The Possible Histological Defensive Effect of Vitamin C Against MSG Thymus toxicity of Adult Male Albino Rat

Original Article

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ABSTRACT

Introduction: Monosodium Glutamate (MSG) as a flavour enhancer is still being widely used in a range of diet formulations. Several studies have recently revealed undesirable side effects of its long-term intake. The immune system is a potential target of toxicity following chemicals exposure. Thymus is a primary lymph organ which produces immunocompetent T cells. It enhances the maturation of lymphoid precursors into T cells to establish the T cell pool during life in human.

Aim of the Work: To evaluate the toxic effect of MSG on the thymus of adult male albino rat and the possible protecting role of vitamin C when administered concomitantly.

Materials and Methods: Forty adult male albino rats were used for this work and were divided randomly into four equal groups; Group 1 used as control, Group II received 100 mg/kg vitamin C, Group 3: received 3 g/kg MSG and Group 4 received both MSG and vitamin C concomitantly. Both drugs were given once daily orally. After 60 days the thymus glands were obtained and processed for histological, immunohistochemical using caspase 3, morphometric and statistical studies.

Results: MSG administration resulted in marked decrease in thymic cellularity with significant increase in caspase 3 positive cells in association with ultrastructural changes in the thymocytes and epithelial reticular cells. Concomitant administration of vitamin C with MSG reduced the previous histological alteration with decrease in caspase 3 positive cells.

Conclusion: Monosodium glutamate affects the histological structure of the thymus gland and its concomitant administration with vitamin C diminished its thymic toxicity.

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Key Words: MSG, thymus, vitamin C.

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INTRODUCTION

The general health of the world's population is under siege from the dramatic changes in life conditions. Environmental and lifestyle changes, including diet are seriously increasing the burden of ill health. Nowadays, many food additives acting either as preservatives or enhancer of palatability are present. (MSG) is one of this commonly used food-additives in industrial foods[1,2,3].

(MSG) is the sodium salt of glutamic acid which is a nonessential amino acid. High-level of MSG is observed in a variety of food resources, involving seaweed, soy sauce, tomatoes, parmesan cheese, and breast milk. The mean consumption of MSG /day is assessed to be 0.3-1.0 g[4]. Those doses possibly disturb neurons and might have harmful effects on behavior.The circulating MSG is disassociated into sodium ion and L-glutamate. In the GIT, dietetic glutamate is widely metabolized by liver into a major energy substrate, plays a role in amino acid metabolism, and is the precursor of several important biologically active molecules[5,6]. MSG acts on the glutamate receptors and releases neurotransmitters which play a vital role in normal physiological as well as pathological processes[7]. Glutamate is the most important excitatory neurotransmitter in the central and peripheral nervous system[8]. Beside to the central nervous system, different organs and tissues contained glutamate receptors such as ovaries, liver, kidney, testis, endocrine glands, hypothalamus and thymus[9]. Glutamate receptors were also discovered in mouse, rat and human lymphocytes[10].

Different studies stated that MSG has a potential toxic effects linked to this food-flavoring property. It was reported that 20 minutes after intake of MSG rich meal (2-4 g/kg body weight) the following symptoms occur; burning sensation at the back of the neck, blistering on arms and occasionally on the anterior thorax, general fatigue, and palpitations. Those symptoms took the term "Chinese restaurant syndrome"[11]. The toxic effects include also CNS disorders[8], obesity[12], disruptions in adipose tissue physiology, hepatic damage[13], congenital rubella syndrome and reproductive malfunctions either in female[14], or in male[15]. Several animal and human studies have proved that usage of even the lowest dose of MSG has toxic effects. Previous studies indicated that MSG augmented apoptosis in rat thymocytes, by decreasing Bcl-2 expression[10].

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Vitamin C is an organic compound with antioxidant properties. Except human, most of the higher animals can synthesize vitamin C. It is necessary for normal growth and development, and has been shown to guard against tissue toxicity and oxidative stress[16]. It has been stated that toxicity of MSG can be diminished by the use of vitamins like A, C, D and E. Quercetin and diltiazem have also been proposed to have protecting role in MSG-induced toxicity[17]. Curcumin displayed a neuroprotective role in nerve cells and cerebral cortex of albino rat against MSG[18].

The primary lymphoid organ; thymus plays a critical role in the growth of T cells. Its definitive inability to balance for the peripheral utilization of mature T-cells may affect significantly the capacity to hold immune responses to novel antigens. The thymus continues its active process of thymopoiesis into the fourth decade of life manifested by the presence of active thymic tissue with DP(double positive) thymocytes, which are greatly reduced after 40yrs of age in human however mice at 2 years of age is fully capable of generating recent thymic immigrants[19]. The thymus begins to shrink and novel T cell production drops to a lower rate (its function declines at a rate of nearly 3% per year throughout adulthood).This process is described to as involution, which is defined as a decrease in the activity, weight and size, of the thymic gland with progressing age. Even though it remains to act as the site of T-cell development and creation throughout adulthood[20], the thymus largely degenerates into fatty tissue in aged adults[21].

The data about effect of MSG on the cells of the primary immune organs and potential protecting impacts of specific antioxidants are still mostly unknown. In the present research, we studied the possible protective role of vitamin C against MSG induced thymic toxicity in adult male albino rat thymus.

MATERIALS AND METHODS

Chemicals

MSG was obtained in the form of L- Glutamic acid Sodium Salt 500 gm (Eastrin Fine Chemicals. LTD) from Gomhoria Company. The powder was dissolved in distilled water to get the desired concentrations.

Vitamin C was obtained in the form of L- ascorbic acid 100 gm tablets (Universal Fine Chemicals PVT. LTD India A.R. grade). It was dissolved in distilled water to get the desired concentrations. Both drugs were given once daily by oral gavage.

Animals

This study was carried out on 40 adult male albino rats, weighing from 150- 200 gm with age range (11-12 weeks). They were housed in clean correctly aired cages under the same environmental conditions with supply of food and water through the entire period of the experiment. All animal procedures in the study followed the guideline for work on experimental animals and approved by Ethical Committee of Faculty of Medicine of Tanta University. The animals were divided randomly into four equal groups (10 rats each):

Group 1(control): served as control group.

Group 2 (vitamin C): received vitamin C only 100 mg/kg body weight/day.

Group 3 (MSG): received MSG 3 g/kg body weight/day (the symptomatic dose).

Group 4 (MSG & Vit C): received concomitantly both MSG and vitamin C (3 g/kg body weight/day and 100 mg/kg body weight/day respectively) .

The drugs and their vehicle were given orally by gastric tube once daily for two months. Weekly recording of animals body weights for adjustment of the dose based on the most recent body weight for each dose group as carried out. The above doses were based on previous reports[22,23,24]. The animals were sacrificed after 60 days.

Specimen collection and preparations

For obtaining tissue specimens, perfusion fixation was used. Perfusion was conducted for 20 minutes till the animal organs hardened and acquired a yellowish tint[25].

For light microscope examination

The specimens were fixed in 10 % neutral buffered formalin, washed, dehydrated, cleared then embedded in paraffin. Serial sections of 5µm were stained with Hematoxylin and Eosin according to Bancroft and Gamble, (2008)[26].

For immunohistochemical detection of active caspase3 immune reaction

Anti-Cleaved Caspase-3 antibody [E83-77] (ab32042) was used. Incubation of sections were done with the primary cleaved caspase-3 antibody at 4°C overnight. Detection of primary binding was done using a horseradish peroxidase-conjugated goat anti-rabbit antibody (Vector Laboratories, Burlingame, CA, USA) and visualized by development with 3, 3- diaminobenzidine (DAB, Sigma). All sections were counter stained with haematoxylin. Immune reaction was detected in the cytoplasm of the examined cells[27]. Negative control was done by skipping the step of adding of primary antibody. A section of the kidney was used as a positive control and was provided by (Biocare medical number CP229A)

For transmission electron microscopy

Tissue samples were put in 2% buffered glutaraldehyde fixative, washed with PBS and fixed in 1% osmium tetroxide. Dehydration was done by using ascending grades of alcohol and embedding was completed via epoxy resins. Ultrathin sections (50–60 nm) were prepared by ultramicrotome (Leica Microsystems, Vienna, Austria) to be installed on copper grids and stained with uranyl acetate and lead citrate[25]. The sections were examined using a

Jeol JEM- 100 Transmission Electron Microscope (Jeol, Tokyo, Japan) In Electron Microscopic Unit, Faculty of Medicine, Tanta University (Egypt).

Digital morphometric study (Computer Assisted digital image analysis)

Slides were photographed using Olympus® digital camera installed on Olympus® microscope with 1/2 X photo adaptor to assess the following parameters,:

a. Cortical thymic thickness: This was done on H&E stained sections using 10X objective by measuring in um the length of a line drawn from the outer limit of the cortex passing perpendicular till corticomedullary junction.

b. Area percentage of anti caspase 3 positive immunoreactions in immunostained sections using 4Xobjective.

Ten different non overlapping randomly selected fields from each slide of each rat of each group were examined. The results of the images were analysed on Intel® Core I7® based computer using VideoTest Morphology® software (Russia).

Statistical analysis

Data analysis were done using one-way analysis of variance (ANOVA), then Tukey's test to compare between the groups using the statistical package for the social sciences (SPSS) (version 11.5; SPSS Inc., Chicago, Illinois, USA). The values were expressed as mean ± SD. Differences were considered as significant if *P values* were less than 0.05 and highly significant if *P values* were less than 0.001[28].

RESULTS

Light microscopic results for Hematoxylin and eosin stains

H &E stained sections of the control and vitamin C treated groups showed the normal histological structure of thymus gland. It was covered by connective tissue capsule and was formed of multiple incomplete lobules separated by thin connective tissue septa. Each lobule was formed of darkly stained cortex and lightly stained medulla. The cortex was occupied by multiple overlapped thymocytes displaying dark basophilic nuclei separated by blood capillaries. The lightly stained medulla demonstrated many blood capillaries, less densely packed thymocytes and vesicular nuclei of epithelial reticular cells. Other epithelial reticular cells were embedded in acidophilic material forming Hassel, s corpuscles (Figure 1 (A,A1)).

Examination of sections obtained from group III (MSG treated group) revealed loss of the normal histological architecture of the thymus gland. Prominent narrow cortex was evidently seen around cellularly depleted corticomedullary junction and lightly stained wide medulla. The epithelial reticular cells were clearly observed forming Hassal's corpuscles (Figure 1 (B,B1)).

However, sections obtained from group IV (protective group) demonstrated restoration of the normal histological architecture of thymus to be more or less similar to control group (Figure 1 (C,C1)).

Immunostaining results with Anti-Cleaved Caspase-3 antibody

The control group revealed negative immune reaction for caspase 3 in both cortex and medulla except for few scattered thymocytes that showed mild reaction in their cytoplasm (Figure 2 (A,A1)). MSG treated group demonstrated wide spread strong positive cytoplasmic caspase 3 reaction in many thymocytes and epithelial reticular cells of cortex and medulla (Figure 2 (B,B1)). However, group IV showed apparent decrease in this reaction where few scattered thymocytes depicted mild faint reaction for caspase 3 in their cytoplasm (Figure 2 (C,C1)).

Electron microscopic results

Thymus gland of both control (I) & vitamin C (II) treated groups showed the same ultrastructural features. Cortex and medulla were seen densely packed with variable sized thymocytes separated from each other by cytoplasmic processes of epithelial reticular cells. The thymocytes contained large heterochromatic nuclei and surrounded by thin rim of cytoplasm (Figure 3 (A,B,C,D,E,F)). Some of them were seen in mitosis (Figure 3(C)). Different types of epithelial reticular cells were seen also in the same regions. Type I epithelial reticular cells were seen in sub capsular region, connective tissue septa and perivascular spaces of the cortex and the medulla. They had elongated or rounded euchromatic nuclei with prominent nucleoli (Figure 3 (A&E)). Type II epithelial reticular cells were observed in the outer and mid cortical regions (Figure 3 (A)). Some epithelial reticular cells displayed autophagosomes formed of double-membranes sequestering vesicles and vacuoles surrounded by intact mitochondria and rER (Figure 3 (B)). While type III epithelial reticular cells were detected in close contact to dividing thymocytes deeper in the cortex, (Figure 3 (C)). Type IV epithelial reticular cells were found in the deeper part of the cortex and more abundant in the medulla. They had numerous processes rich in tonofilaments (Figure 3 (D)). Type V epithelial reticular cells were seen at the cortico-medullary junction in addition to blood capillaries that were seen lined by continuous endothelium surrounded by continuous basal lamina and type I epithelial reticular cells forming blood thymic barrier (Figure 3 (E)). Type VI epithelial reticular cells were depicted in the medulla disclosing central euchromatic nuclei, rER, electron dense granules and, multiple desmosomes and were surrounded by multiple thymocytes (Figure 3 (F)).

Thymic gland of group III (MSG treated group) showed considerable ultrastructural alterations spanning both cortex and medulla. Cortical region revealed decrease cellularity and wide interstitium containing cellular debris. Variable sized thymocytes were widely separated and

revealing nuclear chromatin margination and micronucleus formation (Figure 4 (A,B)). The epithelial reticular cells showed small electron dense pyknotic nuclei and rarified cytoplasm with ill defined organelles in addition to fragmented processes (Figure 4 (A)). Another epithelial reticular cell in the same region most probably type II contained shrunken irregular electron dense nucleus and dilated proliferated rough endoplasmic reticulum (Figure 4 (C)). Continuous blood capillaries at the corticomedullary junctions were lined by swollen endothelium containing distended mitochondria with cristolysis. They were surrounded by discontinuous basal lamina and wide subendothelial space. The processes of the surrounding epithelial reticular cells were swollen, disrupted leaving capillary bare area and had attenuated, ill-defined desmosomes. Wide interstitium filled with cellular debris and fat droplets was also observed (Figure 4 (D)). The medulla showed wide interstitium full of cellular debris. Medullary thymocytes had discontinuation of their cell membranes with the release of their cellular contents into interstitium. Their nuclei displayed either peripherally condensed chromatin or micronuclei and fragmentation. Some medullary epithelial reticular cells appeared degenerated, disintegrated while others were multinucleated with marked chromatin condensation (Figure 4 (E,F)).

As regards group IV (MSG & Vitamin C treated group), restoration of the normal ultrastructure of the thymus gland was observed. The cortical region was formed of normal variable sized thymocytes and different types of epithelial reticular cells with intact processes mostly type I, III &IV. Some thymocytes were still widely separated by the processes of normal epithelial reticular cells and some contained small electron dense nuclei, while others contained mitotic nuclei (Figure 5 (A,B)). At corticomedullary junction, normal thymocytes were seen in addition to dividing one. Continuous blood capillaries with intact basal lamina were seen surrounded by epithelial reticular cells type I containing well defined cytoplasmic organelles and surrounded by continuous basal lamina forming blood thymic barrier. (Figure 5 (C)). The medulla contained normal thymocytes and epithelial reticular cells (VI) exhibiting euchromatic nuclei, dilated rER, areas of rarified cytoplasm and surrounded by discontinuous plasma membrane displaying evident desmosomes (Figure 5 (D)).

Morphometric and statistical results

1- Cortical thickness of the thymus glands

The mean total cortical thickness showed significant decrease in MSG treated group compared with control, vitamin c treated and protective groups. Significant improvement could be observed in the vitamin C protected group, when compared with MSG group (Tables 1,2, Histogram 1):

2-Area percentage of caspase 3 positive immunoreactions

MSG treated group revealed highly significant increase in the mean area percentage of caspase 3 positive immunoreaction compared to control ,vitamin C treated and protective groups. Significant improvement could be observed in the vitamin c protected group, when compared with MSG group(Tables 3,4, Histogram 2):

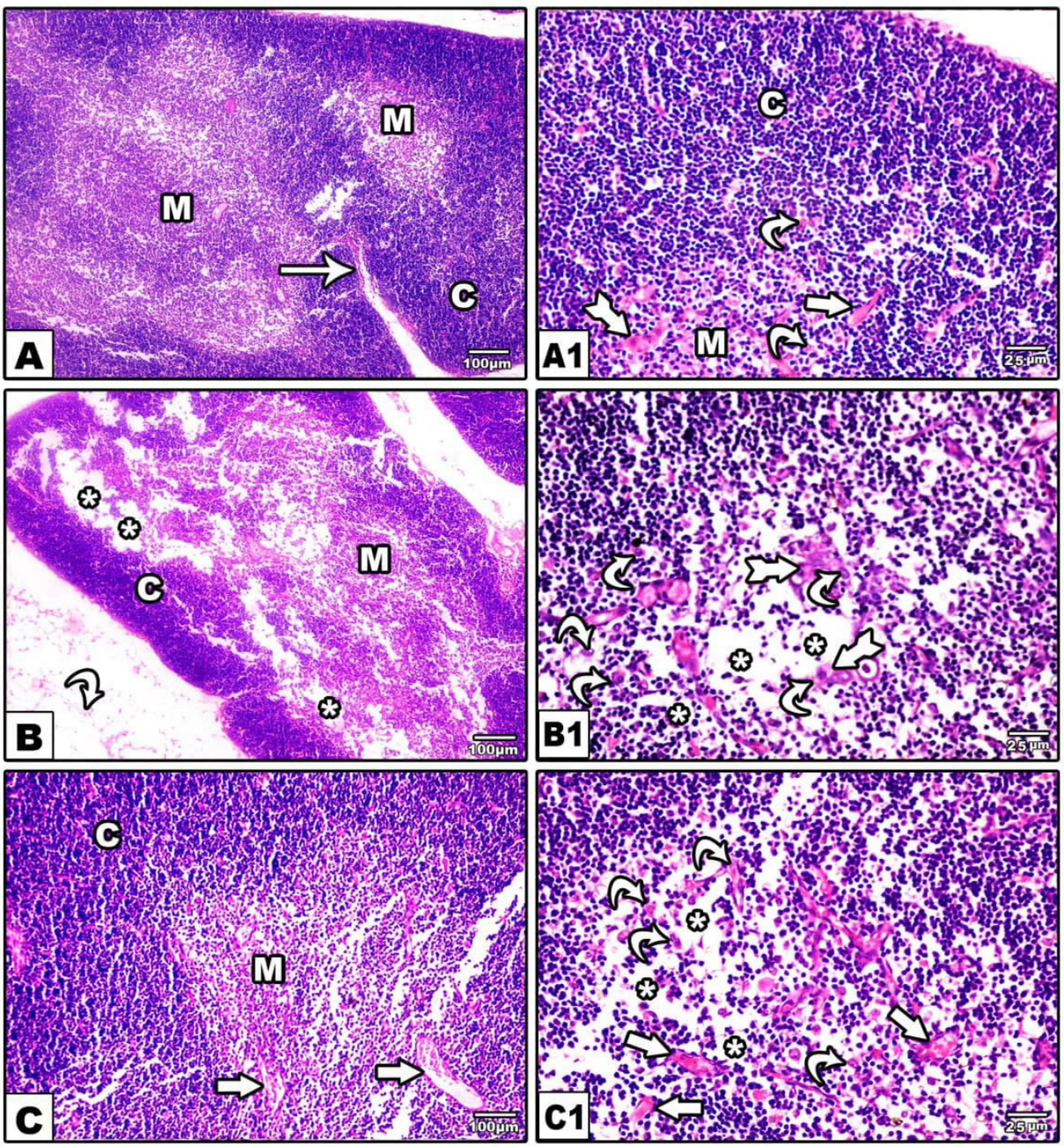


Fig. 1: photomicrographs of H & E stained thymic sections Control group (A &A1); A): shows multiple lobules with darkly stained cortex (C) and lightly stained medulla (M) and thin septa between lobules(arrow). H&E x 200. A1): exhibits darkly stained cortex (C) occupied by multiple overlapped thymocytes displaying dark basophilic nuclei and separated by blood capillaries (thick arrow). Lightly stained medulla (M) demonstrates less densely packed thymocytes and vesicular nuclei of epithelial reticular cells (curved arrows). Some epithelial reticular cells embedded in acidophilic material forming Hassels, corpuscle (bifid arrow) .H&E x 400. MSG treated group (B&B1) ; B): shows narrow cortical zone(C) and wide lightly stained rarified medulla (M) with marked cellular depletion (stars) at corticomedullary junction. Notice the gland is surrounded with thick white adipose tissue (curved arrow). H&E x 200. B1): demonstrates lightly stained medulla having many empty spaces(stars) ,loosely packed thymocytes and epithelial reticular cells with vesicular nuclei (curved arrows) in association with Hassal , s corpuscle(bifid arrows).H&E x400. Protective group (C&C1):C): shows darkly stained cortex (C) and lightly stained medulla (M)with many blood capillaries in between (thick arrows)and less densely packed thymocytes. H&E x 200. C1: lightly stained medulla (M) demonstrating many blood capillaries(thick arrows) ,less densely packed thymocytes and vesicular nuclei of epithelial reticular cells (curved arrows) and multiple empty spaces (stars).H&Ex400.

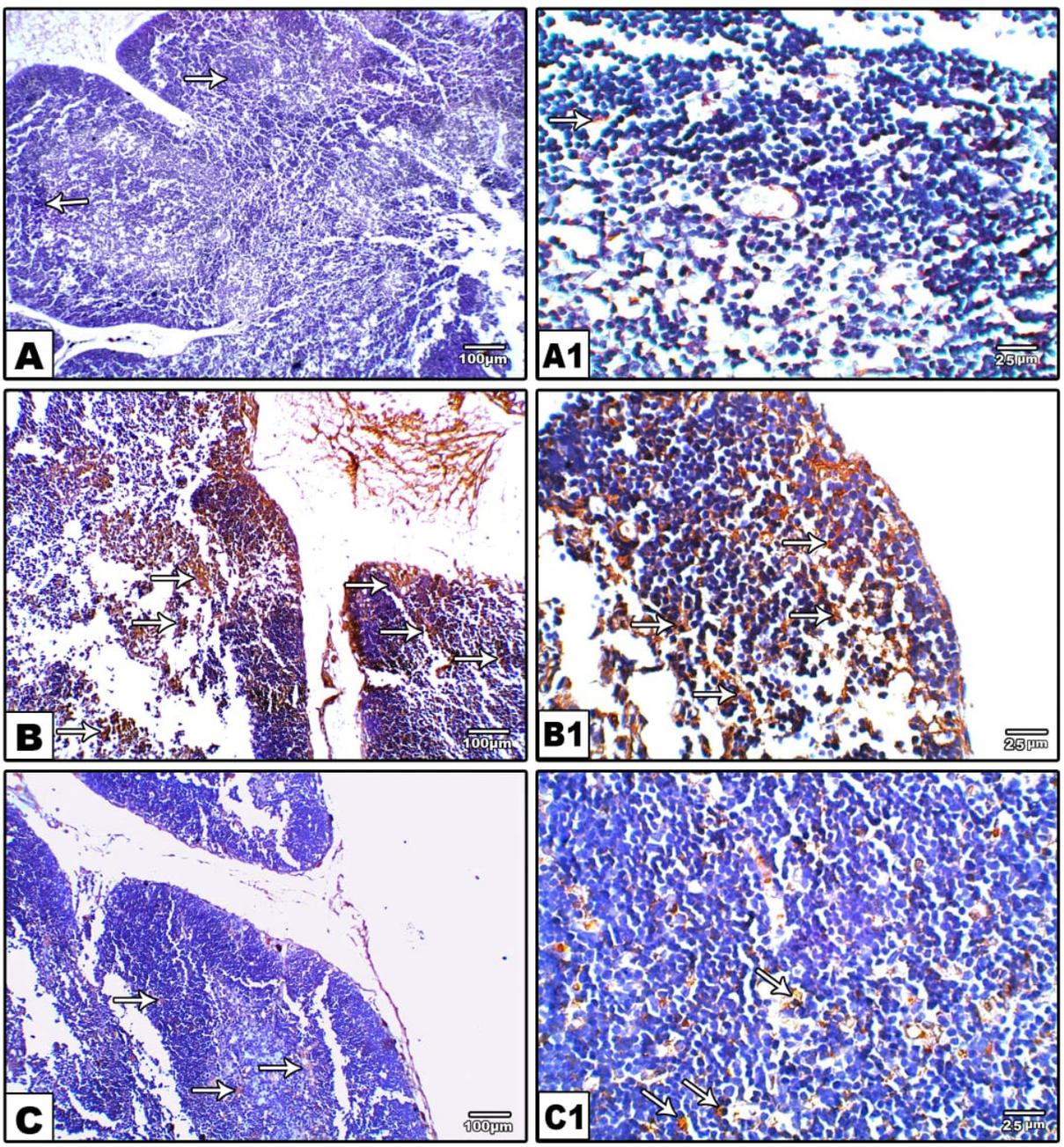


Fig. 2: Photomicrographs of caspase 3-immunoŝtaitned thymic sections Control group (A &A1) showing faint brown cytoplasmic immune reaction in few thymocytes and epithelial reticular cells (arrow) . x100&x400. MSG treated group (B &B1): showing intense cytoplasmic immune reaction in many thymocytes and epithelial reticular cellsx100& x400. Protective group (C&C1): showing mild cytoplasmic immune-reaction in few thymocytes and epithelial reticular cells x 100&x400.

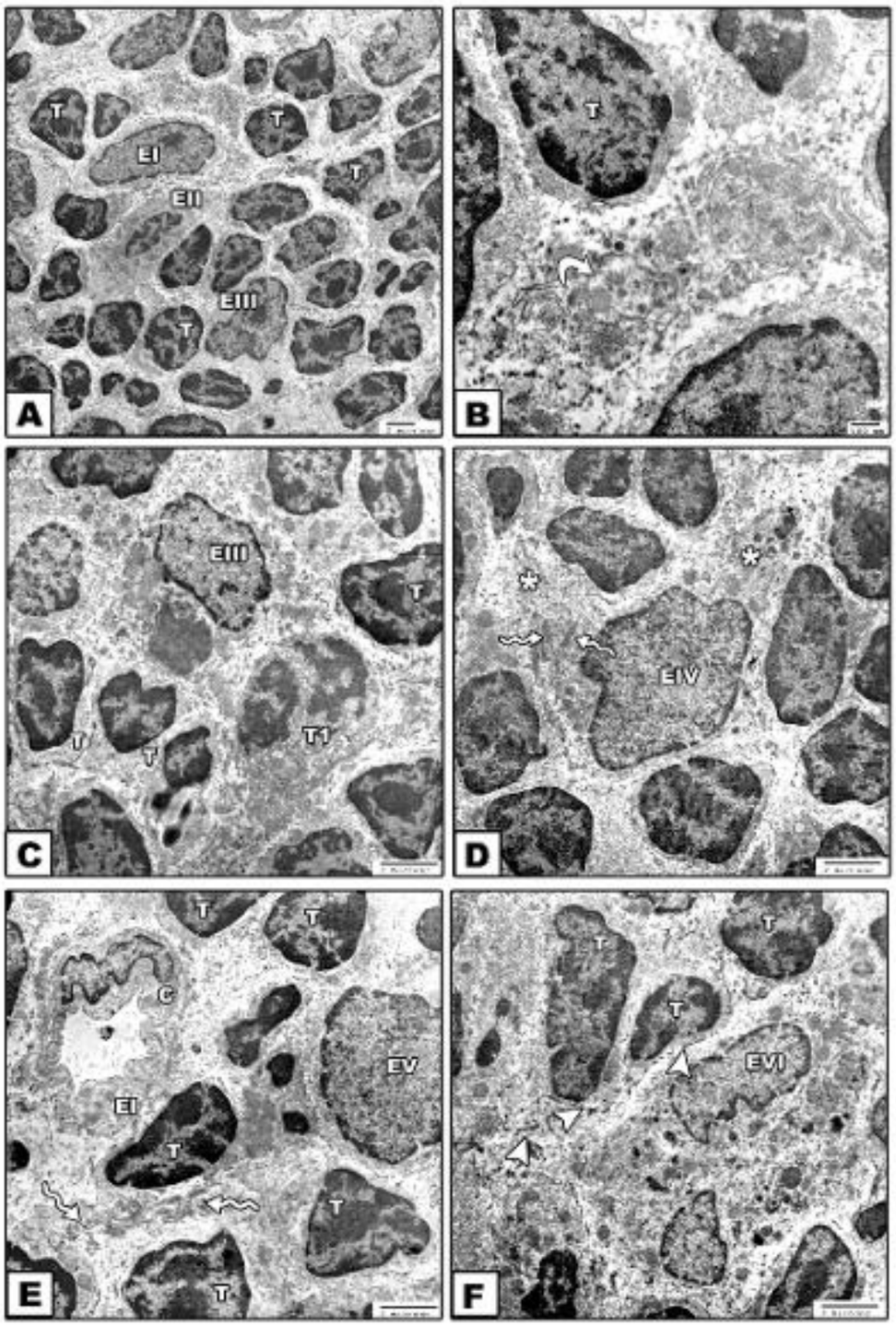


Fig. 3: Electron micrograph of thymic ultrathin sections of control group: **A):** shows cortex containing variable sized thymocytes (T) with heterochromatic nuclei and separated from each other by cytoplasmic processes of epithelial reticular cells. Notice there are three types of epithelial reticular cells (E I, E II and E III) containing euchromatic nuclei with prominent nuclei X5850. **B):** shows epithelial reticular cell containing autophagosome X23400. **C):** demonstrates cortex with different sized thymocytes(T), mitotic thymocytes (T1) and type III ERC(EIII) full of mitochondria. X11700. **D):** depicts epithelial reticular cell type IV (EIV) with euchromatic nucleus, long processes and abundant cytoplasm rich in tonofilaments(hay stack) (wavy arrows).They were surrounded by variable sized thymocytes and wide interstitium (star) in the inner cortex.X11700 . **E):** shows blood thymic barrier formed of continuous blood capillary(C) with continuous basement membrane and surrounded by epithelial reticular cell type I(EI). Epithelial reticular cell type V(E V) was seen in addition to thymocytes (T) with processes of epithelial reticular cells in-between (wavy arrows) containing tonofilaments at the corticomedullary junction . X11700 **F):** shows medulla with epithelial reticular cell type VI (EVI) having euchromatic nucleus, electron dense granules, RER ,mitochondria and multiple desmosomes (arrow heads) surrounded by thymocytes (T). X11700

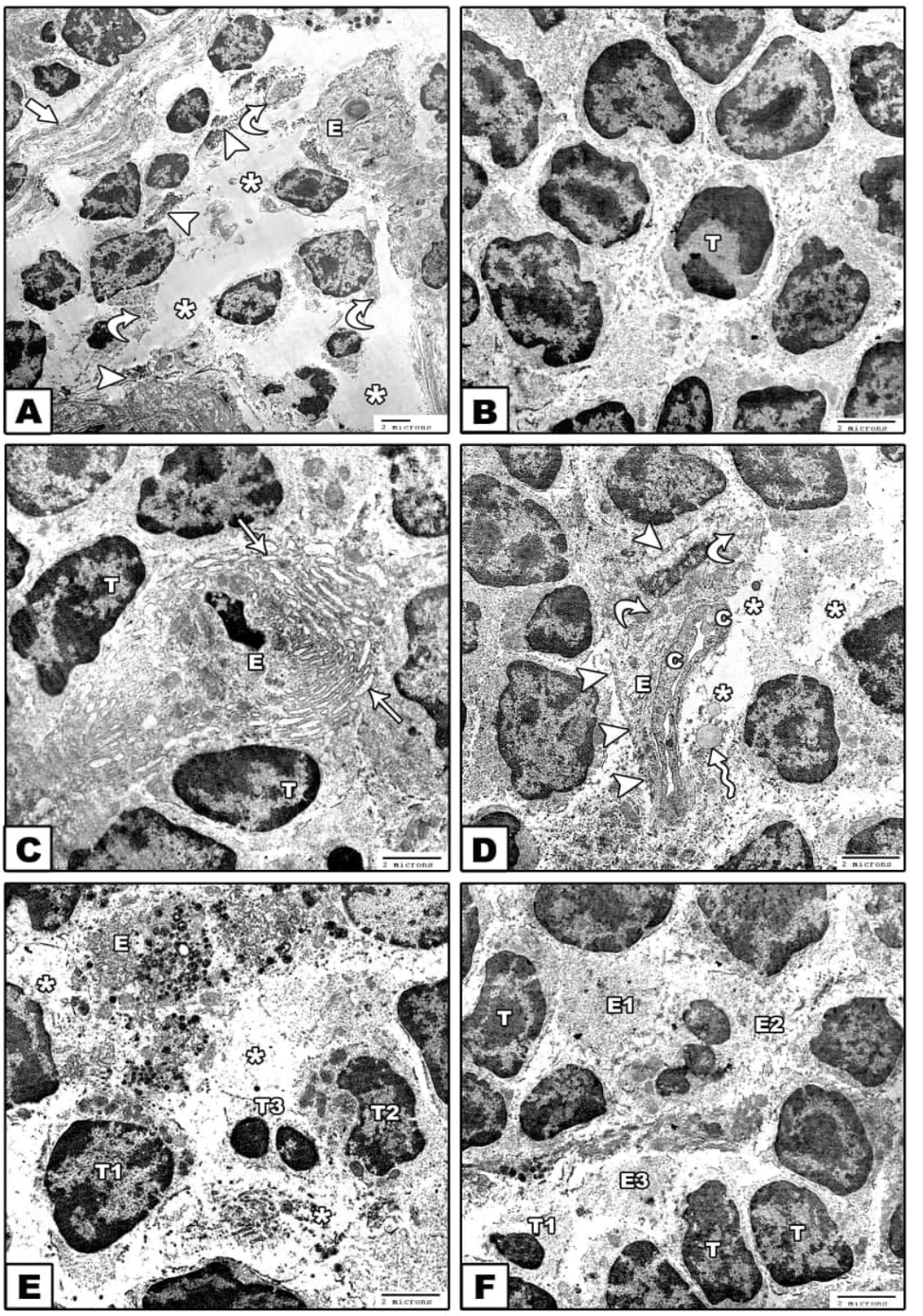


Fig. 4: Electron micrograph of thymic ultrathin sections of MSG treated group **A):**shows cortex having thick CT septa(thick arrow) and decrease cellularity with wide interstitium (stars) containing thymocytes of different sizes and shape and fragmented processes of ERC (curved arrow). ERC type1(E) contains pyknotic nucleus surrounded by rarified cytoplasm of ill-defined organelles is also present. Notice presence of nucleus with abnormal chromatin condensation and fragmentation (X 5850). **B):** demonstrates cortex contained thymocytes of different sizes and shape displaying chromatin margination (T) and micronucleus formation (X 11700). **C):** reveals cortex with epithelial reticular cell II (E) displaying small electron dense irregular nucleus and dilated proliferated RER(arrows) in association with increase in the peripherally condensed chromatin in the surrounding thymocytes (T).X11700 **D):**Corticomedullary junction disclose swollen endothelium (C)of continuous blood capillary surrounded by disrupted processes of epithelial reticular cells(E)leaving bare area. Their cytoplasm contains enlarged mitochondria with cristolysis (curved arrow). The desmosomes appear attenuated and ill-defined (arrow heads). Notice, wide interstitium contains cellular debris (stars)and fat droplets(wavy arrow).X11700 **E):** shows wide interstitium(stars) containing remnant of necrotic ERC (E) with few thymocyte (T1) some of them displaying irregular nuclei with increased peripherally condensed chromatin (T2) or small hyperchromatic one (T3). x11700 **F):** Medulla depicts disintegrated or degenerated epithelial reticular cells (E1,E2 and E3. One of them is a nucleated(E1&E3), while others is seen segmented(E2) with marked chromatin condensation. The nearby thymocyte displays small electron dense nucleus(T1) x11700

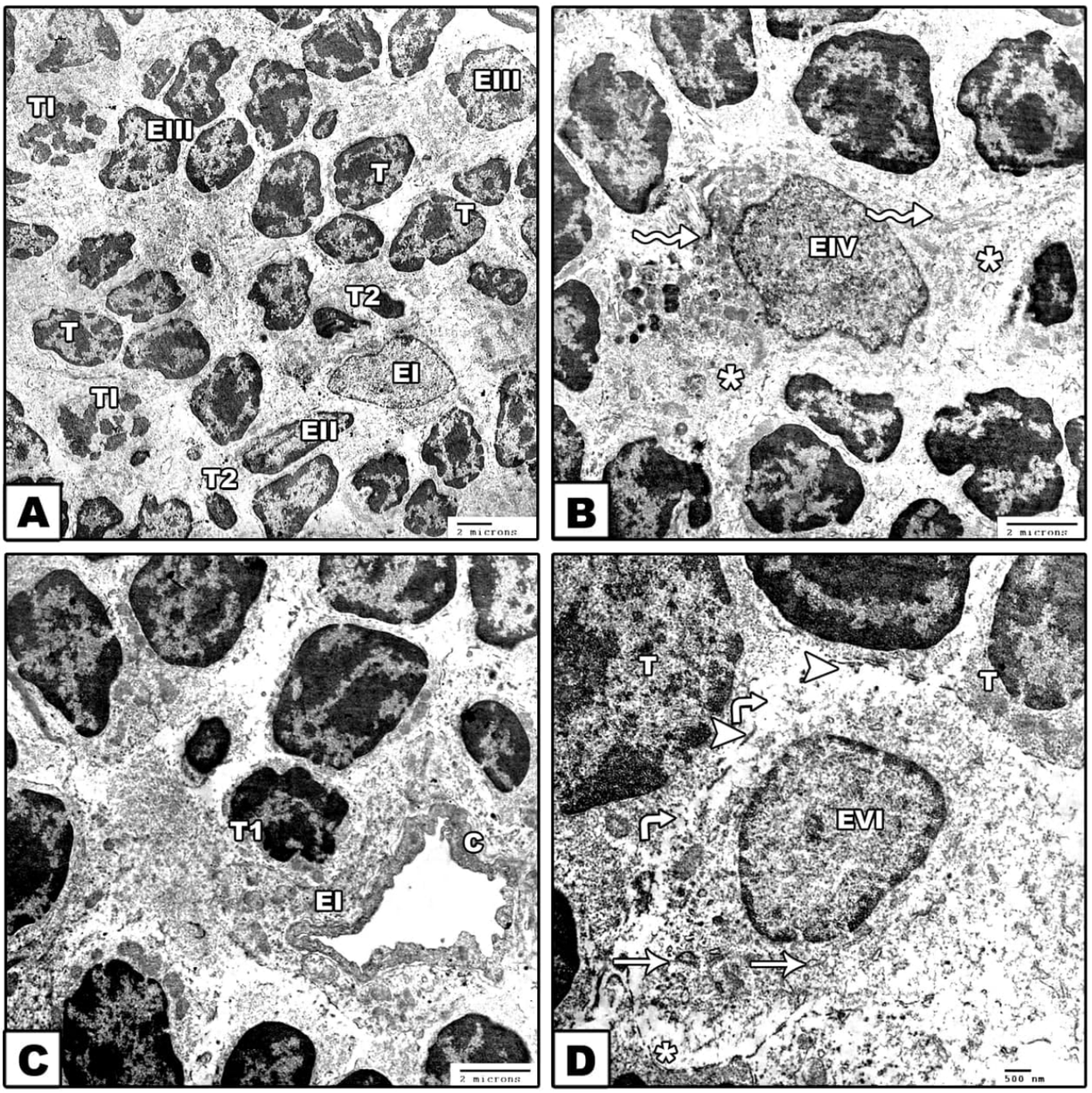


Fig. 5 : Electron micrograph of thymic ultrathin sections of (MSG &Vit C treated group) **A);** shows cortex consisting of thymocytes of different sizes (T) separated by the processes of epithelial reticular cells. Notice the presence of mitotic (T1)and thymocyte with pyknotic nuclei (T2) and the three types of epithelial reticular cells type EI,EII,EIII **B):** discloses inner cortex having epithelial reticular cell type IV (EIV) with euchromatic nucleus, long processes (stars) and abundant cytoplasm rich in tonofilaments(hay stack) and mitochondria(wavy arrows).X2000 **C):** shows the corticomedullary junction containing continuous blood capillary(C) surrounded by epithelial reticular cell type I (E1) with intact basal lamina forming blood thymic barrier. They are surrounded by thymocytes of different sizes (T1).x2000 **D):** reveals medulla containing epithelial reticular cells (EVI) having euchromatic nuclei, dilated RER(arrows), rarified cytoplasm (star) and interrupted plasma membrane(angular arrows). Notice the presence of Desmosomes(arrow head).x3000

Table 1: The range and the mean ± SD of cortical thickness for the different studied groups measured in micrometers

Cortical Thickness	Control	Vit. C	MSG	MSG + Vit. C
Range	840 – 1320	765 – 1025	235 – 425	560 – 702
Mean ± SD	969.00± 99.23	889.20± 00.47	327.60± 72.06	621.00± 66.23

Table 2: Comparison between the mean cortical thickness of the thymus gland in different studied groups represented by the *P value*

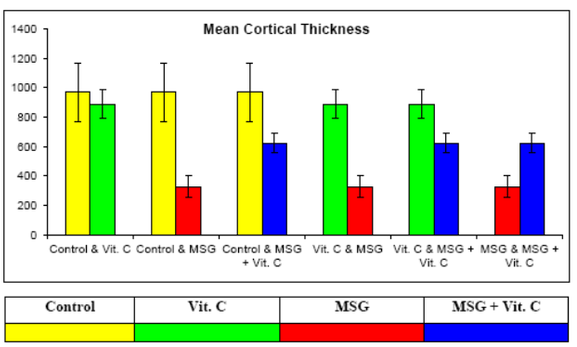
Control &Vit. C	Control & MSG	Control & MSG + Vit. C	Vit. C& MSG	Vit. C& MSG + Vit. C	MSG & MSG + Vit. C
0.316	0.001*	0.001*	0.001*	0.003*	0.002*

Table 3: The range and mean ± SD of the area percentage of positive caspase 3 immunoreaction in immunostained sections of the different studied groups

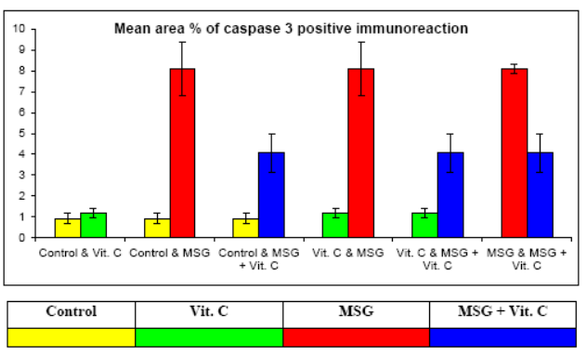
Caspase3 +ve area %	Control	Vit. C	MSG	MSG + Vit. C
Range	0.65 – 1.25	0.85 – 1.42	6.58 – 9.45	3.05 – 5.32
Mean ± SD	0.92 ± 0.24	1.17 ± 0.23	8.10 ± 1.28	4.05 ± 0.93

Table 4: Comparison between the mean cortical thickness of the thymus gland in different studied groups represented by the *P value*

Control &Vit. C	Control & MSG	Control & MSG + Vit. C	Vit. C& MSG	Vit. C& MSG + Vit. C	MSG & MSG + Vit. C
0.632	0.001*	0.001*	0.001*	0.001*	0.001*



Histogram 1: Comparison between the mean cortical thickness of the thymus gland in different studied groups



Histogram 2: Comparison between the mean area percentage of caspase 3 positive immunoreaction of different groups

DISCUSSION

MSG is a widely used food additive. Several studies have lately suggested undesirable side effects after its prolonged intake based on many in *vitro* and in *vivo* animal studies and clinical trials particularly at high doses. MSG can enhance the hazards of hypercholesterolemia, hypertriglyceridemia, obesity and diabetes. Additionally, it can induce oxidative stress, hepatotoxicity and neurotoxicity[29]. The immune system produces major adaptive mechanisms through which the body guards itself from pathogens and other damaging agents[30]. It is a potential target of toxicity following chemical exposure[31]. Thymus is a primary lymph organ which produces immunocompetent T cells[32]. Communication of thymocytes with cellular populations of the cortical and/or the medullary regions of the thymus is required for appropriate T cell growth; particularly thymic epithelial cells (TECs) and dendritic cells (DCs)[33]. The current study evaluated the effect of MSG on the thymus of adult male albino rat when given alone and when administered concomitantly with vitamin C.

Thymus gland of control and vitamin C treated groups of the current study displayed the well known normal structure by both light and transmission electron microscopes. The nuclei of thymocytes displayed different morphologies either seen in interphase stage with characteristic hyperchromatic profile or in mitotic state. Epithelial reticular cells were seen encircling developing thymocytes and disclosed six types distributed in cortex and medulla. They form blood thymic barrier in the cortex and their cytoplasm depicted autophagosome signifying the presence of an active interesting phenomenon of macro autophagy. Similarly, Tsubasa *et al*., (2017)[34] observed the same findings in the thymus of the control rat and they reported its enhancement after ethanol administration.

Macro autophagy is defined as a cellular process involved in both main types of cell death necrosis and apoptosis that involves the engulfment of cytoplasmic material and intracellular organelles within double-membrane vesicles, called autophagosomes as represented in the current study[35]. It is believed that autophagy has a cytoprotective and homeostatic role, as it promotes cell death during normal development[36] and in disease[37]. In addition, a low level of constitutive autophagy has an important housekeeping role in the normal turnover of

long-lived proteins and whole organelles, thereby being crucial for maintaining healthy cells[38,39]. In addition, Chunyan Wu *et al* (2013)[40] elucidated that thymus epithelial reticular cells used autophagy in the process T lymphocytes negative selection. Furthermore, the immune system designates a cellular catabolic process in order to enhance the tolerogenic presentation of self-antigens within the thymic microenvironment. The underlying mechanism of autophagy was suggested to be under the control p53 protein[40-44]. However others added that production of ROS by thymic stromal cells of mice has physiological roles and seem to be necessary for autophagy which is essential for negative selection and thus for the prevention of autoimmune diseases and can eventually lead to thymic atrophy during aging[45].

The current study showed that MSG treated group displayed loss of the thymic architecture with marked cellular depletion particularly at the corticomedullary junction. Immunostained sections of the same group for active caspase 3 demonstrated wide spread strong positive cytoplasmic reaction spanning both thymocytes and epithelial reticular cells of cortex and medulla. These data were verified statistically by considerable significant decrease in the area percentage of cortical thickness and significant increase in caspase3 positive immunoreactivity. Existence of ultrastructural features of different forms of cell death in the same group confirmed and explained the formerly mentioned light microscopic observations. Similar findings proving the toxic effect of MSG on thymus of adult male albino rat were formerly studied by Pavlovic *et al*. (2006)[46] and Hassan *et al*. (2014)[47].

The current study demonstrated electron microscopic features of several forms of cell deaths commonly seen in ERC as well as thymocytes of thymus of MSG treated group. Apoptosis was a common microscopic feature evidenced immunohistochemically by significant increase in caspase 3 immunoreactivity and ultra-structurally by nuclear shrinkage (pyknosis), chromatin margination together with fragmentation (karyorrhexis). Desmosome attenuation and cellular separation secondary to junctional break down were another findings seen in this group.

These results coincided with Pavlovic *et al*. (2006)[46] who elucidated that prolonged giving of MSG to animals led to increase thymocytes apoptosis due to oxidative stress. They reported that MSG administration to animals significantly decreased cell viability of thymocytes in a time-dependent manner. They also attributed MSG induced thymocytes apoptosis to reduction in BCL2 expression secondary to down-regulation of anti-apoptotic (Bcl-2) gene expression which is known as a regulatory proteins that regulate cell death (apoptosis). In addition, Zanfirescu *et al* (2019)[48] reported in their study that there are links between MSG consumption and tumorigenesis, increased oxidative stress and apoptosis in thymocytes, as well as genotoxicity in lymphocytes.

During electron microscopic examination of thymus of MSC treated group several signs of necrotic cell death were seen. These were in the form of disrupted cell membranes, presence of cellular debris and fragmented processes of ERCs in association with total disintegration. Nuclear changes ranged from clumping, shrinkage (pyknosis), fading and up to complete disappearance (karyolysis) were an unusual features seen in this group. In agreement with these findings necrosis by MSG was also observed by Hazzaa *et al* 2020[49].

In trial to understand the mechanism of necrosis in MSG treated group, many factors could play a role in the propagation of necrosis among them, Ca2 and mitochondria. Ca2 controls activation of polylactic acid, calpains and nitric oxide synthase, which induce a series of events leading to necrotic cell death. While mitochondria dysfunction contribute to necrosis by excessive reactive oxygen species (ROS) formation, mitochondrial permeability transition, and ATP depletion[50]. Eguchi *et al*. (1997)[51] and Los *et al*. (2002)[52] found that the intracellular ATP levels direct the form of cell death: a high ATP level leads to apoptosis, whereas a low ATP level leads to necrosis, meaning that an intracellular ATP depletion switches the energy-dependent apoptotic cell death to necrosis. This view could be the cause of necrotic cell death seen in the thymus of MSG treated group as verified by presence of swollen mitochondria with cristolyis and disintegration of the cell organelles. Los *et al*. (2002)[52] & Skulachev(2006)[53] added when a cell is incapable to die by apoptosis, it undergoes necrosis. Additionally ROS formed by monosodium glutamate cause mitochondrial dysfunction, mitochondrial DNA damage and mutation, improper electron transportation, mitochondrial protein nitration, and dysregulated mitochondrial biogenesis[54]. Lotowska *et al*., (2014)[55] correlated mitochondrial configuration with its function. They pointed out that varying degrees of mitochondrial defect indicate decreased intra-mitochondrial protein synthesis and respiratory chain dysfunction and consequently ATP depletion. Furthermore, Lin *et al* (2019)[56] and El – Aarag *et al*. (2019)[57] added that mitochondrial dysfunction lead to excess ROS generation that firmly bind to the phospholipid molecules of the cell membranes, endoplasmic reticulum and mitochondria causing lipid peroxidation and membrane damage. All these facts explained the occurrence of necrosis and the disfigured mitochondria in the present study.

Remarkably, abnormal nuclear profiles in the form of micronucleus formation, binucleation, nuclear segmentation and abnormal chromatin condensation were another attention-grabbing electron microscopic signs seen in thymocyte and ERC nuclei of MSG treated group. These signs could be attributed to type of mitosis-related cell death known as mitotic catastroph (MC).

Mitotic catastrophe (MC) is characterized morphologically by either micronucleation due to chromosomes or chromosome fragments not evenly segregated into the two daughter nuclei or multinucleation

due to mis segregated chromosome fragments enveloped by one or multiple nuclear membranes of different dimension[58]. Many factors were suggested to be responsible for induction of mitotic catastrophe such as DNA alterations, drugs that influence microtubule assembly and stability[59,60], deficiency in cell-cycle checkpoints (especially the ones related to DNA structure analysis and to mitotic spindle) or cell death inducers as p53, p21 and caspases[61]. Previous studies demonstrated that MC is not a separate form of cell death but it represents a pre-stage to necrosis or apoptosis[58,60,62,63,64].

Noteworthy, the presence of swollen disfigured mitochondria in cytoplasm of ERC of thymus of MSG treated group could explain uncommon form of cell death known as mitopotois that occur secondary to mitochondrial dysfunction and oxidative stress[65]. Hasssan *et al* (2014)[47] proved in their study that; MSG significantly increased oxidative stress through increased malondialdehyde (MDA) levels and decreased catalase (CAT) activity in thymic tissues.

Fouad *et al*., (2019)[66] illustrated that DNA damage could be induced by ROS. These free radicals bind to DNA covalently causing DNA oxidation, and formation of DNA adducts, mutations, chromosomal alterations and DNA fragmentation, leading to the accumulation of tumour suppressor gene (p53). This gene first arrests the cell cycle at G1 phase to allow the DNA repair before it is replicated, if the damage is too great to be repaired successfully, p53 triggers apoptosis on top of necrosis[67,68,69]. This explains the different signs of apoptosis and necrosis seen in electron microscopic findings of the group which clarified loss of thymic architecture, cellular depletion and decreased cortical thickness.

Obviously, different types of epithelial reticular cells in MSG treated group displayed altered form of rER in the form of proliferation and dilatation. These changes could be related to endoplasmic reticulum stress secondary to production of ROS by MSG. Quan *et al*. (2020)[70] explained, ROS leads to cellular hypoxia with subsequent reduction in the activity of plasma membrane ATP-dependant sodium pumps followed by intracellular accumulation of sodium and potassium efflux. The accumulation of sodium is followed by water influx causing cell swelling and dilatation of the endoplasmic reticulum.

The blood thymic barrier of MSG treated group revealed continuous blood capillaries at the corticomedullary junctions that were lined by swollen endothelium containing distended mitochondria with cristolysis. They were surrounded by discontinuous basal lamina and wide subendothelial space. The processes of the surrounding epithelial reticular cells were swollen, disrupted leaving capillary bare area and had attenuated, ill-defined desmosomes. These findings could be related to persistent over stimulation of glutamate receptors that can lead to ROS production, DNA damage, lipid peroxidation and caspase activation through triggering Ca2+ influx and subsequent activation of many intracellular enzymes such as protein kinase C[9]. This is confirmed by our results as there was significant increase in caspase 3 immunoreactivity in MSG treated group.

Group IV (MSG & vitamin C) in the current study showed restoration of the normal structure of the thymus gland to be more or less similar to the control group. This revival capacity was proved microscopically and statistically and was in accordance with Pavlovic *et al* (2009)[71] who carried their work on rat thymus.

Pavlovic *et al* (2007)[69] attributed MSG thymus toxicity to oxidative stress and apoptosis induced in rat thymus. This goes in line with our results and previously confirmed by Aulwurm and brand (2000)[72] who stated that thymocytes are highly sensitive to oxidative stress. Victor *et al* (2002) [73] attributed their sensitivity to their high content of cell membrane polysaturated fatty acids and their normal high content of ROS. Ascorbic acid is an essential antioxidant against oxidative process and plays a part in free radical scavenging as an antioxidant as well as plays an important role in biosynthetic process as synthesis of collagen ,catecholamines and leukotrienes. It also has a beneficial effect on the function of T lymphocytes as it increases their antidefense effects and their responsiveness to antigens. So, it may have a role in regulating immune function. In addition, vitamin C modulates the immune system by inhibiting T cell apoptosis signalling pathways[74,75].

Pavlovic *et al* (2009)[71] also added that vitamin C reduced the MSG-induced toxicity in rat thymocytes by up-regulation of Bcl-2 protein expression.In addition, vitamins C preserved important biomolecules when it is oxidized. It also acts as a protective agent through removing oxidized cellular components or repair them.

These observations are in accordance with the findings that vitamin C has a protective role in other organs as El-Meghawry *et al*.,(2013)[76] who declared that concomitant administration of vitamin C along with MSG in rats for 45 days has a protective effect on liver architecture. They attributed it to the reduction in cellular proliferation via decreasing the ki-67 expression and tumor suppressor genes mutation. In the same way, Belin *et al* (2009)[77] and Fromberg *et al* (2011)[78] attributed the anti-proliferative activity of vitamin C to induction of apoptosis, arrest of cell cycle and inhibition of expression of genes involved in protein biosynthesis. In addition; Waiz *et al*. (2015)[79] confirmed the previous results and stated that vitamin C co-administration with MSG, considerably reduced the oxidative stress, hepatic toxicity and the hepatic activity of catalase. In a different study, it was proved that vitamin C attenuated the toxic effect of MSG on the testis, epididymis and decreased the sperm abnormality[80]. These results are in accordance with AnbarkehRahimi *et al*., (2019)[81] who confirmed that MSG has cytotoxic action on the testicular germ cells in rats, and stated that vitamin C antioxidant effect counter act MSG induced cytotoxicity.

CONCLUSION

From the present study it could be concluded that MSG has induced caspase 3 dependent thymotoxic involution effects, including both thymocyte and epithelial reticular cells and co administration of vitamin C reduced this effect in adult male albino rat.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

التأثير الدفاعي النسيجي المحتمل لفيتامين ج ضد سمية الجلوتامات أحادية الصوديوم على الغدة الصعترية للذكور البالغين من الجرذان البيضاء

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المقدمة: لا زالت الجلوتامات أحادية الصوديوم تستخدم على نطاق واسع كمحسن للنكهة في مجموعة متنوعة من طرق تحضير الطعام. حديثا العديد من الدراسات أوضحت أثار جانبية غير مرغوب فيها مع
الاستهلاك طويل المدى للجلوتامات أحادية الصوديوم . يعتبر الجهاز المناعي الهدف المحتمل للسمية بعد التعرض للمواد الكيميائية. الغدة الصعترية هي عضو ليمفاوي أساسي ينتج الخلاياالمناعيةT. هي تعززنضوج السلائف الليمفاوية الى الخلايا Tلانشاء تجمع للخلايا أثناء الحياة في الانسان.
الهدف من العمل: تقييم التأثير السمي لجلوتامات احادية الصوديوم على الغدة الصعترية في ذكر الجرذ الأبيض البالغ. والدور الوقائي المحتمل لفيتامين ج عند تناولهما متزامنين.
مواد وطرق البحث: لقد أجريت الدراسة على أربعين جرذ ذكر ابيض بالغ تم تقسيمها عشوائياً الى أربعة مجموعات متساوية. المجموعة الأولى وقد شغلت منصب المجموعة الضابطة، المجموعة الثانية تلقت ١٠٠مجم/كجم من وزن الجسم فيتامين ج فقط يوميا، المجموعة الثالثة تلقت الجلوتامات أحادية الصوديوم ٣جم /كجم من وزن الجسم يوميا، المجموعة الرابعة تلقت كلا من جلوتامات أحادية الصوديوم متزامنا مع فيتامين ج يوميا لمدة ٦٠ يوماً، وفي نهاية التجربة تم الحصول على الغدة الصعترية و تجهيزها للدراسة الهستولوجية ، الهستوكيميائية المناعية(كسباز ٣) والمورفومترية.
النتائج: تناول الجلوتامات أحادية الصوديوم أسفر عن انخفاض ملحوظ في عدد خلايا الغدة الصعترية مع زيادة ذو دلالة إحصائية في عدد الخلايا الموجبة للكسباز ٣مع التغيرات في التركيب الدقيق في خلايا الغدة الصعترية و الخلايا الشبكية الطلائية، التناول المتزامن لجلوتامات الصوديوم الأحادية مع فيتامين ج يحسن من التغيرات الهستولوجية السابقة مع نقص ذو دلالة إحصائية في عدد الخلايا الإيجابية للكسباز ٣
الخلاصة : الجلوتامات أحادية الصوديوم لديها تاثير على التركيب النسيجي لخلايا الغدة الصعترية كما أن تناول فيتامين ج متزامنا حسن هذا التاثير.