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Original Article

Protective effects of camel milk and vitamin E against monosodium glutamate induced biochemical and testicular dysfunctions

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Summary. *Objective:* The current study was outlined to examine the protective effects of camel milk (CM) against the deleterious effects induced by monosodium glutamate (MSG). *Methods:* MSG was administered either alone or in combination with camel milk or vitamin E for 4 weeks. Serum and testicular tissues were examined using semiquantitative RT-PCR analysis. *Results:* CM normalized the decrease in serum levels of testosterone, luteinizing hormone (LH), sperm profiles and testicular antioxidant activities that were decreased by MSG. 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. All altered genes were normalized and upregulated in presence of CM when compared to the effect of Vit. E. *Conclusions:* The usage of camel milk as a therapy against MSG used in food industry is very indicative and protective.

Key words: monosodium glutamate, camel milk, protection, testicular dysfunction, gene expression, vitamin E.

Introduction

Monosodium glutamate (MSG) is the sodium salt of glutamic acid (1). About 78% of MSG is glutamic acid and 22% are sodium and water (2). Glutamate is one of the most common amino acids found in nature and is the main component of many proteins and peptides of most tissues (2). Glutamate plays an essential role in human metabolism. It is a major component of many protein-rich food products either in free or bound state of animal such as meat, fish, milk and cheese or vegetable origins such as mushroom and tomato (3). Moreover, MSG is widely used as a flavor enhancer especially in Chinese and Japanese foods and restaurants (4,5). MSG provides a flavoring function similar to the naturally occurring free glutamate which differ from sweet, sour, salty and bitter (6). Recently MSG was used as food additive as a flavoring or hydrolysed vegetable protein (7). MSG influences the appetite positively, and induces weight gain as it stimulates oro-sensory receptors and improves the palatability of meals. However, reports indicated that to a certain degree, MSG is toxic to human and experimental animals (7). MSG shows symptoms such as numbness, weakness, flushing, sweating, dizziness and headaches (8). In over doses, MSG exacerbate numerous conditions, including asthma, urticaria, atopic dermatitis, ventricular arrhythmia, neuropathy and abdominal discomfort (8). MSG induced neurotoxic effects that caused brain cell damage, retinal degeneration, endo-

crine disorder and some pathological conditions such as addiction, stroke, epilepsy, brain trauma, neuropathic pain, schizophrenia, anxiety, depression, Parkinson's disease, Al- zheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis (2). MSG is toxic for testis as it induced oligozoospermia and increased abnormal sperm morphology (9). It caused male infertility at histo-pathological level by causing testicular hemorrhage, alteration and degeneration of sperm cell count and morphology (10).

As known, vitamins are essential for all biochemical reactions. They act as antioxidants to protect tissues from oxidative stress due to their easy, effective and safe dietary administration in a large range of concentrations (11,12). Vitamin E (α- Tocopherol [(α-Toc]) is a membrane bound lipid-soluble, chain-breaking antioxidant that protects all cell membranes against lipid peroxidation (13,14). Vitamin E pretreatment has been reported to be beneficial in preventing formaldehyde-induced tissue damage in rats (15,16). The preventive effect of vitamin E on cypermethrin or endotoxin-induced oxidative stress in rat tissues is suggestive of its antioxidant activity (17). Vitamin E is the most known antioxidants that have a protective effect by either reducing or preventing oxidative damage (17). Vitamin E prevents lipid peroxidation by interfering with the propagation of lipid radicals (11). It is a non-enzymatic antioxidant (15,16). Vitamin E inhibits lipid peroxidation by both scavenging lipid peroxyl radicals (15,16), and inhibits oxidative damage induced by heavy metals and pesticides in experimental animals (15).

Camel's milk (CM) exhibits a wide range of biological activities including antimicrobial, antioxidative, antithrombotic, antihypertensive, and immunomodulatory effect (18). It was therapeutically used to treat jaundice, splenic problems, asthma, anemia, piles, and diabetes (19,20). Camel milk contains high levels of lactoferrin which is an iron-binding glycoprotein of the transferring family (21). Camel milk anticarcinogenic, anti-inflammatory and antioxidant activities were proposed to be mainly caused by lactoferrin (22). Camel's milk is effective in food allergy treatments due to its inflammation-inhibiting proteins, and hypoallergenic properties (23). Therapeutic potency of camel milk against several diseases is due to its newly identified nanobodies content (24).

From all established data, we can conclude that the deleterious effects of MSG on liver, kidney and testes were mainly at the histology and pathology levels (25, 26) but no studies about its harmful molecular effects and possible protection by camel milk were reported till now. Therefore, the current study aimed to examine the protective effects of camel milk on deleterious effects of MSG on hepatic and renal biomarkers and antioxidants levels compared to vitamin E action. Moreover, genes of testicular function and steroidogenesis were examined after MSG administration and protection with camel milk compared to vitamin E.

Materials and methods

Chemicals and kits

Monosodium glutamate, agarose and ethidium bromide were purchased from Sigma-Aldrich (St. Louis, MO, USA). The Wistar albino rats were purchased from King Fahd center for Scientific Research, King Abdel-Aziz University, Jeddah, Saudi Arabia. Kits for glutamate pyruvate transaminase (ALT), glutamate oxalacetate transaminase (AST), superoxide dismutase (SOD), malondialdehyde (MDA), catalase, glutathione reductase (GSH), creatinine and urea were purchased from Bio-diagnostic Co., Dokki, Giza, Egypt. The deoxyribonucleic acid (DNA) ladder was purchased from MBI, Fermentas, Thermo Fisher Scientific, USA. Qiazol for RNA extraction and oligo dT primer were purchased from QIAGEN (Valencia, CA, USA).

Animals handling and experimental design

Forty male Wistar rats, 8 weeks old, weighting 170–200 grams were given free access to food and water. Rats were maintained at 12h/12h day and light and at 25 ± 5°C. After 2 weeks of daily handling and acclimatization, rats were assigned for experimental procedures. Rats were allocated into 4 subgroups, with 10 rats per group. Control rats gained free access to food and water. MSG group was given a normal diet together with MSG (2 g/kg/day) for 4 weeks (27,28). MSG + Vit. E group that administered MSG orally (2 g/kg /day) for 4 weeks with Vit E at a dose of (20 mg/kg/day) (29). Camel milk (CM) + MSG group was given MSG for 4

weeks together with camel milk in a dose of 166.6 ml /24 hours / 10 rats according to Althnaian (30). After 4 weeks, all rats were anesthetized using diethyl ether after overnight fasting and blood was collected for serum extraction and biochemical assessments. Testicular tissues were taken on Qiazol for RNA extraction and gene expression (RT-PCR).

Serum separation and biochemistry analysis

Blood was collected from the eye using heparinized capillary tubes inserted into retro-orbital venous plexuses. Blood was left to clot at room temperature then in the refrigerator for 15 minutes and centrifuged for 10 minutes at 4°C and 5000 rpm, supernatant serum was taken and stored at -20 °C till assays. Fasting blood glucose levels were determined using spectrophotometric assay. Serum creatinine, urea, albumin, ALT, AST, triacylgycerol (TAG), total cholesterol and HDL were measured using commercial available kits that are based on spectrophotometric analysis.

Determination of testicular tissue antioxidant activity

For measurements of MDA, catalase and SOD activity, one gram of testicular tissues was homogenized in 5ml of cold buffer (50mM potassium phosphate buffer; PBS, pH 7.4). Cold buffer of catalase activity contains 1mM EDTA and 1mL/l Triton X-100. After centrifugation at 4000 *x g* for 15 minutes at 4°C, the supernatant was removed and stored frozen at -80°C until the time of analysis of catalase and SOD (U/g tissue) and MDA (nmol/g tissue). The activities of MDA, catalase and SOD were determined by ELISA reader (Absorbance Microplate Reader ELx 800TM BioTek®, Seattle, WA, USA). Results were calculated according to the manufacturer's instructions. The assays used for measurement of MDA, Catalase and SOD were based on previous studies (31-33) for MDA, catalase and SOD respectively.

Serum hormone assays

Changes in levels of testosterone and luteinizing hormone (LH) were measured using commercial kits (Testosterone ELISA Kit from abcam, Tokyo, Japan Cat # ab108666) that purchased from Clini Lab., Al-Manial, Cairo, Egypt. Serum LH levels were measured using LH SimpleStep ELISA™ Kit (ab108651)), Osaka, Japan. The instruction manual of each kite was followed as suggested by providers.

Sperm analysis (eosin-nigrosin stain)

The epididymal sperm was collected according to Blash et al (34) with some modification. The testes were removed from the scrotal sac within 5 to 10 minutes, placed in an insulator box and transported to laboratory and processed individually. The parietal tunic was removed leaving the tail of the epididymis exposed. A small lateral incision was made along the tail of the epididymis to open the convoluted tubules and put in petri dish with 3.025 g Tris, 1.7 g citric acid, 1.25 g fructose supplemented with 5.5 mg tylosin, 27.5 mg gentamycin, 16.5 mg lincospectin, and 330 mg spectinomycin per 100 ml. Spermatozoa were sedimented by gentle centrifugation at 800 X g (1200 rpm) for 5 min at 30°C and the pellet was washed twice with TFC medium to remove contaminating epididymal plasma. The cells were dispersed in the same medium and this preparation of spermatozoa was used for the experiments. Individual sperm motility was assessed by bright field microscopy. Diluted sperm was examined microscopically using adjusted hot stage microscope at 38°C. Individual sperm motility percent was determined on a subjective scale of 0–100% to the nearest 5% after examining several microscopic fields. The percentage of live and abnormal sperms was assayed by staining smears with eosin-nigrosin (35). A total of 200 sperm cells were examined randomly. Total sperm abnormalities and live percentage were recorded.

RNA Extraction, cDNA Synthesis and Semi-quantitative RT-PCR Analysis

Total RNA was extracted from testicular tissues in Qiazol reagent (50-100 mg per sample). RNA was extracted using chloroform-isopropanol extraction assay. RNA pellets were washed with 70% ethanol, briefly dry up, and then dissolved in Diethylpyrocarbonate (DEPC) water. For cDNA synthesis, a mixture of 2 µg total RNA and 0.5 ng oligo dT primer in a total volume of 11 µl sterilized DEPC water was incubated in the PeX 0.5 thermal Cycler (Thermo Electronic Corporation, Milford, Ma) at 65°C for 10 min for denaturation. Then, 4 µl of 5X RT-buffer, 2 µl of 10 mM dNTPs and 100 U Moloney Murine Leukemia

Virus (M-MuLV) Reverse Transcriptase (SibEnzyme Ltd. Ak, Novosibirsk, Russia) were added and the total volume was completed up to 20 µl by DEPC water. The mixture was then re-incubated in the thermal Cycler at 37°C for one hour, then at 90°C for 10 min to inactivate the enzyme.

For semi-quantitative RT-PCR analysis, specific primers for examined genes (Table 1) were designed using Oligo-4 computer program and synthesized by Macrogen (Macrogen Company, GAsa-dong, Geumcheon-gu. Korea). PCR was conducted in a final volume of 25 µl consisting of 1 µl cDNA, 1 µl of 10 pM of each primer (forward and reverse), and 12.5 µl PCR master mix (Promega Corporation, Madison, WI). The volume was brought up to 25 using sterilized, deionized water. PCR was carried out using Bio-Rad thermal Cycle with the cycle sequence at 94 ˚C for 5 minutes one cycle, followed by 26 cycles for examined genes and 23 cycles for the reference gene (glyceraldehyde-3-phosphate dehydrogenase; GAPDH). Each PCR cycle consists of denaturation at 94 ˚C for one minute, annealing at the specific temperature corresponding to each primer (Table 1) and extension at 72 ˚C for one minute with additional final extension at 72 ˚C for 7 minutes. Products of PCR were run on 1.5% agarose (Bio Basic INC. Konrad Cres, Markham Ontario) gel stained with ethidium bromide in TBE (Tris-Borate-EDTA) buffer and visualized under UV light and photographed using gel documentation system. The band intensities were densitometrically quantified and calculated using ImageJ software version 1.47 (http://imagej.en.softonic.com/).

Statistical analysis

The data are presented as the mean ± standard error of the mean. Analysis of variance and Fisher post-hoc descriptive test were used to analyze the data using SPSS software version 11.5 for Windows (SPSS, Inc., Chicago, IL, USA). Using the same software, regression analysis was performed. $P<0.05$ were considered to indicate a statistically significant difference.

Results

Serum biochemical, oxidative and antioxidative biomarkers

Administration of MSG constitutively for 4 week induced a decrease in urea, creatinine and increased ALT

Table 1. PCR conditions for examined genes in the testis.

Gene	Product size (bp)	Annealing (°C)	Direction	Sequence (5-3)
ABP	260	58	Sense Antisense	TCCGATACCACCAAGCACAAG TCAGGAAAGCTGGGAACACTG
LHR	272	52	Sense Antisense	AGAGTGATTCCCTGGAAAGGA TCATCCCTTGGAAAGCATTC
$P450_{c17}$	302	55	Sense Antisense	GACCAAGGGAAAGGCGT GCATCCACGATACCCTC
P53	547	55	Sense Antisense	ATCTGGACGACAGGCAGACT AGGCAGTGAAGGGACTAGCA
17β-HSD	653	55	Sense Antisense	TTCTGCAAGGCTTTACCAGG ACAAACTCATCGGCGGTCTT
AR	570	55	Sense Antisense	TTACGAAGTGGGCATGATGA ATCTTGTCCAGGACTCGGTG
FSHR	490	55	Sense Antisense	GAGTCATCCCGAAAGGATCA TAAAATGACTGGCCCAGAGG
Aromatse	389	58	Sense Antisense	GCCTGTCGTGGACTTGGT GGTAAATTCATTGGGCTTGG
β actin	457	60	Sense Antisense	ATGTACGTAGCCATCCAGGC TCCACACAGAGTACTTGCGC

Table 2. Protective effect of camel milk against MSG induced changes on renal and hepatic biomarkers.

Parameter	Urea (mg/dL)	Creatinine (mg/dL)	GOT (U/l)	GPT (U/l)
Control	36 ± 0.3	0.7 ± 0.01	82 ± 3.7	98.6 ± 7.5
MSG	33 ± 1.2*	0.3 ± 0.1*	182 ± 2.9*	186 ± 3.2*
MSG + Vit E	38 ± 1.8#	0.7 ± 0.1#	90.7± 12.8#	86.4 ± 4.1#
MSG + CM	39.9 ± 1.5#	0.67 ± 0.1#	93 ± 4.1#	103 ± 8.2#

Values are means ± standard error (SEM) for 10 different rats per each treatment.
*Values are statistically significant at *p<0.05 Vs. control and #p<0.05 Vs. MSG rats.*

and AST levels and co-administration of camel milk to MSG administered group protect from this significant decrease (Table 2). MSG decreased antioxidants levels. As seen in Table 3, MSG induced oxidative stress as indicated by the increase in MDA and decrease in SOD, GSH-R and catalase. Co-administration of CM with MSG counteracted this decrease and significantly normalized antioxidant levels. The effect of Vitamin E was parallel to the effects of camel milk

Testicular tissue oxidative and antioxidative biomarkers

To confirm the deleterious effects of MSG on testis, we measured testicular MDA levels as an oxidative stress marker and catalase and SOD as antioxidant markers. As shown in Table 4, MSG increased significantly MDA levels and decreased testicular catalase and SOD activities. Co-administration of either Vit. E or CM together with MSG inhibited this decrease and normalized it.

Sperm analysis

MSG induced significant decreases in sperm motility, percentage of live sperms, and an increase in sperm abnormalities. Camel milk supplementation into MSG group improved sperm and percentage of live sperms and decreased sperm abnormalities reported on MSG administered rats (Table 5). Parallel with changes in spermogram, a decrease in serum levels of testosterone and LH were reported in MSG administered rats that were normalized after camel milk sup-

Table 3. Protective effect of camel milk against MSG induced changes on antioxidants levels.

Parameter	MDA (nmol/ml)	SOD (U/ ml)	GSH-R (mg/ dL)	Catalase (U/ L)
Control	11.8 ± 2.6	227.9 ± 33	7.7± 1.1	166.7 ± 18.6
MSG	31.4 ± 3*	117.2 ± 2.2*	4.5± 0.4*	56.7 ± 11.4*
MSG + Vit E	18.3 ± 1#	256.6 ± 35#	6.2 ± 0.07#	169.7 ± 18.3#
MSG + CM	10.9 ± 1.4#	194.7 ± 9.6#	6.3 ± 0.2#	97.5 ± 2.8#

Values are means ± standard error (SEM) for 10 different rats per each treatment.
*Values are statistically significant at *p<0.05 Vs. control and #p<0.05 Vs. MSG rats.*

Table 4. Protective effect of camel milk on MSG induced changes in testicular antioxidant activity in Wistar rats.

	Control	MSG	MSG + Vit E	MSG + Camel milk
MDA (nmol/mg protein)	6.78±1.9	19.7±0.9	8.2 ± 0.6#	9.6 ± 0.3#
Catalase (U/ mg protein)	5.92±.1	3.7±0.2*	4.7 ± 0.04#	4.6 ± 0.06#
SOD (U/ mg protein)	4.3±0.09	2.7± 0.1*	3.9 ± 0.4#	3.6 ± 0.2#

Values are means ± standard error (SEM) for 10 different rats per each treatment.
*Values are statistically significant at *p<0.05 Vs. control and #p<0.05 Vs. MSG rats.*

Table 5. Camel milk protected MSG induced changes in sperm motility and abnormalities.

	Motility%	Live %	Abnormality %
Control	87.89 ± 2.2	83.44 ± 4.6	10.44 ± 2.8
MSG	54.22 ± 3.1*	46.44 ± 3.3*	27.33 ± 3.8*
MSG + Vit E	73.3± 4.1#	77.21± 4.6#	14.33 ± 2.8#
MSG + CM	66.1± 5.3#	66.8 ± 3.7#	14.9 ± 1.7#

Values are means ± standard error (SEM) for 10 different rats per each treatment.
*Values are statistically significant at *p<0.05 Vs. control and #p<0.05 Vs. MSG rats.*

plementation (Table 6). Of note the effect induced by CM is relatively the same reported for vitamin E administered group.

Testicular steroidogenesis and fertility related genes mRNA expression

Next, the effect of MSG on the testis activity at the mRNA expression level was checke. As seen in Figure 1, administration of MSG induced down regulation of genes of steroidogenesis as it decreased significantly the expression of androgen binding protein (ABP), 17 alpha hydroxylase ($P450_{c17}$), 17b-hydroxy steroid dehydrogenase (17β-HSD) and aromatase. Co-administration of CM or vitamin E to MSG groups normalized and even up-regulated the examined genes and retained their expression (Figure 1). In parallel, the genes of fertility markers as androgen receptor (AR), luteinizing hormone receptor (LHR), follicle stimulating hormone receptor (FSHR) and testicular apoptotic gene (P53) were greatly down-regulated in MSG administered rats. Administration of CM or vitamin E together with MSG normalized and up-regulated their expression compared to MSG administered group (Figure 2).

Table 6. Protective effect of camel milk against MSG induced changes in testosterone and LH levels

	Testosterone (ng/ml)	LH (ng/ml)
Control	3.5 ± 0.4	1.7 ± 0.2
MSG	2.1 ± 0.1*	1.0 ± 0.04*
MSG + Vit E	3.8 ± 0.2#	1.56 ± 0.1#
MSG + CM	4.7 ± 0.2#	1.48 ± 0.1#

Values are means ± standard error (SEM) for 10 different rats per each treatment.
*Values are statistically significant at *p<0.05 Vs. control and #p<0.05 Vs. MSG rats.*

Discussion

Monosodium glutamate (MSG) is a commonly-used food processing additive. Several studies showed that MSG is toxic to the various organs such as the

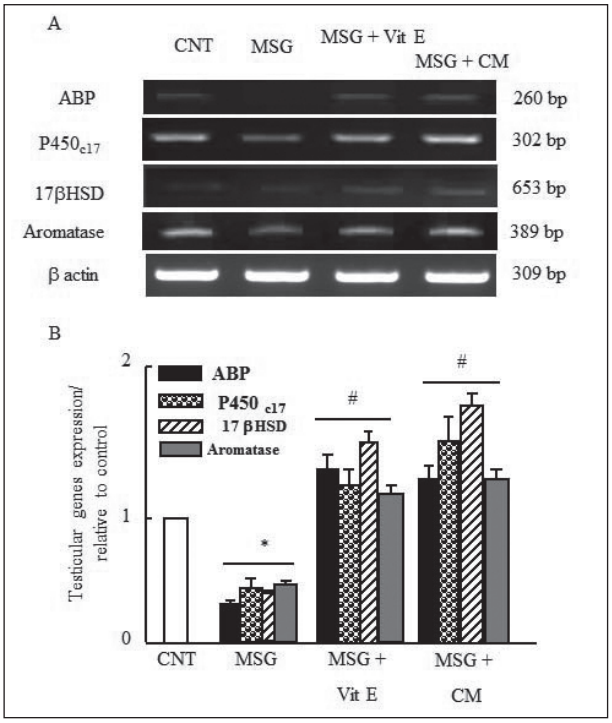

Figure 1. Effect of camel milk on steroidogenesis related gene expressions: Rats groups were treated with contol, MSG, camel milk plus MSG or Vit E plus MSG. Total RNA was extracted from testis tissues and the expressions of genes were analyzed by semi-quantitative RT-PCR analysis. Values are means ± SE of 10 rats. * P < 0.05 Vs control group, # P < 0.05 MSG group.

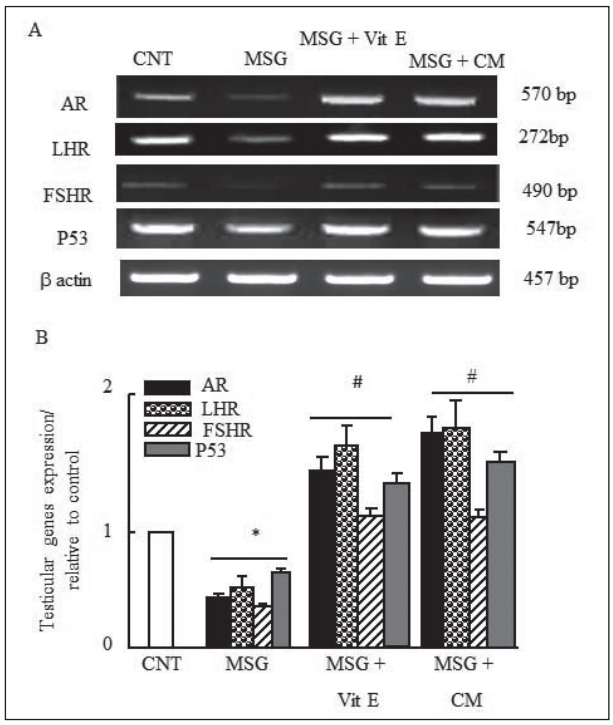

Figure 2. Effect of camel milk on fertility related genes and P53 mRNA expressions: Rats groups were treated with contol, MSG, camel milk plus MSG or Vit E plus MSG. Total RNA was extracted from testis tissues and the expressions of genes were analyzed by semi-quantitative RT-PCR analysis. Values are means ± SE of 10 rats. $^{*}P < 0.05$ Vs control group, $^{\#}P < 0.05$ MSG group.

liver, brain, thymus, and kidneys (36,37). This effect is attributed mainly to oxidative stress caused by MSG (38). Oxidative stress is caused by the excessive production or a decreased elimination of free radicals in cells, the majority of which are oxygen radicals and other reactive oxygen species (ROS) (39).

Camel milk is enriched with various protective proteins such as lysozyme, lactoferrin, lactoperoxidase, immunological properties, growth promotion activity and anti-tumor activity(40,41). Camel milk could play an important role in decreasing oxidative stress by alteration of antioxidant enzymes and nonenzymatic antioxidant molecules levels (23). Current findings confirmed that long term administration of MSG induced biochemical, renal, hepatic and testicular alterations. Moreover, MSG showed testicular dysfunction as presented by the decrease in its antioxidant activity and down regulation in all examined testicular genes.

Both ALT and AST are sensitive markers of liver damage (42,43). Therefore, the increase in the serum liver activity might perhaps be an indication of liver damage. MSG could dissociate easily to release free glutamate. The diminution of glutamate produces ammonium ion that could be toxic unless detoxified in the liver via the reactions of the urea cycle. Thus, the possible ammonium ion overload that may occur as a result of an increased level of glutamate following MSG intake could damage the liver (25).

Our findings are coincided with others at biochemical levels (25,26), as pre-treatment with vitamin E has been reported to confer protection against monosodium glutamate induced-hepatotoxicity and oxidative stress in rats (24, 42). In parallel, dietary antioxidants such as Vitamin C and Vitamin E has a modulator effects on MSG-induced serum urea oxidative damage in the liver and kidney of rats (29). The variation in the level of urea and creatinine are markers of renal dysfunction.

As known, the major indicator of oxidative damage in the body is the increase in lipid peroxidation that initiated by ROS and causes impairment of membrane function (44). The increase in MDA in this study may be attributed to the increase in generation of ROS results from MSG treatment. Similar observations have earlier been reported in studies involving other organs (27,29,36,45). A decrease in antioxidant status is a counter act mechanism by the tissues to restore their activity. A decrease in GSH, SOD and catalase levels in the serum and testis of our experimental animals, are similar to earlier observations that MSG induced oxidative stress in other tissues (36,45,46). The depletion in antioxidants levels reported here correlates with the increase in lipid peroxidation observed in the other tissues (27,29,36).

Antioxidants act as a direct radical scavenger and stabilize membrane structure through the removal of acyl peroxides formed during lipid peroxidation reaction (47). Glutathione depletion is a positive indicator of tissue degeneration and the magnitude of depletion parallels the severity of the damage (48). The increase in the activity of glutathione S-transferase (GST) following MSG administration might have contributed to the depletion of tissue glutathione. GST catalyzes both glutathione-dependent conjugation and reduc-

tion (49). It detoxifies endobiotic and xenobiotic compounds by covalently linking glutathione to a hydrophobic substrate, forming less reactive and more polar glutathione S-conjugate (50). The activities of superoxide dismutase (SOD) and catalase decreased significantly in MSG treated rats and that are coincided with other study (45). Camel milk administration returned this antioxidant activity by decreasing lipid peroxidation and increasing antioxidants levels. The antioxidant activity of CM is due to the presence of lactoferrin in its contents as described in another study (22) and the presence of camel a-lactalbumin (51).

Our results showed that MSG induced testicular oxidative stress. Possibly, the toxic effects of MSG on the sperms and testicular examined parameters might be related to the increased production of free radicals in the rat reproductive organs as decribed histologically in previous study (26). Our current investigation revealed that MSG caused significant decrease in SOD, CAT and GSH-R activities and these findings are greatly in accordance with Fabio et al (42). Here, CM ameliorated these testicular changes as reported for Vit. E and quercetin (29, 42), as CM has been shown to be with antioxidant properties (22,51).

Vit E showed protective effect against MSG. This effect may be due to impaired absorption of MSG in the gastrointestinal tract and/or its antioxidant effect (52). Vitamin E prevents oxidative damage to sensitive membrane lipids by destroying hydroperoxide formation, acting in conjunction with selenium, and protects cellular membranes and lipid containing organelles from peroxidative damage by oxidative stress (53). The recovery of sperm count and motility to the control levels in camel milk- treated rats can be attributed to antioxidant properties of camel milk as supported by serum findings listed in this study. It has been shown that camel milk inhibited oxidative stress by increasing the activities of SOD, catalase and glutathione reductase levels (45,55).

MSG down regulated the expression of steroidogenesis related genes, male fertility hormone receptors and apoptosis related gene (P53). In the testis, LH binds to Leydig cell receptors and initiates the activation of adenylate cyclase, resulting in an increase in cAMP production. Luo and his collaborators (56) reported that, StAR transfers cholesterol from the outer membrane to the inner mitochondrial membrane. Where, the enzyme cytochrome P450 side chain cleavage (P450scc) converts cholesterol into pregnenolone. Ultimately pregnenolone transferred to smooth endoplasmic reticulum. Where, the synthesis of testosterone takes place via the actions of 17β-hydroxysteroid dehydrogenase (17β-HSD). Moreover, StAR is considered to be the rate limiting step in testosterone biosynthesis and reduced StAR is always found in testicular dysfunction (57). The increase in testosterone can be changed to estrogen by aromatase. MSG decreased serum LH and testosterone levels that were confirmed by down regulation on AR and LHR as well as other examined testicular genes. MSG induced testicular oxidative stress and increased ROS production. Of interest, CM had the potential to prevent these changes and provided evidence for such beneficial protective effects at testicular levels. Probably CM interacted with MSG at the intestine and prevented MSG absorption. Moreover, it decreased free radical production, lipid peroxidation (53) as CM has a potential antioxidant activity (22).

In conclusion, CM supplementation had a protective effect against MSG induced oxidative stress and testicular dysfunction. Moreover, CM normalized genes of testis that were down regulated due to MSG administration. Therefore, usage of camel milk as a supplement therapy against toxic materials is very indicative at the testicular levels.

References

1. Eweka AO., Histological studies of the effects of monosodium glutamate on the kidney of adult wistar rats. The Internet Journal of Health, 2007, 6, 2.
2. Samuels, A., The toxicity/safety of MSG: A study in suppression of information. Accountability in Research, 1999, 6: 4,259-310.
3. IFIC., Review of Monosodium Glutamate, Examining the Myths. 1994.
4. Ikeda, K., On the taste of the salt of glutamic acid, Proceedings of 8th Inter. Congress of Appl. Chem., 1917, 38, 147.
5. FDA., Food and Drug Administration Background for Monosodium Glutamate. 1995.
6. Leung, A., Foster, S., Encyclopedia of common natural ingredient used in food, drugs and cosmetics, New York, Wiley, 1996, 373-375.
7. Biodun, D.; Biodun, A., A spice or poison? Is monosodium

glutamate safe for human consumption? National Concord., 1993, 4, 5.
8. Geha, R.S., Beiser, A., Ren, C., Patterson, R., Grammar, L. C., Ditto, A.M., Harris, K.E., Review of allergic reaction to monosodium glutamate and outcome of a multicenter double blind placebo-controlled study. Journal of Nutrition, 2001, 130, 1032S-1038S.
9. Onakewhor, J.U.E., Oforofuo, I.A.O., Singh, S.P., Chronic administration of monosodium glutamate induces oligozoospermia and glycogen accumulation in Wistar rat testes. Africa Journal of Reproductive Health, 1998, 2, 190-197.
10. Oforofuo, I.A.O., Onakewhor, J.U.E., Idaewor, P.E., The effect of chronic administration of msg on the histology of the adult wistar rat testes. Bioscience Res Commun., 1997, 9, 2.
11. Rinne, T., Mutschler, E., Wimmer-Greinecker, G., Moritz, A., Olbrich, H.G., Vitamins C and E protect isolated cardiomyocytes against oxidative damage, Int. J. Cardiol., 2000, 75, 275–281.
12. Al-Attar, A.M., Antioxidant effect of vitamin E treatment on some heavy metals-induced renal and testicular injuries in male mice. Saudi J. Biol. Sci., 2011, 18, 63–72.
13. El-Demerdash, F.M., Yousef, M.I., Kedwany, F.S., Baghdadi, H.H., Cadmium induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats: protective role of vitamin E and b-carotene, Food Chem. Toxicol., 2004, 42:1563–1571.
14. Uzun F.G., Kalender S., Durak D., Demir F., Kalender Y., Malathion induced testicular toxicity in male rats and the protective effect of vitamins C and E, Food Chem. Toxicol., 2009, 47, 1903–1908.
15. Acharya, U.R., Mishra, M., Patro J., Panda, M.K., Effect of vitamins C and E on spermatogenesis in mice exposed to cadmium, Reprod. Toxicol., 2008, 25, 84–88.
16. Uzunhisarcikli, M., Kalender, Y., Protective effects of vitamins C and E against hepatotoxicity induced by methyl parathion in rats. Ecotoxicol. Environ. Saf., 2011, 74, 2112–2118.
17. Paul, M.V., Abhilash, M., Varghese, M.V., Alex, M., Nair, R.H., Protective effects of a-tocopherol against oxidative stress related to nephrotoxicity by monosodium glutamate in rats. Toxicol. Mech. Methods, 2012, 22 (8), 625–630.
18. Saltanat, H., Li, H., Xu, Y., Wang, J., Liu, F., Geng, X.H., The influences of camel milk on the immune response of chronic hepatitis B patients. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi, 2009, 25, 431–433.
19. Knoess, K.H., Milk production of the dromedary. In: Proceeding of the IFS Symposium Camels, Sudan, 1979, 201–214.
20. Rao, M.B., Gupta, R.C., Dastur, N.N., Camels' milk and milk products. Ind J Dairy Sci., 1970, 23: 71–78.
21. Al-Majali, A.M., Bani, Ismail. Z., Al-Hami, Y., Nour, A.Y., Lactoferrin concentration in milk from camel (Camelus dromedarius) with and without subclinical mastitis. Int. J. Appl. Res. Vet. Med., 2007, 5, 120–124.
22. Konuspayeva, G., Serikbayeva, A., Loiseau, G., Narmuratova, M., Faye, B., In: Bernard, Faye, Palmated, Esenov (Eds.), Desertification Combat and Food Safety: The Added Value of Camel Producers. IOS Press, Amisterdam, Ashgabad, Turkmenistan, 2004, 58–167.
23. Al-Ayadhi, L.Y., Elamin, N.E. Evid., Camel Milk as a Potential Therapy as an Antioxidant in Autism Spectrum Disorder (ASD). Based Complement. Alternat. Med., 2013, 1-8.
24. Zafra, O., Fraile, S., Gutiérrez, C., Haro, A., Páez-Espino, A.D., Jiménez, J.I., de Lorenzo, V., Monitoring biodegradative enzymes with nanobodies raised in Camelus dromedaries with mixtures of catabolic proteins. Environ Microbiol., 2011, 13, 960–974.
25. Tawfik, S.A., Al-Badr, N., Adverse Effects of Monosodium Glutamate on Liver and Kidney Functions in Adult Rats and Potential Protective Effect of Vitamins C and E. Food and Nutrition Sciences, 2012, 3: 651-659
26. Hamza, R.Z., AL-Harbi, M.S., Monosodium glutamate induced testicular toxicity and the possible ameliorative role of vitamin E or selenium in male rats. Toxicology Reports, 2014, 1, 1037–1045.
27. Farombi, E.O., Onyema, O.O., Monosodium glutamate-induced oxidative damage and genotoxicity in the rat: modulatory role of vitamin C, vitamin E and quercetin. Hum. Exp. Toxicol., 2006, 25(5):251-9.
28. Igwebuike,U.M., Ochiogu, I.S., Ihidenihu, B.C., Ikokide, J.E., Ikida, I.k., The effects of oral administration of monosodium glutamate (msg) on the testicular morphology and cauda epididymal sperm reserves of young and adult male rats. Veterinarski Arhiv., 2011, 81 (4), 525-534.
29. Onyema, O.O., Farombi, E.O., Emerole, G.O., Ukoha, A.I., Onyeze, G.O., Effect of vitamin E on monosodium glutamate induced hepatotoxicity and oxidative stress in rats. Ind. J. Bioch. Biophy., 2006, 43: 20-24
30. Althnaian, T., Albokhadaim, I., El-Bahr, S. M., Biochemical and histopathological study in rats intoxicated with carbon tetrachloride and treated with camel milk. Springerplus, 2013, 2(1), 57.
31. Satoh, K., Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clinica Chimica Acta, 1978 , 90: 37-43.
32. Aebi, H., Catalase in vitro. Methods in Enzymology, 1984, 105, 121-6.
33. Nishikimi, M., Appaji, N., Yagi, K., The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Biophy. Res. Commun., 1972, 46: 849-854.
34. Blash, S., Melicanm D., Gavinm W., Cryopreservation of epididymal sperm obtained at necropsy from goats. Theriogenology, 2000, 54(6), 899-905.
35. Campbell, R.C., Dott, H.M., Glover, T.D., Nigrosin eosin as stain for differentiating live and dead spermatozoa. J. Agric. Sci., 1956, 48, 1-8.
36. Diniz, Y.S., Fernandes, A.A., Campo, K.E., Mani, F., Ribas, B.O., Novelli, E.L., Toxicity of hypercaloric diet and monosodium glutamate: oxidative stress and metabolic shifting in hepatic tissue. Food Chem.. Toxicol., 2004, 42(2), 319-325.

37.Pavlovic, V., Pavlovic, D., Kocic, G., Sokolovic, D., Sarac, M., Jovic, Z., Ascorbic acid modulates monosodium glutamate induced cytotoxicity in rat thymus. Bratisl Lek Listy. 2009;110(4):205–209
38.Sharma, A., Wongkham, C., Prasongwattana, V., Boonnate, P., Thanan, R., Reungjui, S., Cha'on, U., Proteomic analysis of kidney in rats chronically exposed to monosodium glutamate. PLoS One. 2014;9(12):e116233.
39.Bashan N., Kovsan, J., Kachko, I., Ovadia, H., Rudich, A., Positive and negative regulation of insulin signaling by reactive oxygen and nitrogen species. Physiol Rev. 2009; 89(1):27–71.
40.Mona, E., Ragia, O., Abeer, A. and Mosa, T. Biochemical effects of fermented camel milk on diarrhea in rats. New York Science Journal 2010,.3(5),
41.Jilo, K. , Tegegne, D., Chemical composition and medicinal values of camel milk. International Journal of Research Studies in Biosciences, 2016 (4), 13-25
42.Fabio, R., Seiva, F., Gustavo, L., Chuffa, A., Braga, C.P., Paulo, J., Amorim, A., Fernandes, A.A.H., Quercetin ameliorates glucose and lipid metabolism and improves antioxidant status in postnatally monosodium glutamate-induced metabolic alterations. Food Chem. Toxicol., 2012, 50, 3556–3561.
43.Al-Mamary, M., Al-Habori, M., Al-Aghbari, A.M., Baker, M.M., Investigation into the toxicological effects of catha edulis leaves: a short-term study in animals. Phytoetherapy Research, 2002, 16, 127-132.
44.Selvakumar, E., Prahalathan C., Sudharsan, P.T., Varalakshmi, P., Chemoprotective effect of lipoic acid against cyclophosphamide-induced changes in the rat sperm. Toxicology, 2006, 217, 71-78.
45.Diniz, Y.S., Faine, L.A., Galhardi, C.M., Rodrigues, H.G., Ebaid, G.X., Burneiko, R.C., Cicogna, A.C., Novelli, E.L.B, Monosodium glutamate in standard and highfiber diets: metabolic syndrome and oxidative stress in rats. Nutrition, 2005, 21, 749-755.
46.Singh, P., Mann, K.A., Mangat, H.K., Kaur, G., Prolonged glutamate excitotoxicity: effects on mitochondrial antioxidants and antioxidant enzymes. Mol. Cell Biochem., 2003, 243(1-2), 139-145.
47.Price, A., Lucas, P.W., Lea, P.J., Age dependent damge and glutathione metabolism in ozone fumigated barley: a leaf section approach. J. Exptl. Bot., 1990, 41, 1309-1317.
48.Andersen, J.K., Oxidative stress in neurodegeneration: cause or consequence. Nature reviews; Neuroscience, 2004, Suppl 5: 518-525.
49.Ketterer., B., Taylor., J., Meyer, D., Pemble, P.. Coles, B., Chulin, X., Spencer, S., Some functions of glutathione transferases. In: Structure and function of glutathione transferases. K.D., Tew, C.B., Pickett, T.J., Mantle, Mannervik, J.D., Hayes, eds. CRC Press, Boca Raton, Florida. 1993.
50.Neuefeind, T., Reinemer, P., Bieseler, B., Plant glutathione S- transferases and herbicide detoxification. Biol. Chem., 1997, 378, 199-205.
51.Salami, M., Yousefi, R., Ehsani, M.R., Razavi, S.H., Chobert, J.M. Haertle, T., Enzymatic digestion and antioxidant activity of the native and molten globule states of camel a-lactalbumin: possible significance for use in infant formula. Inter. Dairy J., 2009, 19 (9), 518-523.
52.Giadinis, N., Koptopoulus, G., Roubies, N., Siarkou, V., Papasteriades, A., Selenium and vitamin E effect on antibody production of sheep vaccinated against enzootic abortion (Chlamydia psittaci), Comp. Immunol. Microbiol. Infect. Dis., 2000, 23, 129–137.
53.Gupta, S., Gupta, H.K., Soni, J., Effect of vitamin E and selenium supplementation on concentrations of plasma cortisol and erythrocyte lipid peroxides and the incidence of retinal fetal membranes in crossbred dairy cattle. Theriogenology, 2005, 64, 1273–1286.
54.Soliman, M.M., Hassan, M.Y., Mostafa, S.A., Ali, H.A., Saleh, O.M., (2015). Protective effects of camel milk against pathogenicity induced by Escherichia coli and Staphylococcus aureus in Wistar rats. Mol. Med. Rep., 2015, 12(6), 8306-12.
55.Zhu, W.W., Kong, G.Q., Ma, M.M., Li, Y., Huang, X., Wang, L.P., Peng, Z.Y., Zhang, X.H., Liu, X.Y., Wang, X.Z., Short communication: Camel milk ameliorates inflammatory responses and oxidative stress and downregulates mitogen-activated protein kinase signaling pathways in lipopolysaccharide-induced acute respiratory distress syndrome in rats. J. Dairy Sci., 2016, 99 (1), 53-6.
56.Luo, D.Y., Yang, G., Liu, J.J., Yang, Y.R., Dong, Q. Effects of varicocele on testosterone, apoptosis and expression of StAR mRNA in rat Leydig cells. Asian. J. Androl., 2011, 13, 287-291.
57.Wang, H., Wang, Q., Zhao, X.F., Liu, P., Meng, X.H., Yu, T., Ji, Y.L., Zhang, H., Zhang, C., Zhang, Y., Xu, D.X., Cypermethrin exposure during puberty disrupts testosterone synthesis via down regulating StAR in mouse testes. Arch. Toxicol., 2010, 84, 53-61.

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