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Research Article

Monosodium glutamate induced histological change in the Zona Fasiculata of rats' adrenal and the possible amelioration effect of vitamin C supplementation

Fadia K Abdo[1], Zeinab A Hassan[1,2], Dalia A Mohamed[*1,3], Hanaa S Mousa[1]
[1]*Department of Histology and Cell Biology, Faculty of Medicine, Zagazig University, Zagazig 44519, Egypt*
[2]*Department of Anatomy,Taibah University, Saudi Arabia*
[3]*Department of Anatomy and Histology, Qassim University, Saudi Arabia*

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[*] Corresponding author. E-mail: daliafarag@gmail.com Tel.: 00966546857998/00201223168887

Abstract

Monosodium glutamate in gesting (MSG) is steadily increasing worldwide as a flavour enhancer and food additive. On the other hand, vitamins C has antioxidant properties and can play an important role in preventing or improving many diseases. So, the aim of the present study is to study the impact of MSG administration on the histological structure of the zonafasciculata (ZF) of adult albino rat adrenal cortex and to clarify the possible amelioration effect of vitamin C co-supplementation. Thirty adult male albino rats were divided equally into three groups: group I; negative and positive (received100mg/kg vitamin C) control subgroups. MSG-treated group were administered 2 mg/g body weight MSG via gastric tube andascorbic acid supplemented group were given the same dose of MSG, followed by vitamin C at a dose similar to the positive control group. Tissue sections were obtained and proceeded for light and electron microscope examination. Plasma ACTH and cortisone were estimated. Morphometric and statistical analysis of the results were performed. Plasma ACTH and corticosterone levels in the MSG-treated group were significantly increased comparing to control and MSG-treated group receiving vitamin C. Histologically, in the MSG-treated group, ZF contained highly vacuolated cells and congested blood vessels. The reticular fibres were increased in MSG-treated group decreased in ascorbic acid supplemented group. Ultrastructurally, ZF contained cells with shrunken nuclei and numerous macrophages containing many lysosomes. On the other hand, the cellular architecture of ascorbic acid supplemented group was less affected and congested blood sinusoids were still detected. The reticular fibres were decreased in ascorbic acid supplemented group. Oral administration of MSG caused histological and functional degenerative changes in the ZF of adrenalin adult male albino rat which was ameliorated by supplementation of vitamin C. So, it is recommended to minimize consumption of foodstuffs containing MSG and to eat foods rich in vitamin C after performing more researchers to be sure of these effects on humans.

Keywords: MSG, vitamin C, adrenal cortex, histology, ultrastructure

Introduction

Monosodium glutamate (MSG) is non-essential L-form of glutamatic acid that widely distributed in various foods. It is widely used in manufacturing of canned food, bouillon cubes, frozen food, potato chips, salad dressing, and fast food. It is present in fresh sausages, bottled soy or oriental sauces, synthetic meats, hams, luncheon chicken , flavored tuna and vegetarian burgers (Bojanić *et al.,* 2009). It is believed to impart a fifth, unique taste termed "umami" in addition to the sweet, salty, sour, and bitter taste stimuli. Umami is the meaty mouth-filling rich taste. MSG also enhances the palatability of foodstuffs and disguise unwelcome tastes. (Nayanatara *et al.,* 2009; Bojanić *et al.,* 2009; Savcheniuk *et al.,* 2014). It is also presented in yeast extracts and other food constituents without inscription on the label. So, it could be ingested accidentally (Egbuonu *et al.,* 2009). In 1959, MSG was classified as "generally recognized as safe" substance by the Food and Drug Administration (FDA) But in 1995, FDA declared that MSG might be cause a diversity of clinical disorders comprising asthma, urticaria, angiooedema, rhinitis and obesity which is called the 'monosodium glutamate symptom complex' (Williams and Woessner, 2009).

MSG induced rat obesity by metabolic change characterized by boosting adipocyte capacity to transport glucose and to synthesize lipids resulting in increased insulin sensitivity. It was postulated that the central lesions produced by MSG treatment interrupt the hypothalamic-pituitary-adrenal axis that lead to increase the secretion of adrenalsglands (Savcheniuk *et al.,* 2014).

It has been proved that MSG causes arcuate nucleus damage (Hermanussen *et al.,* 2005) Several studies have linked MSG to many neurological disorders as anxiety, schizophrenia, epilepsy, depression, and degenerative disorders such as Parkinson's disease and Alzheimer's disease (Hlinák *et al.,* 2005; Narayanan *et al.,* 2010).It was established that MSG induces testicular degeneration, sperm abnormalities and decreases the capacity of prostate (Egbuonu, *et al.,* 2010; Ismail, 2012). MSG caused degenerative and atrophic changes in the oocyte and zonagranulosa of the ovary (Eweka, *et al.,* 2010; Ahmed, 2011).

MSG resulted in degenerative and atrophic changes in fundic gland of stomach and Brunner's glands of the duodenum (Eweka *et al.,* 2007). In the liver, inflammation, and centrilobularhaemorrhagic necrosis could be reported (Eweka *et al.,* 2011; Fárr *et al.,* 2010). Moreover, hypertrophy of the epithelial cells lining the bronchioles and

deformation of pneumocytes type I and partial loss of the cytoplasmic organelles of pneumocytes type II was also documented (Ahmed, 2004).

Monosodium glutamate induced cell death by excitotoxic pathway and the oxidative pathway. Oxidative stress is associated with ischemia that caused several clinical disorders such as neurodegenerative diseases, cancer, diabetes, atherosclerosis, chronic inflammation (Linand Beal, 2006). Apoptosis (programmed cell death) is a fundamental and complex biological process in which caspases are crucial mediators that play an important role (Vermeulen *et al.*, 2005).

Ascorbic Acid (Vitamin C) is an antioxidant (Iqbal *et al.*, 2004). It occurs naturally in fresh fruits and vegetables.Natural and synthetic ascorbic acid are chemically similar and there are no differences in their biological effects or bio-availability (Naidu, 2003). It was reported that ACTH stimulates secretion of vitamin C from cells of ZF leading to increased vitamin C concentrations in adrenal vein but not peripheral vein (Padayatty *et al.*, 2007). So, this work aimed to study the impact of MSG administration on the histological structure of the zona fasiculata (ZF) of adult albino rat adrenal cortex and to clarify the possible amelioration effect of vitamin C co-supplementation.

Materials and methods

Animals

Thirty adult male albino rats (220-235 g) were used in this work. They were obtained from the Animal House, Faculty of Medicine, Zagazig University. They were kept at room temperature and allowed food and water adlibitum. All experimental procedures were done in agreement with the guide lines of the Institutional Animal Care and Use Committee of Faculty of Medicine, Zagazig University. Rats were equally divides into three groups (10 rats each).

Monosodium Glutamate

Mono sodium glutamate (MSG) was obtained from Alqahira Pharmaceutical Company in the form of powder. Animals were administrated2g/kg body weightof MSG, so 2000 mg for each kg and since every rat weighs about 200 g (1/5 kg), so every rat was given 2000/5→400 mg MSG dissolved in 2 ml saline

Ascorbic acid (vitamin C)

Vitamin C was obtained from Memphis Pharmaceutical Company in the as cevarol 500 mg tablets. Animals were administrated100 mg /kg body weight ofascorbic acid since every rat weighs about 200 g (1/5 kg), so every rat was given 100/5→20 mg ascorbic acid dissolved in 2 ml saline.

*Experimental design (*Narayanan *et al.*, 2010)

Rats were divided equally into three main groups:

Group I: served as control group and were subdivided into 2 equal subgroups:

Group Ian: given saline by gastric tube for 3 weeks. Group Ib: given 100mg /kg body weight ascorbic acid via gastric tube for 3 weeks. Group II (MSG -treated): given 2 mg/g body weight MSG via gastric tube for 3 weeks. Group III (Ascorbic acid supplemented group): Animals were given the same dose of MSG, followed by 100 mg /kg body weight ascorbic acid via gastric tube for three weeks.

All rats were weighedand then anaesthetized with ether inhalation. Venous blood samples wereobtained from orbital vein to measure serum corticosterone and adrenocorticotrophic hormone (ACTH). Both adrenal glands were resected carefully and prepared for light and electron microscope examination.

Hormonal assay

Venous blood samples wereobtained from rats' orbital veins the day before sacrifice to measure serum corticosterone (9a.m. and 6 p.m.) and ACTH. The results were statistically analyzed.

Histological study

Light microscope: 10 % formol saline were used for sample fixation and paraffin sections (5 µm thick) were prepared for Haematoxylin and Eosin stain (H&E) (Bancroft and Gamble, 2008)

Electron microscope: minute samples ($1mm^3$) were fixed in 2.5 % phosphate buffered glutaraldehyde (pH 7.4) then postfixed in 1 % osmium tetroxide 4˚C, dehydrated and embedded in epoxy resin**.** Semi-thin sections (1 µm thick) were stained with 1% toluidine blue and examined by light microscope. Uranyl acetate and lead citrate were used in staining of ultra-thin sections (El-Drieny *et al.*, 2009)**.** Ultrathin sections were examined and photographed with JEOL JEM 1010 electron microscope in Electron Microscope Research Laboratory of Histology and Cell Biology Department, Faculty of Medicine, Zagazig University.

Morphometric study

The whole adrenal cortex thickness and were measured. Sections with H&E were morphometrically analyzed using image analyzer computer system (Leica Qwin using 500 image analyzer computer system (England), Pathology Department, Faculty of Density, Cairo University).

Statistical analysis

The morphometric and the serological results and were statistically analyzed using SPSS version 15.Data were presented as mean ±SD, andP values less than 0.05 were considered as significant while P value less 0.001were considered highly significant (Ribeiro *et al.*, 2006).

Results

Sereological results

Serum ACTH level

The mean values of serum ACTH levels were 3.37±0.221 µg/dl in the control group, 11.03±0.334µg/dl in MSG-treated group, and 8±0.258 µg/dl in Ascorbic acid supplemented group (Table 1).

Statistical analysis of these results revealed that ACTH level was significantly increased in MSG-treated group in comparing to the control and ascorbic acid supplemented group. In addition, ACTH level in ascorbic acid supplemented group was significantly increase in comparison to the control and significantly decreased in comparison to MSG-treated group.

Table 1: Statistical comparison among groups I, II and III as regard ACTH by one-way ANOVA test

Parameter	Group (Mean ±SD)			F	P
	I	II	III		
ACTH (µg/dl)	3.37 ± 0.221	11.03 ± 0.334	8 ±0.258	1967.78	<0.001**

**= Highly significant (ANOVA- test)

Table 2: Statistical comparison among groups I, II and III as regard a.m. and p.m. serum corticosterone level by one-way ANOVA test

Parameter	Group (Mean ±SD)			F	P
	I	II	III		
A.M serum corticosterone level (µg/dl)	5.18 ± 0.179	8.017 ± 0.123	7.03 ± 0.112	1045.17	<0.001**
P.M serum corticosterone level (µg/dl)	8.45 ± 0.28	14.064 ± 0.308	11.2 ± 0.285	985.023	<0.001**

**= Highly significance (ANOVA- test)

Serum corticosterone level

The mean values of serum a.m. corticosterone levels were 5.18 ±0.179 µg/dl in control group, 8.017 ±0.123 µg/dlin MSG-treated groupand7.03 ±0.112µg/dlin ascorbic acid supplemented group (Table 2 & Bar chart 2).The mean values of serum of serum p.m. corticosterone levels were 8.45 ±0.28 µg/dl in control group, 14.064 ±0.308 µg/dl in MSG-treated group, and 11.2 ±0.285 µg/dl in ascorbic acid supplemented group (Table 2).The mean values of serum of serum p.m. corticosterone levels were 8.45 ±0.28 µg/dl in control group, 14.064 ±0.308 µg/dl in MSG-treated group, and 11.2 ±0.285 µg/dl in ascorbic acid supplemented group (Table 2).Statistical analysis of these results revealed that p.m. serum corticosterone level in MSG-treated group and III was increased significantly comparing to control. While, p.m. serum corticosterone level in ascorbic acid supplemented groupwas significantly decreased compared to MSG-treated group.

Statistical analysis of these results revealed that both a.m. and p.m. serum corticosterone level was increased significantly in MSG-treated group and III comparing to control while ascorbic acid supplemented group showed significantly decreased in a.m. and p.m. serum corticosterone compared to MSG-treated group.

Histological results

Haematoxylin and eosin stained sections of control rats' adrenal gland showed that the adrenal gland was covered by a thin capsule and contained cortex and medulla. The adrenal cortex was formed of three zones; zonaglomerulosa, ZF and zonareticularis (Fig. 1-A).Cells of ZF were arranged in longitudinal parallel cords separated with blood sinusoids in-between. Their cytoplasm was acidophilic and vacuolated. Its cells contained rounded vesicular nuclei with prominent nucleoli (Fig.1-B).MSG-treated group showed that parallel arrangement of the ZF cells was lost. Most cells had darkly stained nuclei and their cytoplasm appeared pale with extensive vacuolation. Dilated capillaries were observed between cells of ZF(Fig.1-C).Ascorbic acid supplemented group showed that the adrenal cortex restored its normal architecture.ZFcells were arranged in parallel columns separated by blood sinusoids. Their nuclei were dark and their cytoplasm showed minimal vacuolation (Fig.1-D).

Silver stain showed reticular fibers in the capsules and between cells of zona glomerulosa and zona fasciculate in control group(Fig. 2-A).MSG-treated groupshowed thick capsules and dense reticular fibers inbetweenzona fasciculate cells (Fig.2-B).Ascorbic acid supplemented group showed moderate amount of reticular fibers in the capsule and between cells of ZFFig. 2-C).

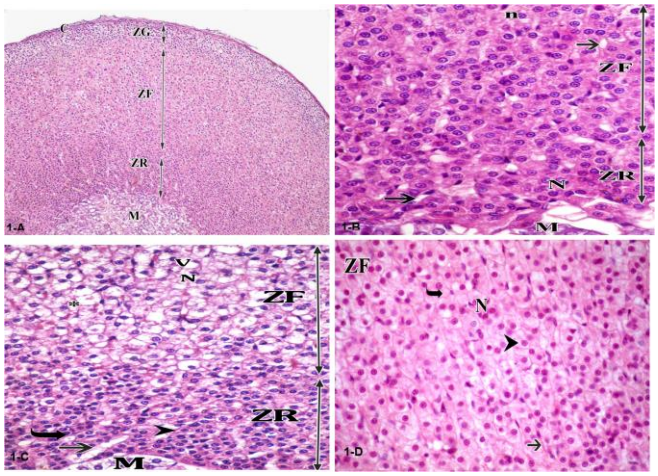

Fig. (1): Photograph of adrenal gland (1-A) showing control adrenal cortex and part of adrenal medulla (M). It is divided into 3 zones; zonaglomerulosa (ZG), zona fasciculata (ZF) and zonareticularis (ZR). [1-B] higher magnification shows cells of ZF arranged in long straight columns separated by blood sinusoids (arrow). They show acidophilic, vacuolated cytoplasm and rounded vesicular nuclei with prominent nucleoli (n). [1-C] showing ZF of MSG-treated rat showing loss of parallel arrangement (ZF). Most cells appear pale with extensive vacuolation (arrowhead) and contain dark nuclei (arrow). [1-D] showing ZF of ascorbic acid-supplemented group showing cells are arranged in parallel columns separated by blood sinusoids (arrow). They contain dark nuclei (N) and acidophilic cytoplasm (curved arrow). Minimal vacuolation are also seen in some cells (arrowhead).

Semithin sections of ZF cells of control group stained bytoluidine blueappeared polyhedral and ere separated by blood sinusoids. Most of cells had rounded nuclei with prominent nucleoli and numerous unstained cytoplasmic lipid droplets (Fig. 2-D).In MSG-treated group, the nuclei of the ZF cells were darkly stained and some of them were shrunken and the cytoplasm contained numerous unstained lipid droplets. The blood sinusoids were dilated and congested. Many macrophages were seen lining the sinusoids (Fig.2-E).Ascorbic acid supplemented group

showed that cells of zona fasciculate cells had rounded nuclei with regular outline and prominent nucleoli while others were small, irregular and dark. Numerous unstained cytoplasmic lipid droplets were also seen. Dilated congested blood sinusoids wers noticed between these cells (Fig.2-F).

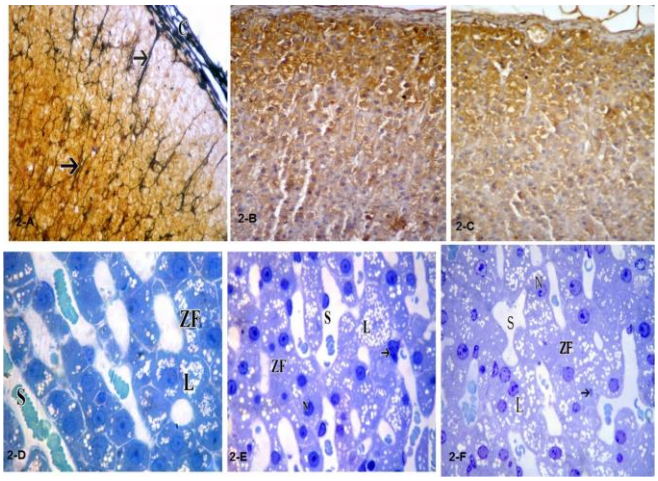

Fig.(2): Silver stain ofcontrol rat's adrenal cortex[2-A] showing capsule (C) and reticular fibers (arrows) between cells of zona glomerulosa (ZG) and zona fasciculate (ZF).[2-B] showing MSG-treated rat showing thick capsule(C) and dense reticular fibers (arrows) in ZG and (ZF).Loss of ZF architecture is observed.[2-C] showing thick capsule(C) and dense reticular fibers (arrows) in ZG and (ZF).Loss of ZF architecture is observed.[2-D]: Semithin section in control rat's adrenal cortexshowing cell of ZF separated by blood sinusoids (S). The cells are large and polyhedral with rounded nuclei and prominent nucleoli (arrowhead) and numerous unstained cytoplasmic lipid droplets (L).[2-E]:ZF of MSG-treated rat showing cells contain many darkly stained nuclei (N);some of them are shrunken (arrow head). The cytoplasm contains numerous lipid droplets (L). The blood sinusoids (S) are dilated and congested. Macrophages are seen lining the sinusoids (arrows).[2-F]:ZF of ascorbic acid-supplemented groupshowing some nuclei (N) are spherical with regular outline and prominent nucleoli while others are small, irregular and dark (arrow). There are numerous unstained lipid droplets (L) inside cells. The blood sinusoids(S) are dilated and congested.

Ultrastructurally, control group revealed euchromatic nuclei with regular outlines and prominent nucleoli in cells of ZF. The cytoplasm contained many mitochondria and lipid droplets (Fig.3-A). In MSG-treated group, some cells of ZF had shrunken appoptotic nuclei while others contained electron dense nuclei. Their cytoplasm contained mitochondria and lipid droplets. Dilated sinusoids and infiltrating lymphocyte were also seen (Fig. 3-B, C]. Macrophages contained many lysosomes [Fig.3-D].ZFof ascorbic acid-supplemented group revealed cells had euchromatic nuclei with prominent nucleolus. Many lipid droplets and lysosomes were found in their cytoplasm. The blood sinusoids (S) were still congested [Fig.3-E].

Discussion

The adrenal gland is the most common endocrine organ linkedto chemically induced injuries. Adrenal cortical cells are a major site of lipid storages used for steroid genesis (Rosol *et al.*, 2001).

Many environmental toxins and food additives have been accused to induce variousdamaging effects. Concerning food additive, they used for food preservationandtaste enhancers. Monosodium glutamate (MSG) is considered the most commonly used food flavor enhancer. Unfortunately, it was proved to cause deleterious health effects (Fárr *et al.*, 2010).

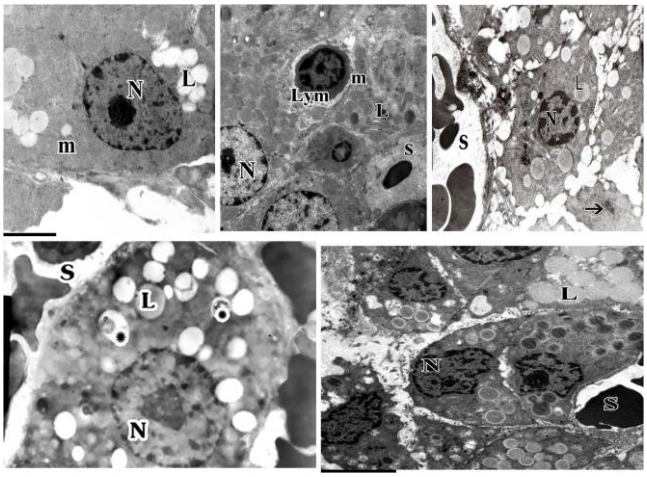

Fig. (3): A transmission electron micrograph of control rat's ZF [3-A] showing a cell haseuchromatic nucleus (N) with regular outline and prominent nucleolus. The cytoplasm contains many mitochondria (m) and lipid droplets.[3-B,C, D]:ZF of MSG treated group] showing infiltrating lymphocyte (lym).). Shrunken electron dense nucleus (N). Apoptotic nucleus (arrow) is also noticed. The cytoplasm contains mitochondria (m) and manylipid droplets (L). Dilated blood sinusoid is also seen(S).Macrophage is also found. Their cytoplasm contained many lysosomes. [3-E] ZF of ascorbic acid-supplemented group showing a cell from ZF. The nucleus (N) is euchromatic with prominent nucleolus. The cytoplasm contains many lipid droplets (L) The blood sinusoids (S) are congested.

Previous researches proved harmful effects of MSG on various body organs, but there was no enough researches discussing its effect on the adrenal cortex especially ZF. On the other hand, ascorbic acid as an antioxidant plays a crucial role in ameliorating these effects. So, this current work aimed to study the structural variations of the ZF after MSG administration andassess the role of vitamin C supplementation

In the present work, the function of the ZF was assessed by measuring plasma ACTH and corticosterone in the serum of rats in all groups. Corticosterone hormone concentration was also measured also at 8 a.m. and 8 p.m. Statistical analysis of these results revealed that corticosterone level in the MSG -treated group (II) was significantly increasd comparing to the control group. Macho et al.1999referred the elevation in corticosterone level to the increased production by the glands and decreased rate of clearance in the liver. Glutamate is excitatory neurotransmitter; it can stimulate the hypothalamo-pituitary-Adrenal (HPA) axis and consequently elevates ACTH (Zelena *et al*, 2004*)*. It was observed that hypersecretion of ACTH is associated with hyperplasia of basophilic pituitary cells. This could be explained by neurotoxic effect of MSG not only on hypothalamic growth hormone releasing hormone. In

addition, MSG induced corrticotrophin-releasing factor (CRF) which stimulates the release of ACTH by pituitary gland causing rise in the serum concentration of ACTH and corticosterone (Bojanović *et al.*, 2007).

Light microscope examination of the ZF of MSG - treated group revealed irregular cellular orientation accompanied by swelling and vacuolation of its cells. Dilated blood sinusoids were also seen. Statistically, analysis of morphometric results indicated a significant decrease in cortical thickness in comparison to control group. Tsou et al.(2004)who confirmed that lipid peroxidation following oxidative stress lead to loss of membrane integrity and cell degeneration. In addition, ROS promote alterations in protein properties. Thus affect the receptors function, enzymes, antibodies and transport proteins and causes alterations in the DNA (Halliwell, 2007).

Silver-stained sections revealed increased amount of reticular fibers in the MSG-treated group. These changes were related to tissue damage which is followed by a complex set of correlated cellular and humoral reactions that remove or neutralize deleterious agents, eliminate the damaged tissue, and promote healing (Cotran *et al.*, 1994). Most of these reactions happened in the connective tissues and most healing eventually depends on the deposition of collagen. Reticular fibers were reported to increase with oxidative stress in experimentally induced diabetic rats (Ribeiro *et al.*, 2006).

Examination of the same group revealed many lipid droplets in cytoplasm of ZF cells. Impaired steroidogenesis is an important mechanism of toxicity in the adrenal cortex. This effect was related to the inhibition of cholesterol biosynthesis or metabolism and disruption of cytochrome P-450 enzyme. Consequently these two mechanisms will lead to increased cytoplasmic lipid in the form of discrete droplets (Rosol *et al.*, 2001).

In the present work, ultrastructural examination of MSG-treated group confirmed the light microscopic observations; the affected cells showed shrunken electron dense nuclei. It was reported that MSG has a direct toxic effect on cell function which is caused mainly by imbalance in homeostasis of cysteine, the precursor of glutathione (GSH), leading to depletion of intracellular GSH levels and reduced ability to protect against oxidative injury in the cell and, ultimately cell damage. Moreover, lipid peroxidation may eliminate the active sulfhydryl group of GSH and other enzymes. Thus oxidative stress and accumulation of free radicals seems to be responsible for MSG toxicity (Diniz *et al.*, 2004; Farombiand Okwudiri, 2012).

MSG caused increase in the popularion of l macrophages in ZF .Their cytoplasm contained numerous multilamellar bodies. Similar findings were observed by Liu *et al.* (1989) who studied the effect on t MSG on the rat alveolar macrophages. Itwas alsomentioned that IL-1 and TNF- α have a direct, ACTH-independent, stimulatory effect on corticosterone secretion in rats(Ozbek and Ozbek, 2006) Also, it was reported that MSG treatment induced endocrine disorders and altered cell-mediated immune responses in general and dysfunction of lymphocytes and macrophages in particular. In addition, cytokines released by macrophages, such as tumour necrosis factor (TNF) - α, interleukin (IL)-1 and IL-6, may influence adrenocortical steroidogenesis through medullary catecholamines (Obochi *et al.*, 2009).

In the present study, light microscope examination of ascorbic acid-supplemented group showed relatively preserved architecture of adrenal cortex. Moderate vascular dilatation and congestion were observed. Hartel *et al.*, (2004) stated that ascorbic acid primarily that exerts its effect on host defense mechanisms and immune homeostasis by being the most important physiological antioxidant Sagun *et al.*, (2005) found that the oxidized form of vitamin C, dehydroascorbic acid (DHA) enters mitochondria via facilitative glucose transporter 1 (Glut1) and accumulates as ascorbic acid which in turn scavenges mitochondrial ROS and inhibited oxidative mitochondrial DNA damage.

In the present study, ultrastructural examination of the same group revealed less affection of cellular ultrastructure. Most of the cells showed euchromatic nuclei and their cytoplasm contains many lipid droplets. Few cells showed electron dense nuclei and mitochondria with ruptured cristae. Ibrahim *et al.*, (2011) reported that vitamin C was to scavenge aqueous reactive oxygen species (ROS) by rapid election transfer that inhibits lipid peroxidation. Further study showed that administration of MSG increased serum alanine aminotransferase and aspartate aminotransferase dose dependently. These elevated parameters were reduced after pretreatment with vitamin C. The histological changes induced by MSG were also ameliorated. Moreover, vitamin C decreases lipid peroxidation either directly or indirectly by regenerating vitamin E, the major lipid-soluble antioxidant (Adikwu and Deo, 2013).

In conclusion, this study has verified that oral administration of MSG resulted in histological and functional degenerative changes in zona fasiculata of the ZF of adult male Albino rats, and the supplementation of ascorbic acid was proved to reduce the harmful effects of MSG on the suprarenal cortex. Therefore, it is advised to minimize the intake of foodstuffs containing MSG and to consume foods rich in ascorbic acid. Additionally, we recommend more research on the effect of MSG on the ZF and to correlate the results with the clinical practice to avoid its harmful effects.

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