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Protective Effect of L-Carnitine Against Reproductive Toxicity of Monosodium Glutamate In Male Albino Mice

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ABSTRACT

Monosodium glutamate (MSG) is commonly used as food enhancer which causes a wide range of toxic effects including reduced male fertility. This study was carried out to investigate the protective effect of L-carnitine against monosodium glutamate (MSG)-induced testicular toxicity in adult male mice. Sixty adult male albino mice (age 10 weeks) were used in the present study and divided into six groups. Group I used as control group (10 mice). Group II (10 mice) received 150 mg/kg/day L-carnitine. Group III& IV (10 mice each) was received 0.3 and 0.6 mg MSG/g body weight. Group V and VI (10 mice each) received 0.3 and 0.6 mg MSG /g body with 150 mg L-carnitine/kg body weight. All animals were treated orally by gastric intubation for 35 days. Reproductive performance, gonadosomatic index (GSI), sperm count, motility, and morphology as well as comet assay were studied. The study demonstrated that L-carnitine ameliorated MSG alterations in testicular weight, sperm count, sperm motility, and sperm morphology together with DNA degradation. This study indicated that the treatment of mice with L-carnitine enhances MSG reproductive toxicity by improving sperm quality and DNA status.

Keywords: Glutamate, Mice, Sperm, Reproductive Toxicity, L-carnitine.

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INTRODUCTION

Monosodium L- glutamate (MSG) is the most common food additive used as a flavor enhancer. The median lethal dose (LD_{50}) in rats and mice is 15.000–18.000 mg/kg body weight [(1)]. Although MSG has proven its value as an enhancer of flavor, different studies have hinted at possible toxic effects related to this popular food-additive. These toxic effects include CNS disorder, obesity, disruptions in adipose tissue physiology, hepatic damage, CRS and reproductive malfunctions ([2]). Results from both animal and human data have demonstrated that administration of even the lowest dose of MSG has toxic effects. The average intake of MSG per day is estimated to be 0.3-1.0 g ([3]). MSG could cause symptoms such as headaches, weakness, sweating, dizziness, flushing and numbness. Moreover to these MSG symptom complex, consumption of MSG has been unproven to cause or intensify numerous conditions, including ventricular arrhythmia, asthma, atopic dermatitis, urticaria, neuropathy and abdominal discomfort ([4]). Many authors revealed that MSG intake causes a disrupted energy balance by increasing the palatability of food and disturbing the leptin-mediated hypothalamus signalling cascade, potentially leading to obesity ([5,6]). MSG at doses of 0.6 and 1.6 mg/g of body weight may cause an adverse effect on the hepatic and renal functions in rats ([7]). MSG has a testicular toxic effect by causing a significant oligozoospermia and increase sperm abnormality in a dose-dependent manner in male Wistar rats ([8]). It has been associated in male infertility by causing testicular hemorrhage, degeneration and alternation of sperm cell count and morphology ([9]). Igwebuike *et al*. ([10]) used a gavage needle to administer MSG solution for 6 weeks to male albino rats and reported a disruption of hypothalamic-pituitary-testes regulatory axis, as well as a lowered serum testosterone. Moreover El-Sawy *et al*. ([11]) reported that rats were administered orally MSG (2 g/kg/day) for 4 weeks showed reduced serum levels of testosterone, luteinizing hormone (LH), sperm profiles and testicular antioxidant activities. At the molecular levels, MSG down-regulated the mRNA expression of steroidogenesis related genes and receptors of androgen, LH and follicle stimulating hormone. MSG induced testicular apoptosis.

Antioxidants are naturally found in semen include vitamins E and C, folate, zinc, selenium, carnitine and carotenoids. These antioxidants act as free radical scavengers that help to overcome ROS ([12]). L-Carnitine is an amino acid; approximately 25% is synthesized from lysine and methionine and naturally is produced in the body and is a water-soluble antioxidant that mostly derived from the human diet. Carnitine at extracellular and intracellular levels may play a significant role in sperm energy metabolism, and provide the primary fuel for sperm motility during epididymal passage ([13]). The healthy epididymides contains Carnitines in both free and acetylated forms for use of spermatozoa via

mitochondrial β-oxidation of long chain fatty acids, as a main transferring system of the acyl to the mitochondrial CoA, and by decreasing fatty acid oxidation restore the phospholipid composition of mitochondrial membranes ([14, 15]).

L-carnitine is essential for the β-oxidation of fatty acids in mitochondria to generate ATP ([16,17]). It was found to possess strong antioxidant, anti-inflammatory and antiapoptotic properties ([18,19,20]). L-carnitine can protect from hepatotoxic, neurotoxic, renal impairment and genotoxic effects. Biochemically and histopathologically with a corresponding reduction of oxidative stress ([21]). L-carnitine has also been shown to play an important role in the control of the male reproductive system and normal function of the testis, where it is highly concentrated in the male reproductive system, especially in the epididymis ([22]). It acts on male gamete maturation and seems to have a key role in: providing readily available energy for sperm motility ([22,23]), sperm DNA repair ([24]), germ cell recovery ([25]), protecting sperms against oxidative damage, reducing apoptosis of spermatogenic cells and inhibiting sperms aggregation ([26]). Nutritional supplementation with L-carnitine improves sperm quality and/or quantity in the testis of rat ([27]) and mice ([28]) exposed to X-ray irradiation. Furthermore, it has been shown that L-carnitine has protective effects on the testis of atherosclerotic rats ([29]). In addition, L-carnitine has a protective effect on di (2-ethylhexyl) Phthalate ([30]), ischaemia-reperfusion ([20]) and etoposide ([31]) induced testis injury in rats. So the aim of the present study was to go more through the toxic effects of two sublethal doses of monosodium glutamate on semen quality and determine the possible reversibility of these effects using L-carnitine as antioxidant.

MATERIALS AND METHOD

Chemicals

Materials used in this research were Monosodium glutamate (MSG) was obtained from FLUKA chemical Co. (USA) and it was liquefied in distilled water. L-Carnitine was purchased from MEPACOMEDI FOOD the L-carnitine powder which obtained from capsules (each one contain 350mg L-carnitine) was dissolved in distilled water solution .

Experimental animals

Experimental animals used in this research were male mice (*Mus musculus*) approximately aged 7-8 weeks with average weight of 25–30 g animals were derived from Vacsera, Egypt (www.mohp.gov.eg/vacsera). Mice were acclimated to lab environment for 10 days in under normal condition of lightening and ventilation. Animals were housed in cages, and given the standard diet and water ad-libitum the study period.

Experimental Design

Sixty mature male *Mus musculus* mice weighting 28±2 g were used in the present study.

Mice were randomly assigned to 6 groups of 10 animals each. Animals of different studied groups were exposed to oral doses of MSG and/or L-carnitine as follows:

Group (1): Control group, ten mice were orally administrated with 0.2 ml of distilled water.

Group (2): Mice were orally administered with oral daily dose 150 mg/kg of L- carnitine

Group (3): Mice were orally administered with a daily dose of 1/60 LD_{50} of MSG (low dose, 0.3 mg MSG /g body weight)

Group (4): Mice were orally administered with a daily dose of 1/30 LD_{50} of MSG (high dose, 0.6 mg MSG /g body weight)

Group (5): Mice were orally administered daily with low dose of MSG concurrent with oral dose 150 mg/kg L-carnitine

Group (6): Mice were orally administered daily with high dose of MSG concurrent with oral dose of 150 mg/kg of L-carnitine

Animals of all control and treated groups were orally treated for 35 days.

Reproductive performance study

The fertility and the reproductive performance of both control and treated groups were studied at the end of treatment. Each male of different studied groups was housed with virgin untreated female 1:1 for 10 days (to complete two estrus cycles). Vaginal plugs were observed daily, the day on which the vaginal plug is detected considered as day zero of gestation. The mating index is calculated as the number of males mated and resulting in a vaginal plug over the number of males cohoused with females multiplied by 100 and expressed as the percent. The fertility index is expressed as the number of males which sired a litter over the number of males resulting a vaginal plug multiplied by100 and expressed as the percent ([32]).

Evaluation of body & testes weights and Gonadosomatic index

During treatment period the body weight of each animal was recorded weekly. The testis was dissected out, cleaned and weighed. The gonadosomatic index (GSI) was calculated by dividing the testis weight by the body weight of each animal and expressed as the percent ([33]).

Sperm analysis

The mice were euthanized 24 h after the last dose. The epididymis was excised and placed in a pre-warmed petri dish containing 0.2 ml of calcium and magnesium free Hank's solution at 37˚C. The tissue was minced with scalpels for approximately 1 min. and placed in 37˚C incubator for 15 min. The epididymis was processed for sperm motility, viability, count, and sperm anomalies.

Sperm motility:

The sperm motility was evaluated according to the method of Ekaluo *et al*. ([34]), two drops of

sperm suspension were put on a microscope slide and covered with cover slip and examined under microscope at 40x magnification. The number of progressively motile sperms was recorded and divided by the total number of spermatozoa counted and expressed as percentage .

Sperm viability

The sperm viability was determined using Eosin-Nigrosin staining technique ([35]). An equal volume of sperm suspension and Eosin-Nigrosin, stain were mixed; air-dried smears were prepared and examined for each sample. Normal live sperms appeared unstained, while dead ones stained pinkish. The percentage of viable sperms was calculated based on the number of live sperm cells out of the total number of cells examined.

Sperm count:

Epididymal sperm count was performed according to Ekaluo *et al*. ([36]) using the improved Neubauer hemocytometer. Heads of sperms were counted manually under light microscope. Data were expressed as total number of sperm/ml.

Sperm anomalies:

The sperm suspension was mixed with 1% eosin Y solution (10:1) for 30 min. Air-dried smears were prepared for examination of sperm anomalies. Slides were examined for counting the number of sperm with abnormal head, mid-piece and tail for every 200 spermatozoa per each slide. Abnormal sperms was calculated and expressed as the percentage according to Ekaluo *et al*. ([37]).

Comet Assay:

The comet assay was performed to determine DNA damage as designated by Singh *et al.* ([38]) with minor modifications. Regular agarose (RA) and low melting point agarose (LMPA) were prepared at 0.75% and 0.5% respectively in Ca++ and Mg++ free PBS, 110 µL of RA were added to fully frosted microscope slides. 75 µL of LMPA containing 105 cells were added. Finally, a top layer of 75 µL LMPA was added. Slides were immersed in lysis solution for at least 1 h at 4°C, and were then left in alkaline buffer for 15 minutes to allow the expression of alkali-labile sites and DNA unwinding. The slides were electrophoresed at 25V and 300 mA for 20 minutes, washed with neutralizing buffer and stained with ethidium bromide (2g/ml in distilled water). Observations were made at 400X magnification using a fluorescent microscope (Olympus) equipped with an excitation filter of 515-560 nm and a barrier filter of 590 nm. When possible, fifty cells per animal were analyzed for DNA migration. The tail length was measured from the trailing edge of the nucleus to the leading edge of the tail, using a calibrated scale in the ocular. The severity of DNA damage was measured comparing comet tail lengths (µm) with the diameter of the nucleus of undamaged cells observed in the same field.

Statistical analysis

Statistical analysis was performed using the analysis of variance (ANOVA) and Duncan's Multiple Range test to determine differences among treatment means at significant level of $p<0.05$ and highly significant at $p<0.01$, Standard errors were also estimated ([39]). All statistics were run on the computer using SPSS program. All curves were fitted with the computer program office (2007). The paper has been approved by scientific and ethical committee of Zoology Department, Faculty of Science in University of Fayoum 2009.

RESULTS

Effect of MSG and L-carnitine treatment on body & testis weights and Gonadosomatic Index

Table (1) demonstrates the effects of MSG on both body and testes weights and gonadosomatic index of male mice. The body weight of high dose only is significantly increased. Meanwhile, the testis weight and gonadosomatic index of all MSG treated groups is significantly reduced when compared with control group ($P<0.01$). The reduction in testis weight and gonadosomatic index goes back to normal levels when recovered with L-carnitine treatment. The mice individually treated only with L-carnitine revealed normal testis weight and gonadosomatic index when compared with controls.

Table 1: Final body weight, total testes weights and gonadosomatic index in male albino mice treated with sublethal doses of MSG individually or co-administered with L-carnitine as antioxidant for 35 days.

Animal groups	Final body weight (g)	Total testes weight (g)	Gonadosomatic index %
Group 1: Control	30.80±1.55 C	0.25±0.02 A	0.82±0.07 A
Group 2: L-carnitine (150mg/kg/day)	29.00±1.59* C	0.23±0.02 A	0.81±0.08 A
Group 3: MSG Low dose (0.3 mg/kg/day)	33.10±1.37* B	0.22±0.02* C	0.67±0.08** B
Group 4: MSG High dose (0.6 mg/kg/day)	34.30±1.33** A	0.20±0.02** D	0.57±0.07** C
Group 5: L-carnitine + MSG low dose	31.60±1.43 C	0.25±0.02 A	0.81±0.06 A
Group 6: L-carnitine +MSG high dose	29.10±1.17 C	0.25±0.02 A	0.87±0.07 A
ANOVA	**	**	**

Data are represented as mean of 10 samples ±SE

Means with the same letter for each parameter in the same column are not significantly different, otherwise they do (Duncan multiple range test).

**P<0.01 Highly Significant.

Reproductive performance

The reproductive performance of male mice treated with MSG is significantly reduced in mice treated with low and high doses (10% and 0%) respectively when compared with (90%) in both control and L-carnitine treated group. While co-administration of L-carnitine with both low and high doses of MSG improves the percentage to normal values (90% and 85%, respectively). The fertility index is dramatically reduced to (0%) in MSG treated groups compared with (100%) in both control and L-carnitine group. Meanwhile, concurrent administration MSG and L-carnitine recovered the fertility index to nearly the normal values (95% & 90%) as shown in Figure (1).

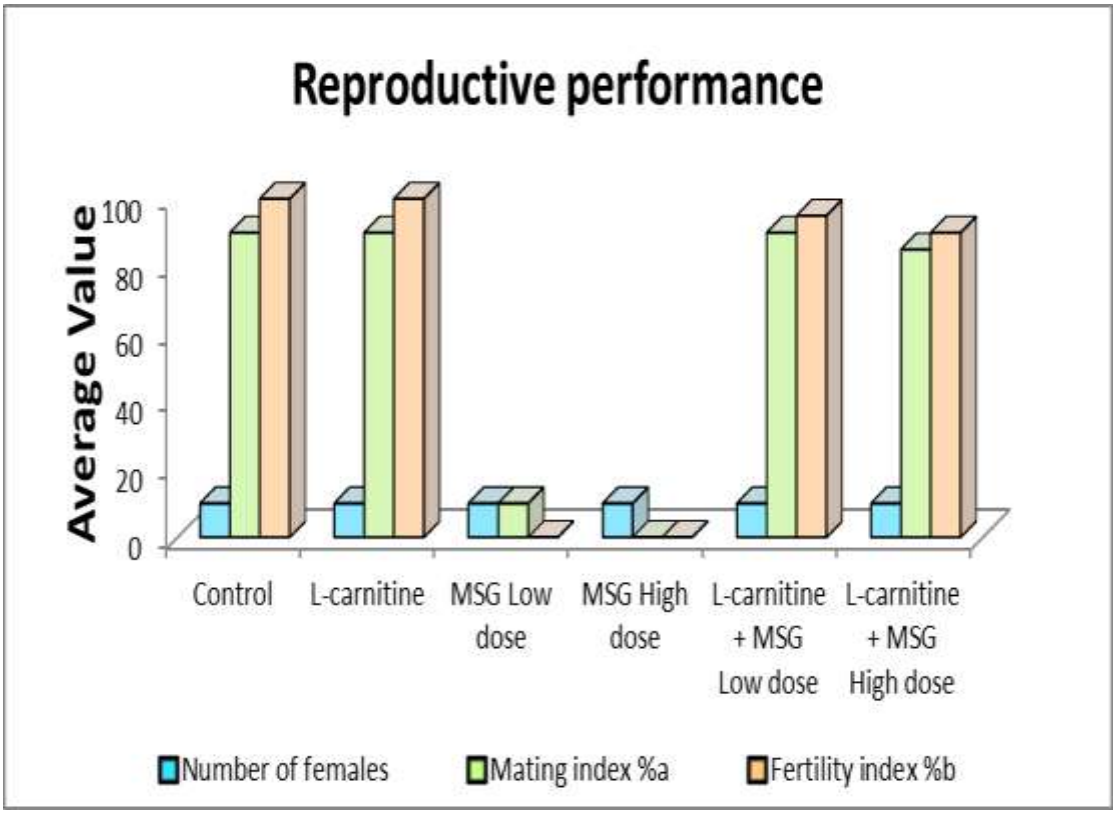


Figure 1: Reproductive performance in male albino mice treated with sublethal doses of MSG individually or co-administered with L-carnitine as antioxidant for 35 days.

Effect of MSG and L-carnitine treatment on sperm count, motility and viability

The data of table (2) demonstrates that the sperm count, motility and viability are significantly reduced in MSG-treated mice. The epididymal sperm count in MSG-treated groups is significantly decreased compared with the control compartment. This reduction is recovered to the normal level via co-administration of L-carnitine (150 mg/kg) ($p<0.01$). The MSG treatment is significantly decline the sperm motility in a dose-dependent manner ($p<0.01$). While, concurrent administration of L-carnitine with MSG is significantly enhanced the sperm motility to normal rates ($p<0.01$). Also, the percentages of epididymal viable sperms is significantly reduced in MSG-treated mice in comparison with control one. The administration of L-carnitine alone (150 mg/kg) and in combination with MSG significantly improves the percent of viable sperms compared to control mice ($p<0.01$).

Table 2: Assessment of Sperm count (X10^6/mm^3), motility and viability of male albino mice treated with_sublethal doses of MSG individually or co-administered with L-carnitine as antioxidant for 35 days.

Animal groups	Count ×10^6	Progressive Motility %	Viability %
Group 1: Control	68.6 ± 1.1 A	70.0 ± 1.1 A	70.6 ± 1.2 A
Group 2: L-carnitine (150mg/kg/day)	67.5 ± 1.0 A	71.2 ± 0.8 A	71.7 ± 0.7 A
Group 3: MSG Low dose (0.3 mg/kg/day)	56.9 ± 1.1** B	40.0 ± 1.2** B	49.8 ± 1.2** B
Group 4: MSG High dose (0.6 mg/kg/day)	57.9 ± 1.0** C	30.9 ± 1.2** C	48.0 ± 1.1** C
Group 5: L-carnitine + MSG low dose	67.3 ± 1.1 A	69.1 ± 1.0 A	69.5 ± 1.1 A
Group 6: L-carnitine +MSG high dose	67.2 ± 1.2 A	69.0 ± 1.0 A	69.7 ± 1.0 A
ANOVA	**	**	**

Data are represented as mean of 10 samples ±SE

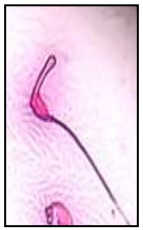
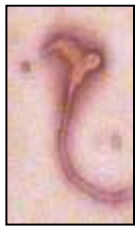
Means with the same letter for each parameter in the same column are not significantly different, otherwise they do (Duncan multiple range test).

**P<0.01 Highly Significant.

Evaluation of sperm anomalies

The sperm abnormalities listed in (Table3) showed a highly significant increase in the incidence percent of sperms anomaly (P<0.01) in all the MSG groups compared with the corresponding control and L-carnitine treated groups. On the other hand, treated groups with MSG combined with L-carnitine showed a significant increase in number of normal sperms and decreased incidence of sperm abnormality.

Table 3: Types of the recorded sperm abnormalities in male albino mice treated with low and high doses of MSG individually or co-administered with L- carnitine as antioxidant for 35 days.

Treatment and doses	Types and percent of sperm abnormalities											
	Percent of head abnormalities				Percent of mid-piece abnormalities				Percent of tail abnormalities			Percent of total abnormalities
	Amorphous head	Hookless	Hammer shape	Double headed	Bent	Coiled	Cytoplasmic droplet	Deformed	Doubled tail	Short tail	Coiled tail	
												
Group 1: Control	2.40 ±0.84 B	1.60 ±0.70 C	2.21 ±0.92 C	0.10 ±0.32 C	2.36 ±1.38 C	1.06 ±0.75 C	0.84 ±0.6 C	0.52 ±0.42 C	3.18 ±1.11 C	1.60 ±0.70 C	1.60 ±0.70 C	15.04 ±6.66 C
Group 2: L-carnitine (150mg/kg/day)	2.93 ±0.89 B	1.63 ±0.69 C	1.53 ±0.92 C	0.10 ±0.33 C	3.00 ±1.45 C	1.5 ±0.75 C	1.05 ±0.65 C	0.5 ±0.45 C	2.23 ±1.16 C	1.40 ±0.74 C	2.10 ±0.70 C	14.92 ± 6.87 C
Group 3: MSG Low dose (0.3 mg/kg/day)	12.20 ± 0.62 A	7.36 ± 0.72 B	10.10 ±0.93 A	1.31 ±0.33 B	3.90 ±1.29 B	1.90 ±0.43 B	1.50 ±0.39 B	0.50 ±0.29 B	22.90 ±1.13 B	3.90 ±0.59 B	3.80 ±0.74 B	65.46 ±6.35 B
Group 4: MSG High dose (0.6 mg/kg/day)	13.00 ± 0.77 A	10.53 ± 0.74 A	4.10 ±0.93 B	3.19 ±0.33 A	5.03 ±1.46 A	3.02 ±0.33 A	1.00 ±0.33 A	1.01 ±0.46 A	29.10 ±1.10 A	4.90 ±0.55 A	4.60 ±0.74 A	74.45 ±6.63 A
Group 5: L-carnitine +MSG Low dose	3.50 ±0.88 A	2.20 ±0.73 C	3.10 ±0.83 C	0.50 ±0.32 C	2.00 ±1.23 C	1.00 ±0.73 C	0.70 ±0.43 C	030 ±0.23 C	3.30 ±1.09 C	2.10 ±0.70 C	1.90 ±0.74 C	18.60 ±6.50 C
Group 6: L-carnitine +MSG High dose	3.40 ±0.84 A	2.83 ±0.63 C	2.01 ±0.75 C	0.43 ±0.31 C	3.50 ±1.45 C	1.50 ±0.75 C	1.50 ±0.85 C	0.50 ±0.45 C	2.28 ±1.14 C	2.20 ±0.71 C	2.10 ±0.61 C	18.74 ±6.46 C
ANOVA	**	**	**	**	**	**	**	**	**	**	**	**

Data are represented as mean of 10 samples ±SE. Means with the same letter for each parameter in the same column are not significantly different, otherwise they do (Duncan multiple range test). **P<0.01 Highly Significant

Comet assay

In mice testes of all groups, the quantitative and qualitative extent of DNA damage in the cells was measured by the length of DNA migration and the percent of migrated DNA. All comets were quantified by three comet parameters (percent of tail DNA, tail moment and the olive tail moment). Comet assay revealed that, MSG induced statistically significant (P<0.05) and highly significant (P<0.01) increase in the average of the comet percent from 8.6±0.39 and 8.39±0.03 in control and L-carnitine groups to 15.9±0.41and 17±0.39 in low and high doses of MSG treated groups. While in groups treated with L-carnitine and low and high doses of MSG showed nearly normal comet assay (8.96±0.36 and 9.41±0.37). Tail length was highly significant increase in low and high MSG treated groups (3.87±0.41 and 5.5±0.41) than control and L-carnitine treated groups (2.72±0.20 and 2.54±0.41); while the co-administered groups (L-carnitine with low and high doses of MSG) showed non-significant changes in comparison with the control (2.64±0.41 and 2.92±0.41). The percent of DNA in comet tail showed highly significant increase in low and high doses of MSG treated groups (30.73±0.37 and 33.10±0.41) comparing with the control (17.03±1.02) and L-carnitine group (17.42±0.29), however the L-carnitine with low and high doses of MSG showed non-significant change (17.96±0.4 and 18.36±0.4) in comparison with control group (17.03±1.02). Tail moment showed highly significant increase in low and high doses of MSG treated groups (1.4±0.41 and 1.52±0.41) compared with the control (0.77±0.13) and L-carnitine (0.87±0.41) groups while the L-carnitine with low and high doses of MSG treated groups showed non-significant change (0.83±0.4 and 0.9±0.4) in comparison with the control group (0.77±0.13) (Table 4 and Fig. 2).

Table 4: Comet assay in the sperm of male albino mice treated with sublethal doses of MSG individually or co-administered with L-carnitine as antioxidant for 35 days.

Animal groups	Comet %	Tail length	% DNA in tail	Tail moment
Group 1: Control	8.6±0.39 C	2.72±0.20 C	17.03± 1.02 C	0.77± 0.13 C
Group 2: L-carnitine(150mg/kg/day)	8.39±0.03 C	2.54±0.41 C	17.42±0.29 C	0.87±0.41 C
Group 3: MSG Low dose(0.3 mg/kg/day)	15.9±0.41 B	3.87±0.41 B	30.73±0.37 B	1.4±0.41 B
Group 4: MSG High dose(0.6 mg/kg/day)	17±0.39 A	5.5±0.41 A	33.1±0.41 A	1.52±0.41 A
Group 5: L-carnitine+ MSG low dose	8.96±0.36 C	2.64±0.41 C	17.96±0.40 C	0.83±0.40 C
Group 6: L-carnitine + MSG high dose	9.41±0.37 C	2.92±0.41 C	18.36±0.40 C	0.9±0.40 C
ANOVA	**	**	**	**

Data are represented as mean of 10 samples ±SE

Means with the same letter for each parameter in the same column are not significantly different, otherwise they do (Duncan multiple range test).

**P<0.01 Highly Significant.

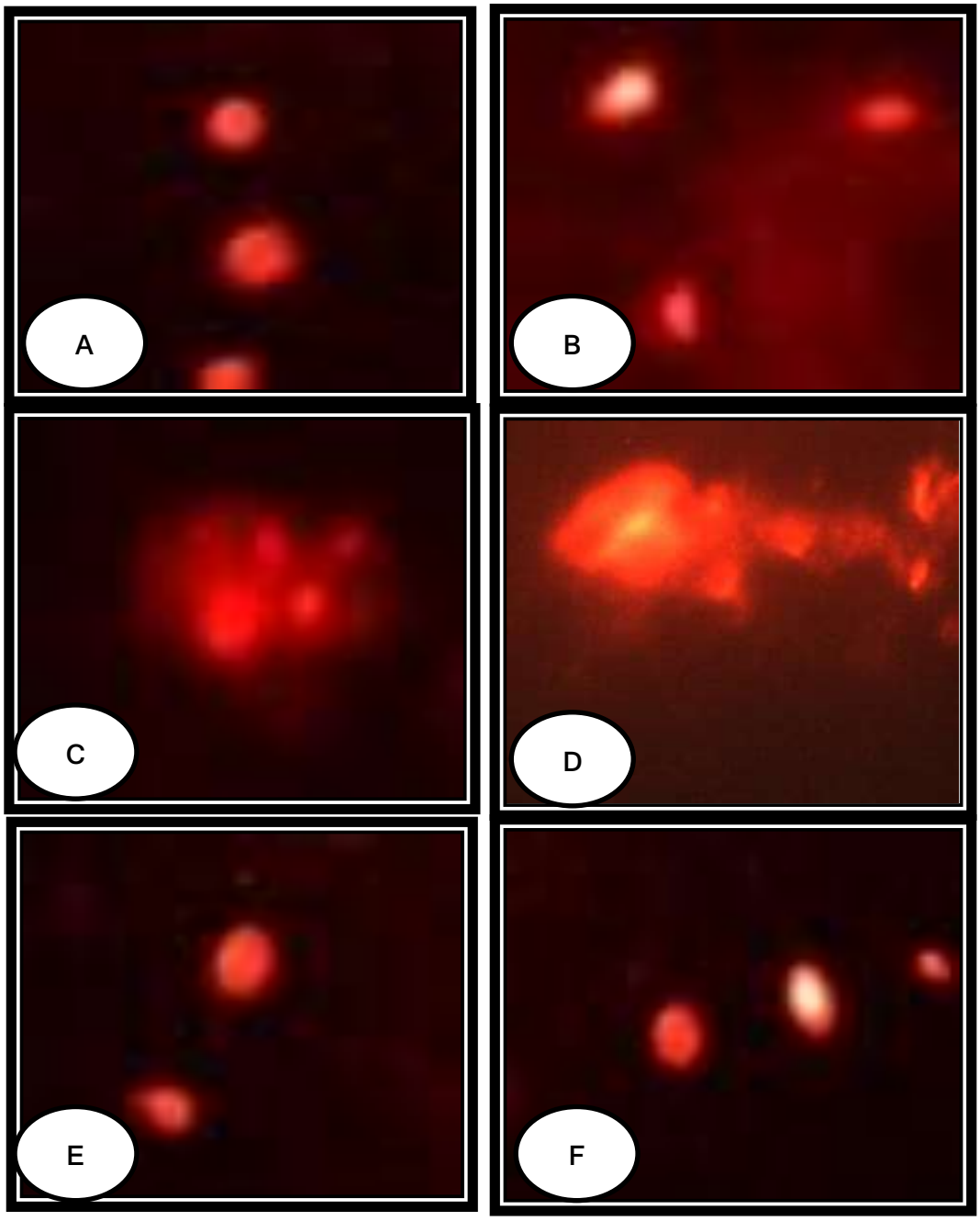


Figure 2: Photomicrographs of comet assay of testes of male albino mice showing DNA damage in treated groups with sublethal doses of MSG individually or co-administered with L- carnitine for 35 days. A control, B L-carnitine, C MSG low dose, D MSG high dose, E MSG low dose with L-carnitine and F MSG high dose and L-carnitine.

DISCUSSION

In the current study, mice treated with low and high doses of MSG showed a significant increase in their body weights in dose dependent manner compared with control and L-

carnitine treated groups. Elevation of the body weight may be attributed to the metabolic changes caused by MSG. In such case all metabolic processes are probably reduced and the anabolic processes are accelerated leading to the body weight increase. These results are in line with the studies carried by Abd-El Aziz and Ashoush ([40]) who revealed that rats treated with MSG caused a significant increase in their body weight gain after 90 days. Also, Nosseir *et al.* ([41]) showed similar findings in rats after daily intraperitoneal injection with MSG (4 ml/kg body weight) for 14 days. Likewise results of Yuan *et al.* ([42]) indicated that MSG could induced fat accumulation and obesity after intraperitonneal injection of male mice offspring by MSG (4 mg/kg body weight) from the first postnatal day until 14th day (prepuberty), or to 28th day (puberty), or day 56 (adult). In addition, oral administration of rats with 4 mg/g body weight for 2 or 4 weeks increased body weight of in both treated groups ([43]). Also, Elbassuoni *et al.* ([44]) found that MSG treatment increases the body weight in rats which given an oral dose of MSG (35 mg/kg/d) for 2 weeks. In this respect, Bloom ([45]) suggested that MSG affects hypothalamus which is responsible for appetite, body temperature, water balance and sleep.

On the other hand, the present work showed that L-carnitine treatment resulted in reduction in the body weight gain of the treated mice. In this concern Pooyandjoo *et al*. ([46]) reported that L-carnitine supplementation shows a positive effect on body weight loss. Carnitine supplementing as weight-loss agent is based on the fact that regular oral ingestion of this drug leads to the increase of its intracellular concentration. This in-turn activates fat oxidation and helps reduction of the body's fat reserves and consequently, resulted in weight loss ([46]). However, the rat model presented by Brandsch and Eder ([47]) did not show a positive effect of L-carnitine supplementation on weight loss and body composition of rats fed an energy-deficient diet.

In the present work, testes weight and gonadosomatic index of MSG treated mice showed significant reduction. This in agreement with the results of Nosseir *et al*. ([41]) who found that MSG cause reduction in testicular size and weight and Yuan *et al.* ([42]) who reported that testicular weight and volume decreased in all male offspring of mice interapertoneally injected with MSG (4 mg/kg body weight) from the first postnatal day (day 0), to day14 (prepuberty), or to day 28 (puberty) or till day 56 (adult). In the same line, daily oral administration of MSG (35, 350 and 3500 mg / kg BW/day), for three months showed significant reduction in absolute testes weight ([40]). On the other hand, Alalwani ([48]) found that the relative testis weight increased in rats treated for 2 months with MSG at doses 30 & 60 g/kg body weight.

The results of the current study showed a zero reproductive index in MSG treated mice although the sperm count is more or less similar to the control group. Likewise, Hamza and

El-Harbi ([49]) noticed significant decrease in the reproductive performance in male mice treated with MSG. Moreover, Hilwani *et al.* ([50]) revealed that intraperitoneal injections of MSG at 250, 500 and 1000 mg/kg of body weight for 14 days induced various implications on reproductive system of male mice which consequently affect fertility potential.

According to Parastie *et al*. ([51]) the sperm morphology must only be considered as an indicator of fertilization potential, not as an absolute indicator of sterility. The evaluation human sperm morphology and their clinical significance is still a controversial aspect of the semen analysis for determination of a male's fertility potential. Menkveld *et al*. ([52]) concluded that if the sperm morphology done correctly with strict application of guidelines outlined by WHO 2010, the sperm morphology measurement still has a very important role in clinical evaluation of male fertility potential. The toxic effect of MSG on testis caused a significant oligozoospermia and increased abnormal sperm morphology in dose-dependent fashion in male Wistar rat as shown by Onakewhor *et al.* ([8]).

Results of the present study revealed a significant increase in the abnormal sperm morphology and a significant decrease in the sperm motility in MSG treated animals compared with control and L-carnitine treated groups. Similar finding is reported by ([53, 10]) they studied the toxic effect of MSG on the sperm parameters and concluded a significant oligospermia and increase abnormal sperm morphology. In the same respect, significant decrease in sperm concentration, motility and live spermatozoa, in addition to the increased sperm abnormalities in male mice after oral administration of different doses of MSG for 90 days ([40]). In addition, the Hilwani *et al.* ([50]) recorded that decreased viability and integrity of sperm membrane of male mice intraperitoneally injected with MSG at 250, 500 and 1000 mg/kg body weight for 14 days. Also, Dong and Robbins ([54]) reported in their review, a lower sperm counts in three of four studies and abnormal sperm morphology in five of six studies carried on MSG fed rats with (0-4 g/kg) on duration of 10 – 56 day. In the same concern, Kadir ([55]) found decreased in sperm motility and normal sperm morphology in rats received daily dose of MSG (1.53 or 3.07 or 6.13 or 12.27 g/kg) for 14 days. Furthermore, Ochiogu *et al.* ([56]) reported reduction in gonadotrophin-releasing hormone, luteinizing hormone, testosterone, which may causes reduction in epididymal sperm reserves after subcutaneous and oral administration of MSG at 0.25-1.00 g/kg to albino rats for 6 weeks. In the same respect, rats treated intraperitoneally with monosodium glutamate (4 mg/kg b.w) for 14 days, showed reduction in all indices of sperm analysis as count, motility and viability ([57]). The data of the present study showed that co-administration of animals with MSG and 150 mg/kg of L-carnitine revealed great improvement of all sperm parameters. In the same line, L-carnitine prevented, to a large extent harmful changes in sperm quality observed following exposure to different toxicants such gamma irradiation in mice ([28]) methotrexate-induced

injury in rat testis ([58]) and cisplatin ([59]). They concluded that L-carnitine enhanced sperm count and motility and reduced sperm abnormalities. Moreover, rats treated with 500 mg/kg b.w. and intraperitoneally injected wit L-carnitine for 16 days improves the reproductive toxicity of cadmium as showed by increase in the number and viability of epididymal sperms ([60]). Also, Yaman and Topcu-Tarladacalisir ([61]) reported that, during the adult period, epididymal sperm count and viability were improved in rats treated with L-carnitine before prepubertal cisplatin injection. In the same respect, Khushboo *et al.* ([62]) found that oral administration 50 and 100 mg/kg L-carnitine into male albino rats for 30 days prevent deleterious effect on sperm quality after long-term copper consumption.

In fact, low levels of carnitine have been suggested as one of the contributing factors for sperm disorders such as azoospermia and asthenospermia ([63]). Similarly, many studies demonstrated that free carnitine levels in the epididymis affect the number, motility and maturity of spermatozoa in rat treated with cisplatin and sacrificed after 72 hrs ([64]). For instance, the spermatozoa make use of the energy provided by carnitine and acetyl carnitine in sperm metabolism which impacts positively on the overall spermatogenic process. Parallel with these results, it was reported by Adewoyin *et al*. ([65]) that sperm quality and function is enhanced with a regular intake of carnitine and acetyl carnitine.

In addition, a previous study from Banihani *et al.* ([66]) showed that a dosage of 0.5 mg/mL of L-carnitine significantly increased the motility of human spermatozoa after *in vitro* incubation and centrifugation. As concerning the metal chelator activity of L-carnitine, it has been shown that L-carnitine can effectively compete for the chelation of calcium ions. In fact, the detrimental effect of the high dosage of L-carnitine may be mainly due to its ability to bind Ca^{2+}, a vital ion needed for sperm motion ([67]).

According to what has been said, it is clear that the use of L-carnitine and its esters, acetyl-L-carnitine and propionyl-L-carnitine, is effective in determining an improvement in sperm parameters and in particular of the total motility and progressive motility, reduces the levels of ROS in seminal fluid, and would be able to improve the quality of the semen. The administration of these molecules in the treatment of male infertility (alone or in combination) is, therefore, a rational and effective therapeutic strategy. However, clinical benefits should not be achieved at high doses, since the evidence of calcium chelator activity of L-carnitine that may determine cell damage and decrease serum calcium ([68]).

Other antioxidant exhibited similar protective or ameliorative result against MSG induced testicular toxicity as those observed in our study such as vitamin E and selenium in male rats ([49]), ascorbic acid in male rats ([53, 34, 69]), curcumin in male rat ([70]). Al-Shahari and El-Kott ([71]) reported significant decrease in testis weight, sperm count and significant increase in their

abnormality after MSG administration in 6 mg/g/day dissolved in distilled water for two months.

In comparison with other genotoxicity tests, the comet assay is sensitive for detecting low levels of DNA damage, the requirement for small numbers of cells, flexibility, low costs, simplicity of application, and short time needed to complete an experiment ([72]).

In vivo studies showed that MSG leads to oxidative damage and elevate lipid peroxidation rate ([73, 74, 75, 53, 76, 77]). Excess production or insufficient consumption of reactive oxygen derivatives results in oxidative stress, this also gives rise to DNA mutation, damage or fragmentation and DNA repair and/ or replication disorder following exposure to the reactive oxygen species (ROS) ([78]). In this manner, sperm DNA fragmentation tests have reopened the debate over their usefulness in improving pregnancy outcome. In this regards, two considerations should be disentangled. First, spermatozoa are not simply carriers of paternal chromosomes, but play a role beyond fertilization. For instance, the spermatozoon transcribes genes critical for early embryonic development, inferring that integrity of sperm genome is essential for a successful gestation. Second, if sperm factors play a role in early embryonic development, sperm DNA integrity tests are useful as diagnostic and prognostic markers, especially in the context of recurrent pregnancy loss ([79]).

In the current study, the MSG treatment at two sublethal doses, showed clear DNA degradation in the nuclei since the increasing in percentage of tail DNA is observed in comparison with the control and L-carnitine treated groups. These results coincided with the results of Ismail ([77]) when orally administered male rats with MSG 8g /kg for 90 days and found an increase in the tail moment in the testes cells of animals treated with MSG compared to the control group. Conversely, animals co-administered with L-carnitine and MSG displayed enhancement in DNA integrity. In the same manner, Lewis *et al.* ([80]) mentioned that carnitine may shield the spermatozoa from oxidative stress of DNA and membrane damage by reducing the reactive oxygen and reduces the detrimental effect of sperm lipid peroxidation. Carnitine has been shown to protect cells against mitochondrial and free radical-related nuclear DNA damage and improve mitochondrial functions by reducing stress mediated DNA damage through reducing the production of oxidants and enhancing antioxidant status ([19]). In this respect, carnitines act as safeguard to sperm and cell membrane against reactive oxygen species induced DNA fragmentation and apoptosis ([65]).

Acetyl L-carnitine protect cells against free radical induced nuclear DNA damage and improve mitochondrial functions by reducing stress-mediated DNA damage through reducing production of oxidants and enhancing antioxidant status ([81, 82,19]). Several studies demonstrated that free carnitine levels in the epididymis affect the number, motility and maturity of spermatozoa ([83 ,22]).

CONCLUSION

Hence it is recommended that the reproductive toxicity MSG in male albino mice could be ameliorated by L-carnitine supplementation. Furthermore, carry out other investigations using different parameters as antioxidants measurements and other immunohistochemical markers to evaluate the protective effects of different antioxidants against the reproductive toxicity of MSG. This study spots light organization of human health responsible for food safety to use common salt and natural products as flavor instead of MSG.

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