.

Body weight was recorded at the beginning of the experiment for all animals of the studied groups and after 2 months of MSG and/or vitamin C treatment before they were sacrificed.

**Tissue Processing:**

For histological examination: At the end of the study, all rats were anaesthetized with diethyl ether inhalation then sacrificed by cervical dislocation. A median longitudinal incision was performed in the lower abdomen; the ovary was identified by its connection to the free end of the fallopian tube, which was connected to the uterus, and then a bilateral oophorectomy was performed. Each ovary was divided into small pieces; one was immediately fixed in Bouin’s solution for the paraffin sections and light microscopic study. After paraffin embedding, 5µ thick sections were cut and stained with H&E. The sections were studied for qualitative assessment (Bancroft and Gamble, 2002).

**For caspase-3 immunohistochemical study:** formalin-fixed, paraffin-embedded ovarian sections were de-paraffinized, hydrated and then treated with 0.2% hydrogen peroxide in phosphate buffer solution (PBS) to block endogenous peroxidase for 30 min. The sections were then incubated overnight in a humid chamber with the primary anti-rat antibody against caspase-3 (diluted 1:100), (New Markers, Lab Vision, Fremont California, USA). The sections were then rinsed three times in PBS and incubated with goat anti rabbit peroxidase-conjugated secondary antibody (Peroxidase-labelled streptavidin) for one hour at room temperature and rinsed again three times in PBS. The immune-reactivity was visualized by applying streptavidin peroxidase as 3,3 Diaminobenzidine (DAB)-hydrogen peroxide as a chromogen. Counterstaining was performed by haematoxylin. Positive caspase-3 cells have dark brown granules in their cytoplasm. For negative control, the primary antibody was replaced by phosphate buffer solution (Stirling, 1994).
Morphometric study

The morphometric measurements were carried out using the Leica Qwin 500 (Leica, London, UK) image analyzer computer system (Central Laboratory, Faculty of Medicine, Tanta University). The caspase index was scored (from 0 to 4+) after having examined ten high-power fields, from each slide of all animals of each group, to count the percentage of caspase-3 positively stained cells. A score of (0) indicated that there was 0-10% positive cells, (1+) indicated 11-25% positive cells, (2+) indicated 26-50%, (3+) indicated 51-75% and (4+) indicated more than 75% positive cells. Immuno-reactivity in more than 10% of cells was considered positive i.e. score equal to or above +1 (Dharmapatni et al., 2009).

Statistics:

Data were expressed as means ± standard deviation. Anova test was used for comparison among different groups in quantitative data. Schefte test was used to compare each group versus another. P<0.05 was considered significant.

RESULTS

Table (1) and figure (1) show the data of the initial and final body weights of adult female rats of all studied groups. There was no significant difference in the initial body weights in all studied groups.

The mean values of the final weight of MSG- treated rats (group III) were significantly increased compared to other groups (I,II,IV) while administration of vitamin C concomitantly with MSG in group IV significantly reduced this increase. Moreover, the mean values of the final body weights in 4gm/kg MSG-treated rats (338.3 ± 16.53) were significantly higher than the mean values in 100mg/kg MSG-treated rats (296 ± 16.53). There was significant reduction in final body weight in 100mg/kg MSG-vitamin C treated animals (256±19.63) compared to 4gm/kg MSG-vitamin C treated group (277.2±16.18).
Table (1): Mean values of initial and final body weights (gm) among all studied groups (each group 10 rats).

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n= number  B.W= body weight  *significant P<0.05   **highly significant P<0.001
Histopathological Findings:

A. Ovaries of Control and vitamin C treated Groups (I&II): The ovarian sections of both groups showed an outer covering of a single layer of cuboidal to columnar germinal epithelium. Enclosed within this, the ovarian parenchyma showed an outer, highly cellular zone, the cortex with number of follicles at different stages of maturation (Fig.2,4). Some capillaries were extending from the medulla to the cortical area between the follicles. The stroma of the ovary was cellular with fibroblasts like cells with ellipsoidal nuclei and forming a capsular zone (theca). The follicles were limited by a thecal zone. The primordial follicle consisted of a large spherical oocyte enveloped by a single layer of squamous epithelium (follicular cells). Nucleus of the ova was pale stained with eosinophilic cytoplasm, while primary follicle having a cuboidal lining with big ova inside (Fig.2). There were many secondary follicles with formation of cavities within the interstices of the follicular cells.

In mature follicle, the oocyte was situated at one pole of the follicle being encompassed by cumulus oophorus cells (Fig.3). There were several mature (Graffian) follicles occupying the cortex. There were many corpora lutea in most of the ovaries. The cells were larger, paler, arranged in clusters and separated by minimum amount of connective tissue.
B. Ovaries of Group (III) (MSG treated animals): Sections from the ovaries of both subgroups showed that the germinal epithelium was cuboidal to low columnar type. The appearance of the ovarian stroma and the vascular network were similar to that of control and vitamin C treated groups. Furthermore, the ovaries of the female rats treated orally by MSG in a dosage of 100mg/kg contained many atretic follicles and corpora lutea with few primary and secondary follicles (Fig.5), while those of female rats treated orally by MSG in a dosage of 4gm/kg contained many atretic follicles and corpora lutea only without any other developmental stages of the follicles (Fig. 6,7).

C. Ovaries of Group (IV) (Protective group): Ovarian sections from both subgroups showed that the germinal epithelium was cuboidal to low columnar type. The appearance of the ovarian stroma and the vascular network were similar to that of groups I and II. The ovaries of the female rats treated orally by 100mg/kg MSG and 100mg/kg vitamin C showed several follicles at different stages of development including: primary follicles, secondary follicles, Graffian follicles, atretic follicle and corpus luteum (Fig.8), while those of female rats treated orally by 4gm/kg MSG and 100mg/kg vitamin C showed few developmental stages of follicles as Graffian follicle, atretic follicles and corpus luteum (Fig.9).

Immunohistochemical Findings:

A. Ovaries of Control and vitamin C treated Groups (I&II): The ovarian sections showed healthy ovarian follicles with (1+) cytoplasmic immune reaction for caspase-3 (Fig.10,11).

B. Ovaries of Group (III) (MSG treated animals): The ovarian sections of rats treated with 100mg/kg MSG showed atretic follicles with (3+) immunostaining for caspase-3 (Fig.12), while sections of rats treated with 4gm/kg MSG showed atretic ovarian follicles with (4+) caspase-3 immunoreaction (Fig.13).

C. Ovaries of Group (IV) (Protective group): Ovarian sections in rats treated with 100mg/kg vitamin C concomitantly with 100mg/kg MSG showed healthy ovarian follicles with (2+) cytoplasmic immune reaction for caspase-3 (Fig.14), while sections of rats treated with 4gm/kg MSG showed atretic ovarian follicles with (4+) caspase-3 immunoreaction (Fig.15).

As regards the statistical analysis, the frequency of caspase-3 positive cells was significantly increased in MSG treated group (group III) in comparison with control rats (group I) and vitamin C treated rats (group II) (p<0.05). Compared to group III (MSG treated rats), group IV (MSG- vitamin C treated rats) showed significant decrease in caspase-3 positive
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cells (p<0.05). Rats treated with 4gm/kg showed that caspase-3 immuno-reactivity was significantly increased in comparison with 100mg/kg MSG treated animals (3.8±0.42 vs 2.7±0.48). Moreover, 100mg/kg MSG- vitamin C treated rats showed significant decrease in caspase-3 immuno-reactivity compared to 4gm/kg MSG- vitamin C treated rats (1.6±0.51 vs 2.5±0.52, table 2 and figure 16).

Table (2): Mean score values of intensity of cytoplasmic immune reaction for caspase-3 (each group 10 rats).

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n= number *significant P<0.05 **highly significant P<0.001
Fig. (2): A photomicrograph of an ovarian section of a control rat showing cortical and medullary stroma containing capillaries and several follicles at different stages of development, P: primary follicles, SF: secondary follicles, GF: Graafian follicle, AF: atretic follicle and corpus luteum (*H&E x100*).

Fig. (3): A photomicrograph of an ovarian section of a control rat showing a healthy mature ovarian follicle containing an oocyte surrounded by granulosa cells (arrow) (*H&E x400*).

Fig. (4): A photomicrograph of an ovarian section of an adult female rat of the vitamin C treated group showing blood capillaries and several follicles at different stages of development, P: primary follicles, SF: secondary follicles, GF: Graafian follicle, AF: atretic follicle and corpus luteum (*H&E x100*).

Fig. (5): A photomicrograph of an ovarian section in an adult female rat treated orally by MSG in a dosage of 100mg/kg showing many atretic follicles (arrows) and corpora lutea with few primary follicles (PF,arrow heads) and secondary follicles (SF) (*H&E X200*).
Fig. (6): A photomicrograph of an ovarian section of an adult female rat treated orally by MSG in a dosage of 4gm/kg showing many atretic follicles (arrows) and corpora lutea (CL) *(H&E x100).*

Fig. (7): A photomicrograph of an ovarian section of an adult female rat treated orally by MSG in a dosage of 4gm/kg showing an atretic ovarian follicle with thin, disrupted and loosely attached granulosa cells and detached oocyte (O) *(H&E x400).*

Fig. (8): A photomicrograph of an ovarian section of an adult female rat treated orally by 100mg/kg MSG and 100mg/kg vitamin C showing several follicles at different stages of development, P: primary follicles, SF: secondary follicles, GF: Graafian follicle, AF: atretic follicle and corpus luteum *(H&E stain X200).*

Fig. (9): A photomicrograph of an ovarian section of an adult female rat treated orally by 4gm/kg MSG and 100mg/kg vitamin C showing Graafian follicle (arrow), atretic follicles (arrow heads) and corpus luteum *(H&E stain X200).*
Fig. (10): A photomicrograph of an ovarian section of a control rat showing a healthy ovarian follicle containing an oocyte (O) with (1+) cytoplasmic immune reaction for caspase-3 (IHCx400).

Fig. (11): A photomicrograph of an ovarian section of vitamin C treated group showing a healthy ovarian follicle (arrow head) and secondary follicle (arrow) with (1+) cytoplasmic immune reaction for caspase-3 (IHCx400).

Fig. (12): A photomicrograph of an ovarian section of an adult female rat treated orally by MSG in a dosage of 100mg/kg showing an atretic ovarian follicle with (3+) immuno-staining for caspase-3 (IHCx400).

Fig. (13): A photomicrograph of an ovarian section of an adult female rat treated orally by MSG in a dosage of 4gm/kg showing an atretic ovarian follicle with (4+) immuno-staining for caspase-3 (IHCx400).
Fig. (14): A photomicrograph of an ovarian section of an adult female rat treated orally by 100mg/kg MSG and 100mg/kg vitamin C showing a healthy ovarian follicle with (2+) cytoplasmic immune reaction for caspase-3 (IHCx400).

Fig. (15): A photomicrograph of an ovarian section of an adult female rat treated orally by 4gm/kg MSG and 100mg/kg vitamin C showing an atretic ovarian follicle (arrow head) with (3+) cytoplasmic immune reaction for caspase-3 (IHCx400).

Fig. (16): Mean score values of caspase-3 immunoreactivity among all studied groups
**DISCUSSION**

A common example of one of the newly added chemicals used in our foods is monosodium glutamate (MSG). Daily dietary intake of man from reasonably all possible sources did not exceed more than 0.7gm i.e. 0.01 mg/gm of body wt. in an average adult (Diniz et al., 2005).

A number of reports showed induction of oxidative stress in different tissues of experimental animals after administration of chronic doses of MSG (Singh et al., 2003; Diniz et al., 2004; Farombi and Onyema, 2006 and Onyema et al., 2006).

Another mechanism of MSG toxicity is the changed permeability of neural membrane for calcium. The toxic effect of MSG on female reproductive system has been attributed to its direct effect on nuclei of hypothalamus for years. Recent studies have shown that glutamate receptors play very important role in pathogenesis of disorders induced by MSG. Glutamate receptors are present in different tissues: hypothalamus, heart, lungs, liver, kidneys, endocrine system, ovaries, uterus, etc. (Gill et al., 2008).

There was a significant increase in the body weight of MSG treated rats compared with control and vitamin C treated animals. Furthermore, this increase in the body weight was more significant in 4gm/kg than in 100mg/kg MSG- treated animals.

Egbuonu et al. (2010) reported that administration of monosodium glutamate has been associated with increased body weight. On the other hand, Diniz et al. (2005) observed that the grown up pups of the MSG treated group gained more weight than that of the control animals which might be due to higher voluntary intake of the food induced by the flavour enhancing food additive. This corroborates the fact that MSG causes an increase in appetite and thereby leading to increase in weight and obesity (Yeomans et al., 2008). Moreover, Bursey et al. (2011) and He et al. (2011) reported association of MSG with obese persons in an obesity epidemic.

Several lines of evidence indicate that treatment with MSG induced decrease in the levels of dopamine, epinephrine, norepinephrine and serotonin and their primary metabolites in some brain regions (Nakagawa et al., 2000 and Lombardi et al., 2004). These changes are associated with increase in body weight (Camihort et al., 2005 and Moreno et al., 2006). It was suggested that the increase in weight of MSG treated rats may be attributable to the destruction of the ventromedial nucleus (the satiety center) and the lateral hypothalamic areas (the food intake center) in the hypothalamus (Ganong, 1990 and
Chen et al. (2013) had reported that MSG treated rats display typical symptoms of the metabolic syndrome, i.e., excessive abdominal obesity, hypertriglyceridemia, hyperinsulinemia, insulin resistance, and hepatic steatosis, but with lower food intake. Experimentally, hypothalamic obesity is frequently used and can be induced in the neonatal period through the systemic administration of monosodium glutamate “MSG” (Sukhanov et al., 1999 and de Mello et al., 2001).

In the present study, histopathological examination of the ovaries showed that those from MSG treated groups were pathologically affected as compared to ovaries of control and vitamin C treated groups. They contained many atretic follicles and corpora lutea. Moreover, these effects were dose dependent as the ovaries of MSG treated rats in a dosage of 100mg/kg contained few primary and secondary follicles, while those of female rats treated in a dosage of 4gm/kg contained many atretic follicles and corpora lutea only without any other developmental stages of the follicles.

Olney et al. (1972) registered sterility in female mice, which were treated with MSG during the first ten days of their neonatal life. There were twice as more atretic follicles in their ovaries than in animals of the control group. The wall of their uterus was very thin.

Miskowiak et al. (1999) and Diniz et al. (2004) had observed an increased number of primordial follicles and decreased number of Graffian follicles of the ovaries of treated animals. Also, there was decrease in the endometrial thickness of females received perinatal injections of MSG, as compared to the controls.

Additionally, Bojanic et al. (2009) showed cystic degeneration, many atretic follicles with no corpora lutea and fibrotically changed stroma. Arteriolar hyalinosis was found too in ovaries of MSG-treated rats.

On the other hand, Pizzi et al. (1977) reported no histological changes despite observing reduced female fertility. Similarly, Rodriguez-Sierra et al. (1980) reported small follicles with no corpora lutea. While, Miskowiak et al. (1999) reported increased number of primordial follicles and simultaneous reaction in Graffian follicles in MSG treated animals.

However, Das and Ghosh (2011) noticed that MSG treated animals (2mg/gm s.c starting from the 2nd day of neonatal life for 5 days) showed similar stromal architecture compared to controls. There was increase in primary follicles, similar primordial, secondary and Graffian and
few atretic follicles compared to controls which was explained by arrest in the developmental stages due to possible disruption of pituitary-adrenal axis.

The results of Eweka and Om’Iniabohs (2011) showed some cellular hypertrophy of the theca folliculi with complete distortion/destruction of the basement membrane separating the theca folliculi from the zona granulosa. Degenerative and atrophic changes were observed in the oocyte and zona granulosa. These changes were more pronounced in animals received 0.08mg/kg of MSG.

Programmed cell death or apoptosis is an essential component of normal reproductive function and development in the ovary. Most importantly, apoptosis occurs as a result of the exhaustion of the oocyte reserve, either directly through germ cell death or indirectly through follicular atresia. In this respect, apoptosis has been proposed to be the major mechanism that determines the female reproductive life span (Slot et al., 2006).

Apoptosis is the cellular mechanism of ovarian follicular atresia and luteal regression that is triggered by the activation of a proteolytic cascade of cysteine aspartate specific proteases (caspases) (Devine et al., 2000). Caspases exert their action at several levels of signaling during apoptosis, ranging from responding to external factors at the trans-membrane receptor to proteolytic breakdown of cellular components. Among the group of 11 caspases identified, caspase-3 has been recognized as a central player in the execution of apoptosis and may provide a target for regulating cell death (Kumar et al., 2004).

During ovarian follicular development, only limited numbers of follicles are selected for ovulation, whereas the rest undergo atresia at various stages of development (Yu et al., 2004). Yang and Rajamahendran (2000) have suggested that the degenerative changes associated with atresia appear initially in the granulosa cell (GC) layer. The death of GC leads to almost total destruction of the GC layer lining the inner follicular wall and induces the atresia of the follicles (Jolly et al., 1997). Hussein (2005) have reported that the death of GC during follicular atresia in ovaries occurs by apoptosis.

In the present study, there was a significant over-expression of caspase-3 in MSG-treated animals compared to control and vitamin C treated groups. Increased caspase-3 expression in MSG-treated rats was dose dependent. It was more marked in animals treated with 4gm/kg that indicated more liability for cell death with higher dose. These results could be explained on the basis that the active caspase-3 was more predominantly present in atretic fol-
Antioxidants have been reported to play a significant role in the protection against lipid peroxidation (Cordier et al., 2013). Vitamin C (ascorbic acid) has a considerable antioxidant activity: it scavenges reactive oxygen species and may, thereby, prevent oxidative damage to the important biological macromolecules, such as DNA, proteins, and lipids (Konopacka, 2004).

In the present study, there was significant decrease in the body weight in animals treated with vitamin C along with MSG compared to those treated with MSG only. Moreover, this ameliorating effect of vitamin C was more significant in rats treated with 100mg/kg than those treated with 4gm/kg.

There was also marked improvement in the pathological changes occurring in rats treated with 100mg/kg MSG and 100mg/kg vitamin C as cross sections showed several follicles at different stages of development, while the improvement occurred in 4gm/kg MSG and 100mg/kg vitamin C treated group was less as few developmental stages of follicles as Graffian follicle, atretic follicles and corpus luteum were seen. This was explained by the fact that vitamin C is usually concentrated in granulosa cells, theca cells, luteal cells and the oocyte and it has been associated with fertility (Murray et al., 2001).

Furthermore, El-Meghawry et al. (2013) reported that oral therapeutic administration of vitamin C prior to the administration of MSG showed an improvement in pathological changes in comparison with the group of rats taking MSG only due to its ability to protect against the toxicity of MSG through its cyto-protective effect.

These results coincided with those of Okolie and Iroanya (2003) who concluded that vitamin C markedly reduced the histopathological degeneration of tissues by toxic agents.

**CONCLUSIONS**

It could be concluded from the present study that MSG may have some deleterious effects on the oocytes of the ovaries of adult albino rats at high doses and may contribute to the causes of female infertility. Moreover, concomitant administration of vitamin C and MSG proved to have protective role especially in low dose MSG treated rats compared to those treated with high doses.

**RECOMMENDATIONS**

We recommend further long term studies to determine whether MSG induced toxic effects are reversible or not and the
potential protective effects of different anti-oxidants.

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الدور الوقائي لفيتامين سي ضد موت خلايا المبيضين المبرمج الحادث
من تعاطي جلوتانات أحادية الصوديوم في إناث الجرذان البيضاء البالغة

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إن الجهاز التناسلي من أكثر أجهزة الجسم حساسية لمختلف العوامل البيئية الضارة. ويمكن منظور الأكبر في الاستخدام المتزايد لضادات الطعس المختلفة مثل الجلوتانات أحادية الصوديوم. وقد أشارت الدراسات المكربة إلى أن الجرعات العالية من الجلوتانات أحادية الصوديوم يبحث على حدود الإجهاد التانكيكي، والذي يؤدي إلى موت الخلايا المبرمج. لذلك ها هذا البحث لدراسة التأثير الوقائي المحتمل لفيتامين سي على نسبة الجلوتانات أحادية الصوديوم على مبيض الجرذان الإناث البالغين البالغة.

وقد أجريت هذه الدراسة على 10 نسخة من إناث الجرذان البضائع البالغة، وتسميتها إلى أربع مجموعات رئيسية. المجموعة الأولى استعملت كمجموعة ضابطة واحترى على 10 جرذان، المجموعة الثانية (10 جرذان) وتم إعطائها 100 ملليجرام/كجم فيتامين سي، المجموعة الثالثة (20 جرذان) وتم قسمتها إلى مجموعتين فرعيتين تحتوي كل منهما على 10 جرذان. وتم إعطاء المجموعة الفرعية الأولى 100 ملليجرام/كجم من الجلوتانات أحادية الصوديوم، بينما أُعطيت المجموعة الفرعية الثانية الجلوتانات أحادية الصوديوم بجرعة 4 جرام/كجم يوميًا. أما المجموعة الرابعة (20 جرذان) فقد تم تقسيمها أيضا إلى مجموعتين فرعيتين وقد أعطي جرذان هاتين المجموعتين فيتامين سي مع الجلوتانات أحادية الصوديوم بنفس الجرعات السابقة. وتم معالجة جميع الجرذان من طرف المف بواسطة أثوب معدي لدورة شهرين. وتم قضاء وزن الجسم، هذا بالإضافة إلى دراسة هيستوئولوجيا باستخدام صبغة الهيماتوكسيلين والبنيون وكذلك صبغة الكبيس-3 المفهومة

وقد وجدت زيادة ذات دلالة إحصائية في وزن الجسم في الحيوانات التي تم معالجتها بالجلوتانات أحادية الصوديوم (المجموعة الثالثة) مقارنة بالمجموعات الأخرى (الثانية والرابعة). وكانت هذه الزيادة اعتندة على الجرعة حيث كانت ذات دلالة إحصائية أكبر في الحيوانات التي تم معالجتها بجرعة 4 جرام/كجم عنها في التي تم معالجتها بجرعة 100 ملليجرام/كجم. وقد كشفت صبغة الهيماتوكسيلين والأزوئين عن وجود العديد من بسيطات رتقة الأسماك، مع القليل من الخيوصات الأوردة والذاتية وذلك في المجموعة التي تم معالجتها بجرعة 100 ملليجرام/كجم من الجلوتانات أحادية الصوديوم، بينما لوحظ وجد وجود العديد من بسيطات رتقة الأسماك، وعدم وجود أي من مراحل النم لحواف للتسبب في مجموعة التي تم معالجاتها بجرعة 4 جرام/كجم من الجلوتانات أحادية الصوديوم. كما وجد أن هناك زيادة ملحوظة في إظهار صبغة الكبيس-3 في المجموعة التي تم معالجتها بجرعة 4 جرام/كجم من الجلوتانات أحادية الصوديوم مقارنة بالمعالجات الأخرى. وعلاوة على ذلك فقد أسفرت هذه الدراسة عن وجود خصوص دلالة إحصائية في جميع العوامل التي تم دراستها عند إعطاء فيتامين سي بالترافق مع الجلوتانات أحادية الصوديوم والذين كان ملحوظاً باستخدام الجروة الأقل من الجلوتانات أحادية الصوديوم (100 ملليجرام/كجم). و بذلك سيكون للفيتامين سي دوراً وقائياً في تقليل التأثيرات السامة للجلوتانات أحادية الصوديوم على البلاستين.