Original Article

Impact of Monosodium Glutamate Intake on Heart Structure of Neonate Albino Rats and The Protective Role of Vitamin C

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ABSTRACT

Background: Despite worldwide use of monosodium glutamate (MSG) as a flavor enhancer, its consumption has been an alarm due to daily exposure without definitive safety or exact limit. Vitamin C has an antioxidant activity.

Objective: To clarify effects of neonatal MSG intake on heart structure of male albino rat pups and the possible modifying role of vitamin C and to assess reversibility of these effects.

Material and Methods: 40 male albino rats at postnatal day 1(PD1) were allocated to three groups; control, group B administered MSG (4 mg/gm bw/day) and group C administered previous dose of MSG plus vitamin C (500 mg/kg bw/day) for 10 days. Groups B and C were subdivided according to animal sacrification age into B1, C1 sacrificed at PD10 (after the last dose of treatment) and B2, C2 sacrificed at PD30 (20 days after treatment cessation). At experimental end, left ventricular specimens were processed for histopathological, immunohistochemical and morphometric studies.

Results: Histologically, group B1 revealed massive degenerative changes including marked muscle fibers disruption, degeneration, separation with increased CT cells and fibroblasts, dilated congested capillaries and extravasated hemorrhage. Cardiomyocytes showed nuclear pyknosis, cytoplasmic coagulative necrosis and vacuolation. Previous changes were still present in group B2 but with less degree. MSG plus vitamin C resulted in greatly restored normal cardiomyocyte architecture in group C1 but complete recovery was observed in group C2. The mean area percentage of collagen fibers, vimentin and iNOS expressions showed high significant increase in B1, B2, low significant elevation in C1 but non-significantly different in C2 relative to control group.

Conclusion: MSG induced myocardial toxicity in early postnatal period. Vitamin C greatly ameliorated histopathological and immunohistochemical alterations in cardiac tissue. MSG withdrawal for 20 days showed mild improvement but withdrawal after vitamin C administration was more effective in reversibility of MSG toxicity.

Received: 28 October 2020, **Accepted:** 20 November 2020

Key Words: iNOS, monosodium glutamate, neonate heart, vimentin, vitamin C.

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ISSN: 1110-0559, Vol. 44, No.3

INTRODUCTION

Monosodium glutamate (MSG) is a common food preservative widely used in restaurants, homes and in processed foods in each grocery store or market to enhance flavor. It has a specific taste (umami), which was considered at first a predominant taste in Asia but later on became in Western cultures. It is derived from l-glutamic acid, an amino acid naturally occurring in a variety of food products[1,2,3]. It is found in foods with high protein content like meat and fish. It is also present in certain cheese types such as Roquefort and Parmesan and in some vegetables (broccoli, tomatoes, and mushrooms). Compared to MSG, l-glutamic acid and its disodium salt analog have mild umami properties[4].

It has been reported that MSG improves food palatability by stimulating the orosensory receptors and enhancing appetite[5]. Also, MSG acts on the glutamate receptors and causes release of neurotransmitters playing an important role in the normal physiological processes[6]. However, its consumption has been an alarm due to daily exposure to this chemical substance in foods without definitive safety and exact limit[7].

Previous studies on neonate as well as adult experimental animal models have discussed the adverse effect of daily consumed MSG and some data regarding MSG-induced injury in different organs. Such organs included hypothalamic–pituitary–adrenal axis[8], Kidney[9], liver[5], hippocampus[10] and testis[11,12]. Also, administration of MSG was associated with morphological and biochemical changes in heart tissue, alterations in cardiac rhythm and elevation of heart disease biomarkers[13-16]. Neonatal consumption of MSG resulted in obesity associated with insulin resistance and reduction in glucose tolerance in rodents in later life[17]. Also, MSG administrated at a dose of 2 mg/gm/bw/day during various perinatal period of life led to seminiferous tubular atrophy and reduction in sperm count and in serum testosterone level in adult male rats when compared with control animals[18]. An experimental trial on neonate rats noticed glutamate receptor over activation in the brain induced by MSG subcutaneous administration[19]. In another research,

 DOI: 10.21608/ejh.2020.48021.1381

neuronal degenerative and hippocampal cytoarchitectural changes were observed[20]. At the same time, to best of our knowledge, review literature regarding MSG adverse consequence on the heart structure in early postnatal life in experimental animals was scarce.

Vitamin C has a powerful antioxidant activity. It may prevent the oxidative damage to the important biological macromolecules as lipids, proteins and DNA because it cleans out the reactive oxygen species (ROS)[21]. Vitamin C is also associated with the prevention of degenerative diseases such as certain cancers, cardiovascular diseases and cataract[22]. It is also mentioned that vitamin C helps to decrease high blood pressure, cholesterol levels and prevent atherosclerosis[23].

Vimentin is an intermediate filament protein that is commonly used to label cells of mesenchymal origin. In the cardiac tissue, it is commonly expressed in fibroblasts, endothelial cells, macrophages and in endomysial and perimysial sheaths of the myocardium. Vimentin-immune positive cells commonly increase in number during stressful conditions such as heart failure[24].

Increased inducible nitric oxide synthase (iNOS) expression in the heart causes excess nitric oxide (NO) generation. NO overproduction is cytotoxic and leads to many cardiovascular diseases. Also, iNOS induces superoxide anion generation that reacts with NO to produce peroxynitrite, a harmful oxidant, resulting in cardiac oxidative stress. Increased iNOS expression has been recorded in heart failure, dilated cardiomyopathy (DCM) and arteriosclerosis[25].

The purpose of this study is to shed light on MSG toxicity on cardiac muscle structure in neonatal male albino rat after early postnatal exposure to it for 10 days and the possible protective role of vitamin C and also to assess is this effect reversible or not? by using histopathological techniques, immunohistochemistry and morphometric Studies.

MATERIAL AND METHODS

Chemicals

1. **The Monosodium glutamate (MSG):** It was obtained from Alkahira Company of pharmaceutical industries as a powder and was dissolved in water. It was administered orally to rats at a dose of 4 mg/gm bw/day dissolved in distilled water[7] for 10 days.
2. **Vitamin C:** It was purchased from Alkahira Company of pharmaceutical industries as a powder. It was administered orally to rats at a dose of 500 mg/kg bw/day dissolved in water[26] for 10 days.

Animals

Forty male albino rat pups at postnatal day 1 (PD1) obtained from the animal house, Faculty of Medicine, Zagazig University were used in this study. The average weight was 9-25 grams. Experimental plans were carried out according to the instructions of the Institutional Animal Care and Use Committee Zagazig University (ZU-IACUC) with Approval number ZU-IACUC/3/F/40/2020

Experimental procedures: The experimental animals were housed through controlled laboratory conditions (at 20-26 ºc room temperature with appropriate humidity) throughout the experimental period. For ensuring adequate nutritional state until weaning, the rats were housed with their mothers in standard plastic cages for lactation.

The rat pups were randomly classified to 3 groups; control, treated and protective groups. The animals were given treatments orally via orogastric tube for 10 days. After last dose of treatment, half of animals were sacrificed and the remaining rats were left to feed standard food without any treatment until 30 day old age. Therefore, each group was further subdivided into 2 subgroups according to the age of animal sacrification at PD 10 old age (after the last dose of treatment) and at PD 30 old age (20 days after cessation of treatment).

I- Control group (group A): contained 12 pups and were subdivided into 2 equal subgroups:

a. Negative control subgroup: included 6 pups; each of them was kept without any intervention. Three of them were sacrificed at PD10 (A1) and the others at PD30 (A2).

b. Vitamin C-treated (Positive control) subgroup: 6 pups were given orally vitamin C/ day in doses as previously described for only 10 days. Three rats were sacrificed at PD 10 (A3) and the others at PD 30 (A4).

II- MSG treated group (group B): consisted of 14 pups were given orally the previous dose of MSG for only 10 days. Half of animals (7 rats) were sacrificed at PD 10 (group B1) and the other half at PD 30 (group B2).

III- MSG + Vitamin C protective group (group C): 14 pups were given previous doses of MSG and vitamin C for only 10 days. 7 rats were sacrificed at PD 10 (group C1) and the other 7 rats at PD 30 (group C2).

At the start of experiment and just before sacrificing the animals, their weights were calculated and recorded in each subgroup. The Statistical comparisons of mean values of final body weights between control, treated and protected groups of male albino rats were done.

At the end of the experiment, the animals were anaesthetized with intraperitoneal injection of thiopental (50 mg/kg bw)[27]. The thoracic cavity was opened then the heart was dissected out, and placed in 0.9% physiological saline solution to wash away the blood from the heart chambers. Left ventricular specimens were well processed to be examined by histological, immunohistochemical and morphometric studies.

A- Histological study

Specimens for histopathological examination were fixed in 10% neutral formol saline and processed in ascending grades of alcohol (50%, 70%, 90% and 95%). The samples were dehydrated (one hour for each), then bathed in absolute alcohol (100%) for two changes (one hour for each). The samples were embedded in soft paraffin wax at 55 ºC for 2 hours and in hard paraffin at 60 ºC for another 2 hours after clearing in xylene. 5 µm thick paraffin sections were prepared for Hematoxylin and eosin, staining[28] and for Mallory trichrome staining[29] for distinguishing the collagen fibers; all according to standard methods. The histopathological examination and photography using light microscope (Lecia ICC50 W" with a Leica digital camera) were established at Anatomy Department, Faculty of Medicine, Zagazig University.

B-Immunohistochemistry

For the immunohistochemistry studies, paraffin sections of 4µm were deparaffinized in xylene and processed for vimentin and iNOS antibody immunohistochemistry. Sections were rehydrated in a descending series of ethanol and rinsed in phosphate-buffered saline (PBS). Endogenous peroxide was inactivated using 10% hydrogen peroxide (Sigma) in PBS (pH 7.4) for 10 min. The sections were incubated with a 1:200 dilution of anti-vimentin (monoclonal mouse vimentin antibody, Vim 3B4, catalog No. M7020; Dakopatts CA, USA)[30] and with a 1:100 dilution of anti-iNOS (rabbit anti-rats iNOS polyclonal antibody, M-19/Sc 650, Santa Cruz Biotechnology, Santa Cruz, CA, USA)[31].

Slide sections were incubated with biotinylated secondary antibody for 30 min., after washing with PBS (3 times for 5 min./slide), followed by streptavidin–peroxidase complex at room temperature for 10 min. The antigen antibody reaction was visualized after adding 0.05% diaminobenzidine (DAB) reagent. The material was counterstained with Mayer's Hematoxylin then dehydrated and mounted. All slides were examined and photographed at Anatomy Department, Faculty of Medicine, Zagazig University. The negative control sections were done by omitting the primary antibody. The positive control sections were prepared for localization of vimentin immunoreactions (labels the cells of mesenchymal origin as interstitial fibroblasts, endothelial cells, macrophages and in endomysial and perimysial sheaths of the myocardium)[24], and for iNOS expression (cytoplasmic cellular reaction). The immunoreaction sites were stained brown[31].

C- Morphometric Study

The mean area percentage % of the collagen content in the Mallory trichrome stained sections as well as for vimentin and iNOS expressions in the immunostained sections (at magnification of X 400), were randomly morphometrically analyzed using computerized image' analyzer (Lecia Imaging computer System Ltd., controlled by Leica Qwin 500 software, Cambridge, England) at anatomy department, Faculty of Medicine, Zagazig University. Form five different specimens, 5 slides were examined in each group. From each slide, ten readings were obtained and the mean values were recorded for Statistical Analysis.

D-Statistical Analysis

The collected data were computerized and statistically analyzed using Graph Pad Prism 5.01. Quantitative data were expressed as mean ± SD (Standard deviation). Differences between mean values of experimental groups were tested with analysis of variance (ANOVA). Tukey's multiple comparison test was carried out as post hoc test of ANOVA. The results were considered statistically significant when the *P value* <0.05. Different stages of significance were considered. High significance (***) means *P value* < 0.001, Moderate significant (**) at 0.01 >*P value* >0.001 and low significance (*) when 0.05 > *P value* >0.01.

RESULTS

I-Measurement of final body weights

There was statistically significant difference between the mean values of final body weights of the control, treated and protected groups in both PD 10 and PD 30 age groups. The mean value of final body weights of rats receiving MSG only showed moderate significant increase in group (B1, B2) when compared with that of control and group (C1, C2) denoting weight gain (Tables 1,2).

II-Histological results

Histological examination showed no difference in all subgroups of group (A) which displayed normal cardiac muscle structure in the variable stains.

A- Rats sacrificed at PD 10: (Figures 1a-1g)

Hematoxylin and eosin stained sections of a control rat heart revealed normal histological architecture of cardiomyocytes of the ventricular wall. The cardiomyocytes appeared as elongated muscle fibers, separated by narrow connective tissue (CT) spaces then communicating again and anastomosing. They contained an acidophilic cytoplasm and large central oval basophilic vesicular nuclei. Few fibroblasts with flat dark nuclei and small blood capillaries were seen in the narrow CT spaces among the cardiac muscle fibers (Figure 1a).

However, the MSG treated group (B1) revealed massive degenerative changes indicating cardiotoxicity when contrasted with control groups. Marked disruption, degeneration and loss of branching and anastomosis of cardiac muscle fibers were clearly observed. The cardiomyocytes appeared shrunken with small deeply stained nuclei (pyknotic). Some cells exhibited darkly stained acidophilic cytoplasm (coagulative necrosis). Extensive areas of extravasated hemorrhage were seen between muscle fibers (Figure 1b). Moreover, extensive

cytoplasmic vacuolation and nuclear pyknosis of cardiomyocytes were seen. The fibroblasts were apparently increased in number (Figure 1c). The intercellular spaces appeared markedly dilated with wide separation between muscle fibers. Some muscle fibers appeared necrotic. Some cardiomyocytes showed small vesicular nuclei (Figure 1d). The dilated CT spaces showed an excessive amount of CT cells (Figure 1e) and extensively dilated and congested capillaries (Figure 1f).

On the contrary, in group (C1), there was great improvement in the cardiac muscle structure by exposure to vitamin C. Most muscle fibers restored normal architecture. Cardiomyocytes with acidophilic cytoplasm, central oval vesicular nuclei were well demonstrated. However, in certain areas, few cells appeared with vacuolated cytoplasm. Also, dilated and mildly congested blood capillaries were still observed (Figures 1g-1h).

Mallory trichrome staining sections of a control group revealed delicate collagen fibers in the CT spaces between cardiomyocytes and around the wall of blood capillaries. However, the collagen fibers amount was greatly increased in in group (B1) which received MSG only, while was less in amount in group (C1) treated with MSG and vitamin C (Figures 2a-2c).

In addition, vimentin immunostaining sections of a control group exhibited weak brown positive immunoreaction in some interstitial fibroblasts, in vascular endothelium as well as in the endomysium. However, sections from group (B1) displayed extensive vimentin expression whereas sections from group (C1) displayed mild expression. The cardiomyocytes appeared with negative immunoreaction (Figures 3a-3c).

Moreover, with iNOS immunostaining, minimal light brown positive INOS expression appeared in the cytoplasm of some cardiomyocytes in sections of the control group. However, strong expression was apparent in sections of group (B1) while was mild expression in sections from group (C1) (Figures 4a-4c).

B- Rats sacrificed at PD 30 (Figures 5a-5d)

Sections of control rat heart stained with Hematoxylin and eosin revealed normal cardiac muscle structure. Well-developed bundles of cardiac muscle fibers appeared striated, branched and anastomosing forming a complex network. The cardiomyocytes displayed an acidophilic cytoplasm and large oval vesicular basophilic nuclei. Flat nuclei of the fibroblasts and capillaries were seen in the narrow CT space between the muscle fibers (Figure 5a).

However, heart sections from group (B2) also revealed the same histopathological changes of group (B1) but with less degree. The cardiac muscle fibers exhibited moderate separations with many CT cells were observed in the wide CT spaces between the muscle fibers. Other cardiac muscle fibers appeared attenuated and corrugated with focal areas of extravasated hemorrhage in-between. Some cardiomyocytes showed deeply stained acidophilic cytoplasm (coagulative necrosis) and pyknotic nuclei. Other cells exhibited cytoplasmic vacuolation. Simultaneously, normal cardiomyocytes with central vesicular nuclei and acidophilic cytoplasm were well recognized (Figures 5b,5c). On the other hand, the cessation of treatment for 20 days following exposure to vitamin C for 10 days in group C2 resulted in complete recovery from MSG toxicity if compared to control group. Normal cardiomyocytes with acidophilic cytoplasm and central vesicular basophilic nuclei were observed. The narrow spaces between muscle fibers exhibited some fibroblast cells with flat dark nuclei and normal capillaries (Figure 5d).

Mallory trichrome staining revealed scarce amount of collagen fibers in the CT spaces between cardiac muscle fibers and around the wall of blood capillaries in heart sections from both control and group (C2). However, the amount of collagen fibers was apparently increased in group (B2) (Figures 6a-6c).

In addition, by Vimentin immunostaining, minimal vimentin immune-reactions in the interstitial fibroblasts, in vascular endothelium and similarly in the endomysium were clear in heart sections of control and group (C2). However, sections received only MSG of group (B2) displayed increased vimentin expression. The cardiomyocytes appeared with negative immunoreactions (Figures 7a-7c)

Moreover, by iNOS immunostaining, minimal light brown positive INOS expression appeared in the cytoplasm of some cardiomyocytes in heart sections of both control and group (C2). However, increased expression was apparent in sections from group (B2) (Figures 8a-8c).

III-Statistical results: (Table 3, Histogram 1)

A- Rats sacrificed at PD 10

The mean area percentage % of collagen fiber deposition (fibrosis), vimentin and iNOS immunoexpressing in MSG treated group (B1) was highly significant increased relative to the control group. In contrast, MSG plus Vit C treated group (C1) showed a high significant decrease if compared to MSG group (B1) but showed (low) significant increase when compared to control group.

B- Rats sacrificed at PD 30

The mean area percentage of collagen fiber deposition (fibrosis), vimentin and iNOS immune expressing in MSG treated group (B2) was highly significant increased relative to the control group. Meanwhile, MSG plus Vit C treated group (C2) versus to MSG group (B2) showed high significant decrease but had non-significant difference relative to the control group.

In conclusion, the area percentage % of collagen fibers, vimentin and iNOS expressions showed high significant increase in groups (B1, B2) relative to the control group denoting high toxicity. After co-administration of MSG with vitamin C in group (C1), this area % showed high significant decrease when compared to MSG group (B1)

but showed (low) significant increase (*) relative to control group denoting (great) improvement. On the other hand, the cessation of treatment for 20 days following exposure to vitamin C, the previous area % showed non-significant statistical difference in group (C2) relative to the control group denoting complete recovery.

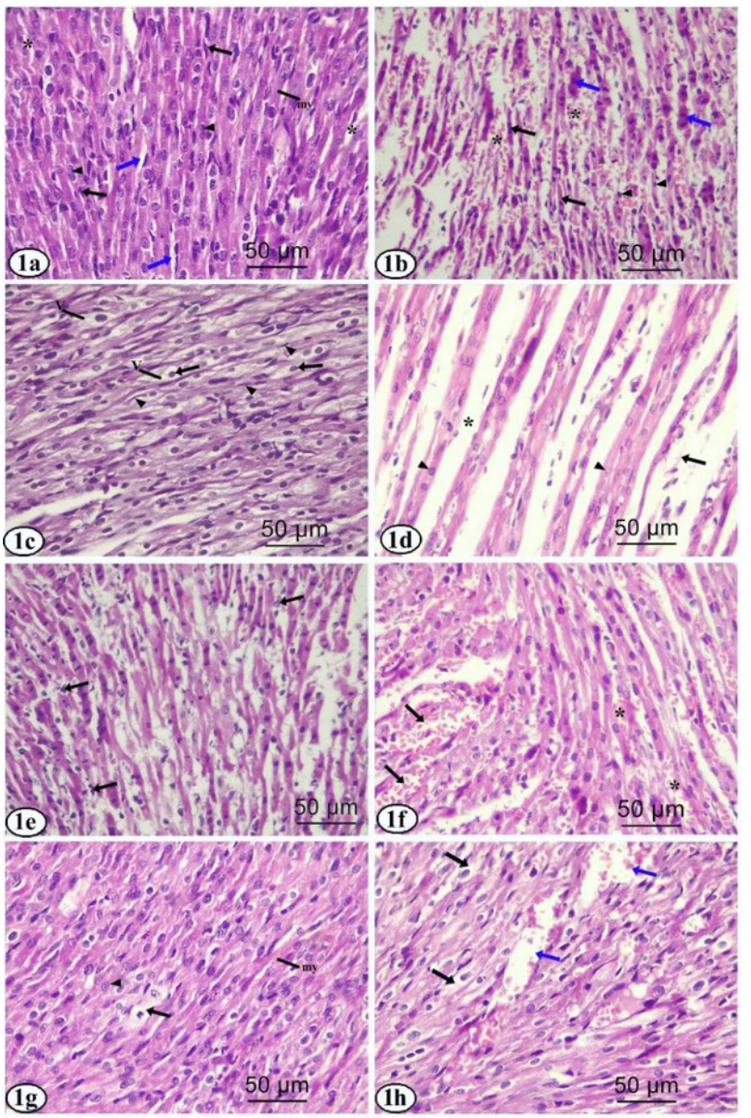


Fig. 1: Photomicrographs of sections of ventricular wall of the heart of PD 10 male albino rats of a control and groups B1 and C1. [a]: Section of a control rat showing normal cardiomyocytes (my) with an acidophilic cytoplasm and large oval basophilic vesicular nuclei (arrowhead). Notice fibroblasts (black arrow) and some blood capillaries (blue arrow) are seen in the narrow CT space (*) between muscle fibers. [b-f]: Section from group (B1). [b]: the muscle fibers appear markedly disrupted, shrunken (black arrow) with extensive areas of extravasated hemorrhage in between (*). Some cardiomyocytes exhibit small deeply stained nuclei (pyknotic) (arrowhead) and darkly stained acidophilic cytoplasm (coagulative necrosis) (blue arrow). [c]: Some cardiomyocytes exhibit pale acidophilic vacuolated cytoplasm (V) and small darkly stained nuclei (arrow). Notice, the fibroblasts (arrowhead) are apparently increased in number. [d]: showing marked muscle fiber separation with wide CT spaces (*), some fibers appear necrotic (arrow). Some cardiomyocytes show small vesicular nuclei (arrowhead). [e]: showing marked increase in CT cells (arrow) in the CT spaces between muscle fibers. [f]: showing extensively dilated and congested blood capillaries (arrow) among dilated CT spaces between muscle fibers. Extravasated blood between muscle fibers (*) is also seen. [g-h]: Sections from group (C1). [g]: showing normal cardiomyocytes (my) with central oval nuclei (arrowhead). [h]: few cardiomyocytes exhibit cytoplasmic vacuolation (black arrow) and some blood capillaries appear dilated and mildly congested (blue arrow) (H&E × 400)

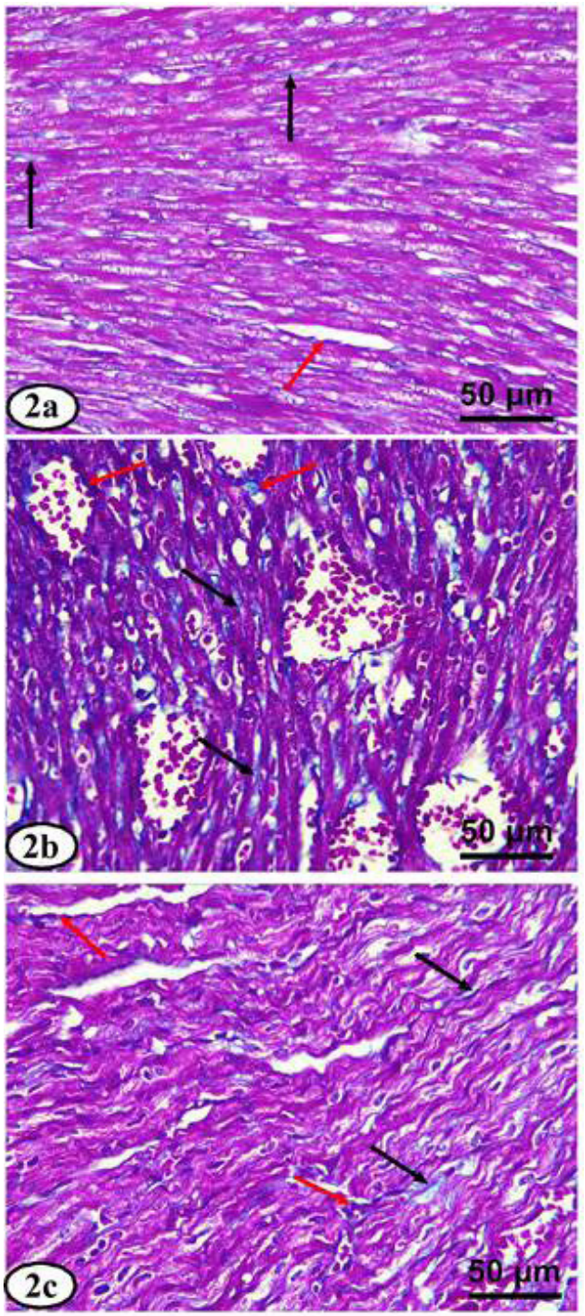


Fig. 2: Photomicrographs of sections of Mallory trichrome staining heart muscles of PD 10 male albino rats of a control and groups B1 and C1. [2a]: Section of a control group showing delicate collagen fibers in the CT spaces between cardiac muscle fibers (black arrow) and around the wall of blood capillaries (red arrow). However, the amount of collagen fiber is markedly increased in group (B1) [2b], while is less in group (C1) [2c]. (Mallory's trichrome stain × 400)

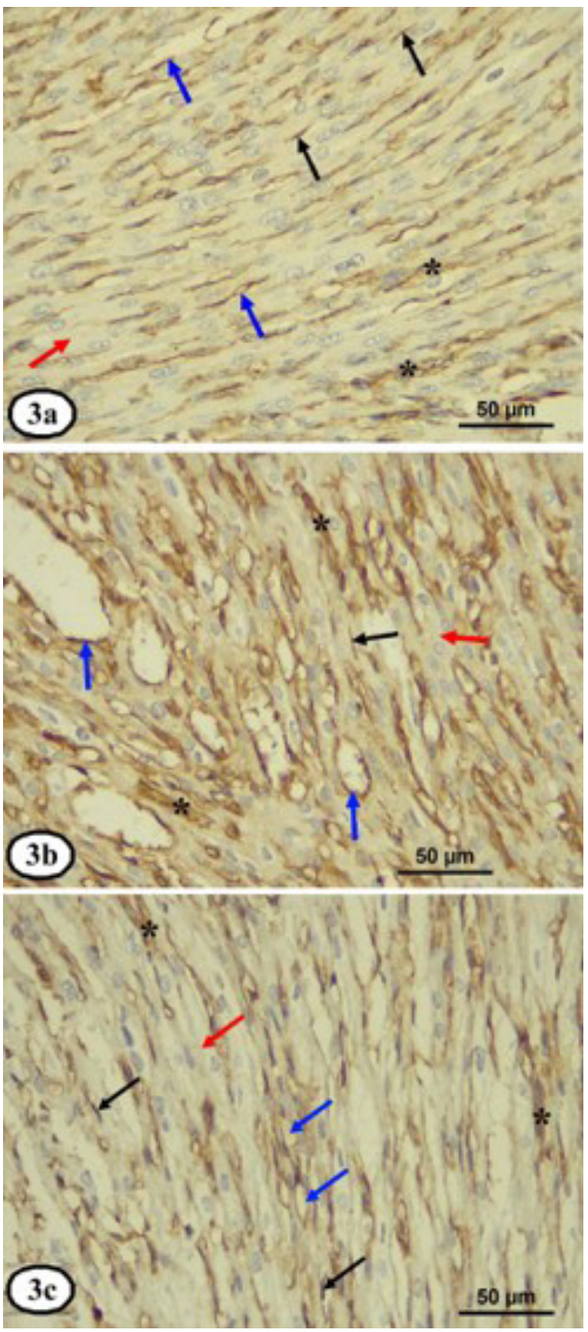


Fig. 3: Photomicrographs of sections of vimentin immunostaining heart muscles of PD 10 male albino rats of a control and groups B1 and C1. [3a]: Section from a control rat heart showing weak brown positive vimentin immunoreactions in the interstitial fibroblasts (black arrows), in the endothelium of blood capillaries (blue arrows) and in the endomysium (*). However, [3b]: section from group (B1) showing extensive vimentin expression. [3c]: Section from group (C1) showing mild vimentin expression. Notice the cardiomyocytes (red arrow) show negative immunoreaction. (Vimentin IHC × 400)

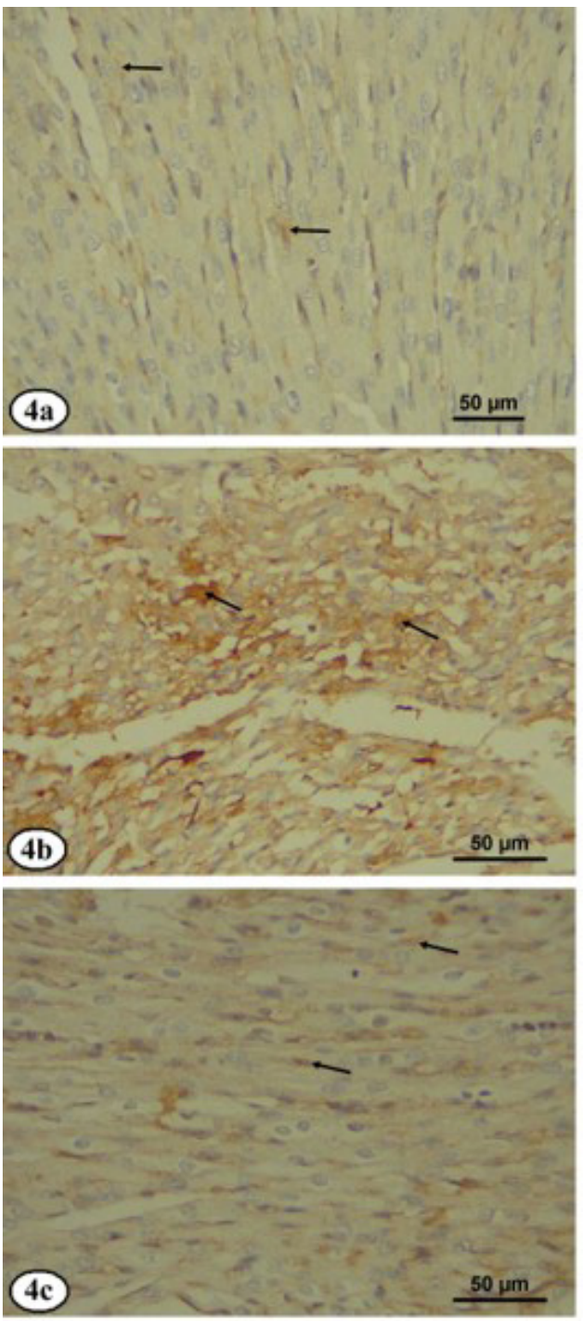


Fig. 4: Photomicrographs of sections of INOS immunostaining heart muscles of PD 10 male albino rats of a control and groups B1 and C1. [4a]: Section from control group showing minimal light brown positive iNOS expression in the cytoplasm of some cardiomyocytes (arrow). However, [4b]: Section from group (B1) showing strong expression. [4c]: Section from group (C1) showing mild iNOS expression. (iNOS IHC × 400)

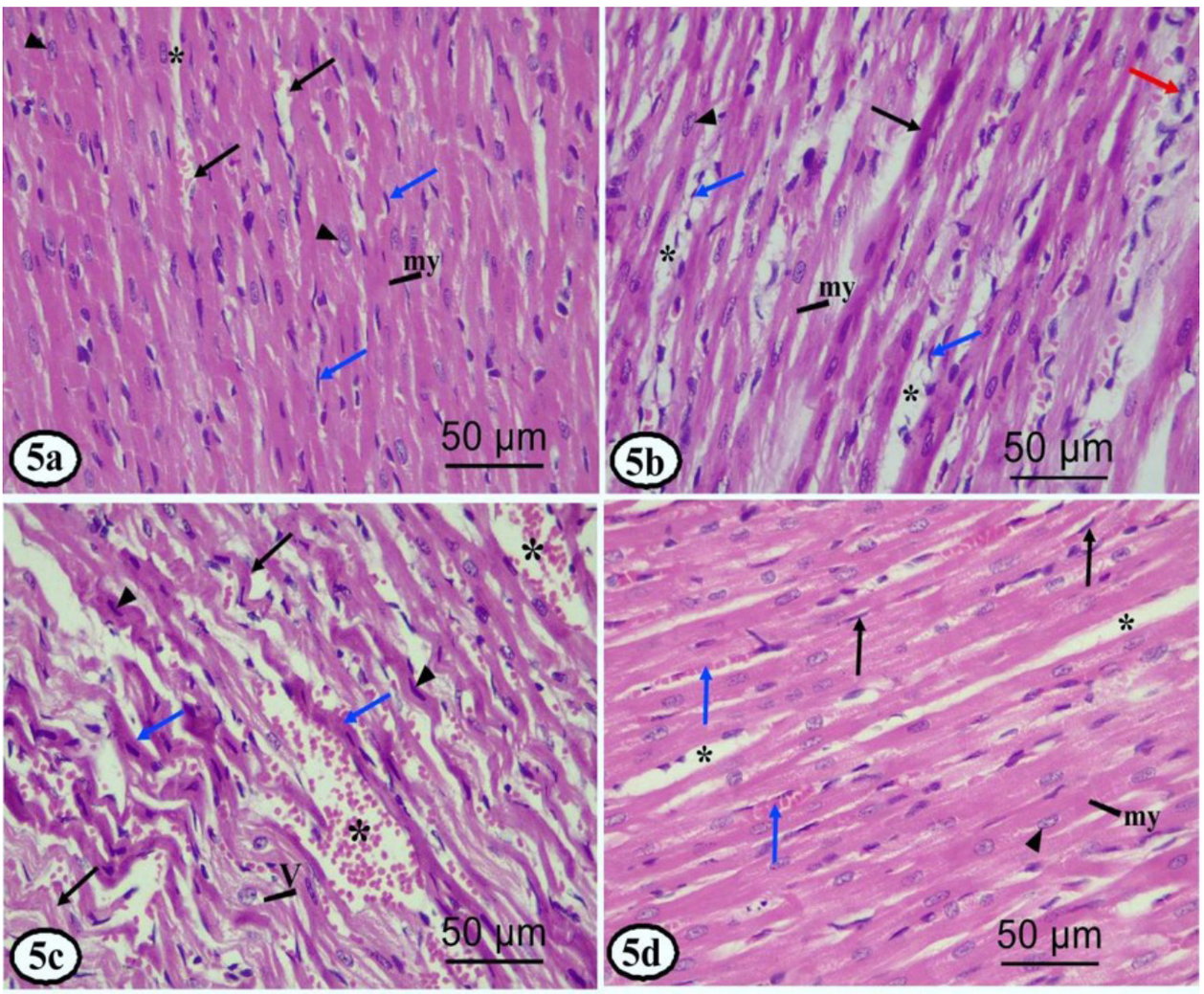


Fig. 5: Photomicrographs of sections of heart muscles of PD 30 male albino rats of control and groups B2 and C2. [a]: Section from a control rat heart showing normal cardiomyocytes (my) of ventricular wall. They appear striated with an acidophilic cytoplasm and large oval nuclei (arrowhead). Flat nuclei of the fibroblasts (blue arrows) and capillaries (black arrows) are seen in the small connective tissue space (*) between muscle fibers. [b-c]: showing sections from group (B2). [b]: showing the cardiac muscle fibers appear moderately separated. Many fibroblast cells with flat dark nuclei (blue arrows) and CT cells (red arrow) are seen in the wide CT spaces (*). Some cardiomyocytes (my) appear with normal central vesicular nuclei (arrowhead) and acidophilic cytoplasm and others show deeply stained acidophilic cytoplasm (black arrow). [c]: The cardiac muscle fibers appear attenuated and corrugated (black arrows). Some cardiomyocytes exhibit deeply stained acidophilic cytoplasm (coagulative necrosis) (blue arrow) and small dark nuclei (pyknotic) (arrowhead) and other cells show cytoplasmic vacuolation (V). Focal areas of extravasated hemorrhage are seen between the moderately separated muscle fibers (*). [d]: showing a section from group (C2). The cardiomyocytes (my) show normal acidophilic cytoplasm and central vesicular nuclei (arrowhead). Some fibroblast cells with flat dark nuclei (black arrow) and small capillaries (blue arrow) are seen in the narrow spaces between muscle fibers (*). (H&E × 400)

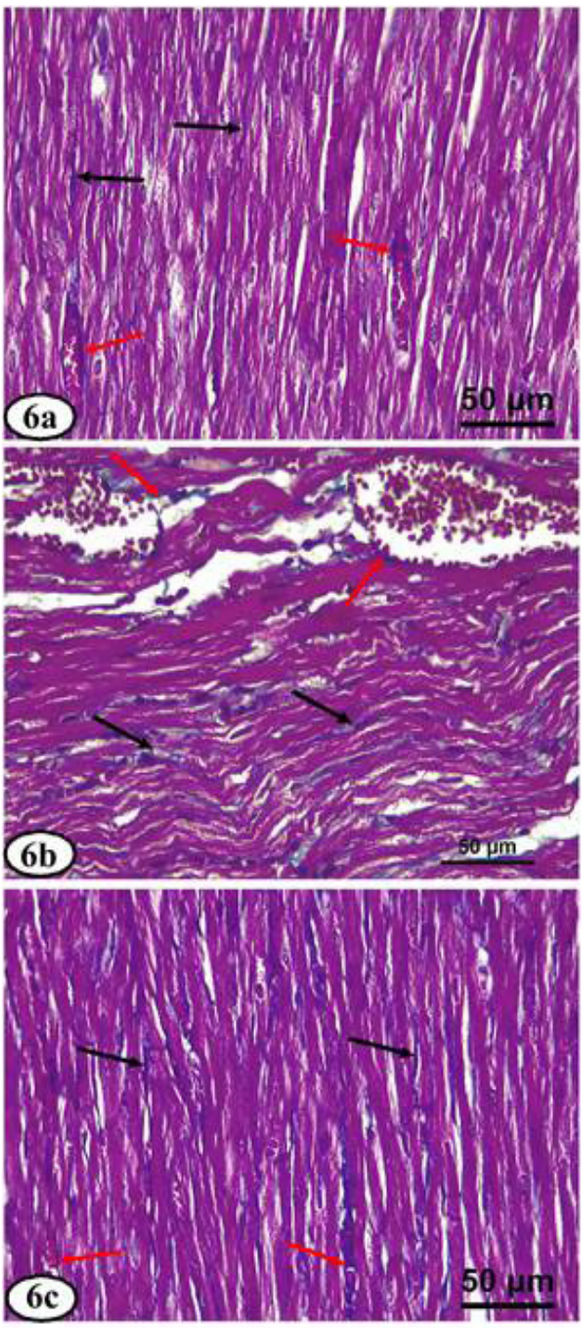


Fig. 6: Photomicrographs of sections of Mallory trichrome staining collagen fibers of heart muscles of PD 30 male albino rats of control and groups B2 and C2. Scarce collagen fibers in the CT spaces between cardiac muscle fibers (black arrow) and around the wall of blood capillaries (red arrow) are seen in a heart sections from control [Fig. 6a] and from group (C2) [Fig. 6c]. However, a section from group (B2) [Fig. 6b] showing increased amount of collagen fibers. (Mallory trichrome stain, × 400)

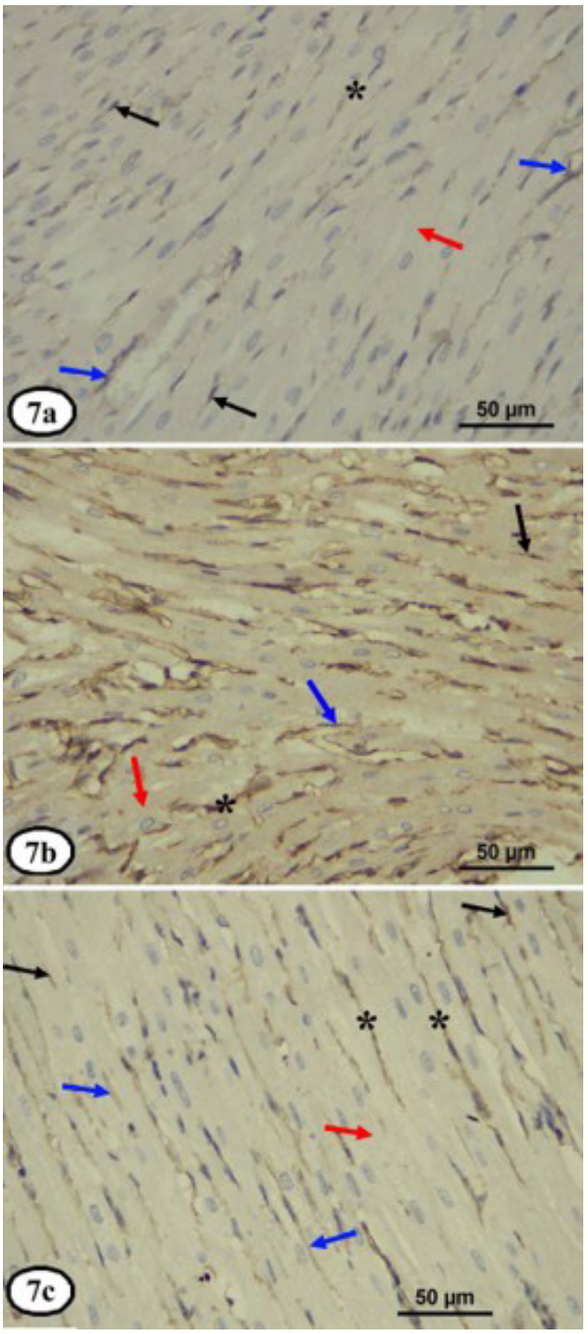


Fig. 7: Photomicrographs of sections of vimentin immunostaining of heart muscles in PD 30 male albino rats of control and groups B2 and C2. Minimal vimentin immunoreactions in the interstitial fibroblasts (black arrows) and in the endothelium of blood capillaries (blue arrows) and in the endomysium (*) are seen in a section from a control [Fig. 7a] and in a section from group (C2) [Fig. 7c]. However, a section from group (B2) [Fig. 7b] shows moderate vimentin expression. Notice, the cardiomyocytes (red arrows) reveal negative immunoreaction. (Vimentin IHC × 400)

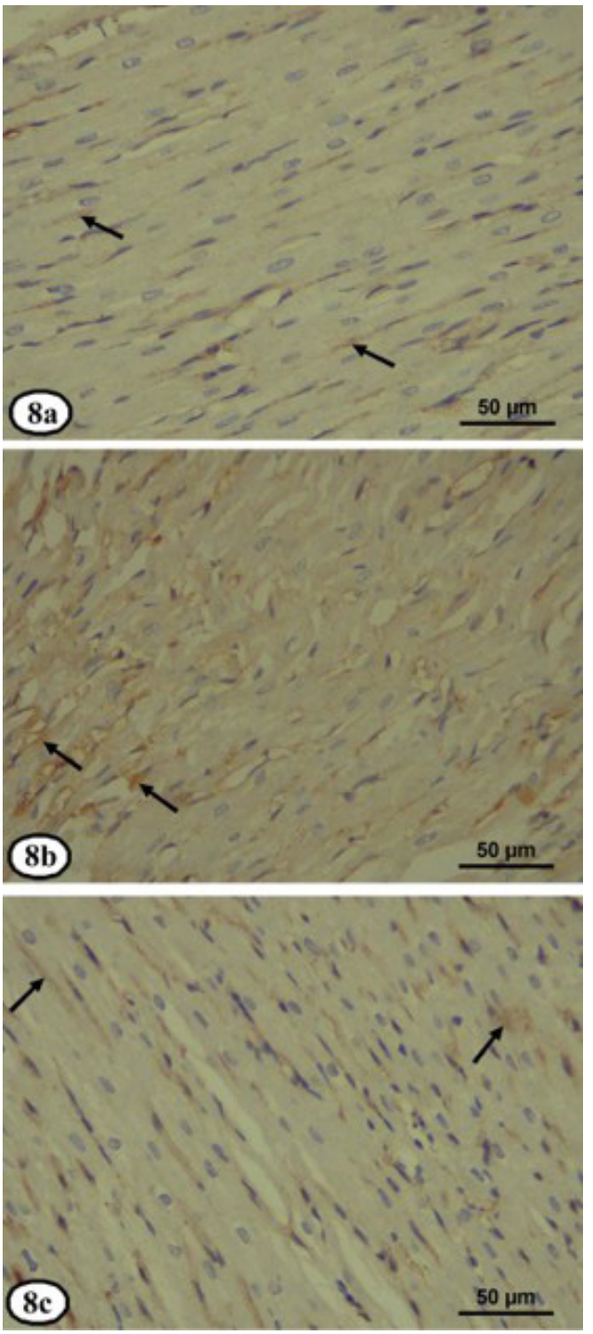


Fig. 8: Photomicrographs of sections of iNOS immunostaining heart muscles of PD 30 male albino rats of control and groups B2 and C2. Minimal light brown positive iNOS expressions are noticed in the cytoplasm of some cardiomyocytes (arrow) in a section from control group [Fig. 8a] and in a section from group (C2) [Fig. 8c]. However, section from group (B2) [Fig. 8b] showing moderate positive iNOS expression. (iNOS IHC × 400)

Table 1: Statistical comparison of mean values of final body weights between control, treated and protected groups of male albino rat pups at postnatal day 10 (PD 10)

Rats sacrificed at PD10	Final body weights		One-way ANOVA	
	Range	Mean ± SD	F	*P value*
Control (Group A)	17-30	25.73 ± 4.37		
MSG (Group B1)	28-42	36.00 ± 5.05	6.217	0.006**
MSG +vit C (Group C1)	19-30	23.30 ± 5.75		

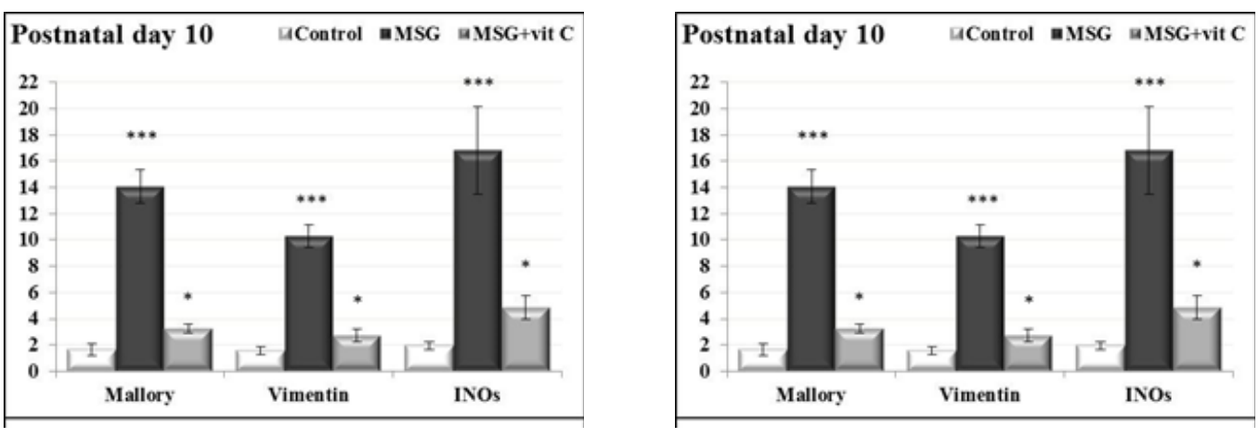
Table 2: Statistical comparison of mean values of final body weights between control, treated and protected groups of male albino rats at postnatal day 30 (PD 30).

Rats sacrificed at PD30	Final body weights		One-way ANOVA	
	Range	Mean ± SD	F	*P value*
Control (Group A)	82-92	65.67 ± 11.45		
MSG (Group B1)	85-105	94.00 ± 6.7	15.873	0.002**
MSG +vit C (Group C1)	60 -80	68.65 ± 5.75		

Table 3: statistical analysis of the mean values of area percentage of collagen fiber deposition and positive immune reaction for vimentin and iNOS in the different studied groups

Postnatal day 10 (PD10)							
Area percentage	Control mean ± SD	MSG mean ± SD	MSG +vit C mean ± SD	ANOVA	Tukey's multiple comparison test		
					MSG vs Control	MSG+vit C vs Control	MSG+vit C vs MSG
Mallory Trichrome	1.64 ± 0.47	14.09 ± 1.25	3.24 ± 0.36	< 0.0001***	***	*	***
Vimentin	1.54 ± 0.29	10.29± 0.87	2.76 ± 0.49	< 0.0001***	***	*	***
iNOS	1.94 ± 0.31	16.84 ± 3.33	4.87 ± 0.89	< 0.0001***	***	*	***
Postnatal day 30 (PD30)							
Area percentage	Control mean ± SD	MSG mean ± SD	MSG +vit C mean ± SD	ANOVA	Tukey's multiple comparison test		
					MSG vs Control	MSG+vit C vs Control	MSG+vit C vs MSG
Mallory Trichrome	4.75 ± 1.18	9.01 ± 1.15	5.58 ± 1.09	< 0.0001***	***	ns	***
Vimentin	2.24 ± 0.59	8.11 ± 1.37	2.23 ± 0.51	< 0.0001***	***	ns	***
iNOS	0.79 ± 0.15	2.63 ± 0.62	1.12 ± 0.38	< 0.0001***	***	ns	***

High significance (***) means *P value* < 0.001, Moderate significant (**) at 0.01 >*P value* >0.001 and low significance (*) when 0.05 >*P value* >0.01.



Histogram 1: The mean values of the area % of the collagen fiber deposition, vimentin and iNOS immune reactions in the different studied groups. Regarding the histogram, all the groups are compared relative to control group.

DISCUSSION

The main finding in the present study were histological and immunohistochemical alterations in the cardiac muscle structure in neonate male albino rat pups indicating cardiotoxicity in MSG treated group if compared with the control group.

In the present work, MSG induced marked vascular congestion and dilatation with extravasated blood in between the muscle fibers. The same findings were recorded by[32,33] .Vascular congestion could be due to vascular obstruction. In the same context, MSG might induce endothelial injury resulting in sequestration of red blood cells and platelets, impairment of circulation and thrombus formation[34]. Small areas of capillary occlusion with subsequent capillary dilatation will increase the intracapillary pressure with subsequent blood leakage due to either increased capillary permeability or capillary wall rupture. Also, MSG might cause cardiovascular stress accompanied by a mild, slowly occurring heart failure followed by passive congestion[35].

In the current work, oral MSG consumption induced disruption, fragmentation, and wide separation of cardiac muscle fibers. Many cardiomyocytes exhibited darkly stained acidophilic cytoplasm (coagulative necrosis) and others showed cytoplasmic vacuolation. Most nuclei appeared pyknotic. Such myocardial abnormalities could result from MSG induced cardiac tissue oxidative stress with reactive oxygen species [ROS] generation[4]. ROS-mediated lipid peroxidation might be the direct cause of cytoplasmic vacuolation detected in cardiomyocytes. Also, such radicals could induce cellular DNA damage, besides lipids and proteins, and lysis of stromal cells with subsequent cell necrosis or apoptosis[36,37]. Furthermore, glutamate receptors play a critical role in pathogenesis of disorders induced by MSG. Following MSG absorption, presence of glutamate receptors and excess glutamate metabolism could induce more lipid peroxidation and less major antioxidant enzymes with subsequent oxidative stress induced tissue damage[38,39]. Glutamate changes the activity of voltage-gated potassium channels leading to increased content of intracellular calcium[40]. Increased calcium influx leads to excessive mitochondrial calcium which can activate death of cell via releasing of proapoptotic factors and increasing generation of ROS[41].

In the present investigation, following MSG exposure, an apparent increase in number of fibroblasts and CT cells in the CT spaces was observed. Also, excess collagen fiber deposition was obvious in the interstitial tissue as well as perivascular. Moreover, the area percentage of collagen fiber deposition showed a high significant elevation in MSG-treated group in contrast to the control group. Similar findings were reported that there was a high significant increase in the percent area of fibrosis in the intertubular interstitial tissue and around the blood vessels in rat testis and kidney upon exposure to MSG[12,39].

It was proposed that, ROS induced lipid peroxidation leads to stimulation and maintenance of an inflammatory reaction in which macrophages interact with matrix generating cells and subsequent increased fibrotic tissue. Moreover, it could induce excess fibro genic cytokines expression which is the key molecules in fibrosis mechanisms as well as increased collagen transcription and translation[42]. Furthermore, ROS generation could stimulate fibroblast transformation into excess fibrous tissue synthetic myofibroblasts[43].

In this work, a significant high increase in the area percentage of positive reaction of vimentin immunostaining in interstitial fibroblasts, vascular endothelium and endomysium in MSG treated group compared to the control one was recorded. These findings are similar to that previously described in cases of dilated cardiomyopathy (DCM) in both human and animal models. Vimentin disorders could add to diversity in intracellular signal transduction, cardiomyocyte function and cardiomyocytes extracellular matrix coupling. Also, concomitant increase in both vimentin-positive cells and fibrosis in certain tissue is an indicator of interstitial tissue increase[44-46]. Altered Vimentin expression might be due to intracellular calcium level changes[47]. A negative association between vimentin expressing in cardiac tissue and the actin–myosin gliding rate was established with subsequent reduction in cardiomyocyte contraction as in heart failure[24].

In healthy adult rat and human, the cardiomyocytes normally, constitute nearly 30-40% of cardiac tissue mass meanwhile non-cardiomyocyte cells, constitute for the remaining 60–70% tissue mass (primarily formed of cardiac fibroblasts). The cardiac fibroblasts are necessary for the extracellular matrix (ECM) homeostasis by maintaining cytokines synthesis degradation balance and by secreting non-rigid collagen type I and III of ECM. Moreover, fibroblasts are capable of proliferation and migration to the injury site and production of great amounts of collagen for damaged tissue repair[48,49]. Furthermore, after heart injury, the cardiac fibroblasts could transform into the myofibroblast phenotype, the cells which are implicated in contribution to fibrosis and progress to heart failure[50,51].

The present work also aimed to predict the possible mechanism of MSG cardiac toxicity, in this context, a trial to localize the distribution of iNOS immuno-expressing in the cardiac tissue was settled. In the control specimens, minimal iNOS immunoreactivity was detected in cardiomyocytes. On the other hand, in MSG-treated rats, positive immunoreaction for iNOS showed a high significant increase when compared with the control group. Previous studies revealed that, the iNOS expression could be increased in DCM, ischemic heart disease[52] and in myocardial infarction[53]. Also, when excess cytokines are released as noticed in chronic heart failure and hypoxia, iNOS leads to liberating of a free radical gas; Nitric oxide (NO). Normally, in almost all mammalian cells, NO is a bioactive product that mediates multiple biological functions such as vasorelaxation[54,55]. In *vivo*, NO could be generated by L-citrulline L-arginine transformation, catalyzed by three NO synthases (NOS) isoforms; neuronal

(nNOS), endothelial (eNOS), and inducible (iNOS)[56]. NO overproduction could contribute to contractile dysfunction, which is a dominant factor in chronic heart failure[57]. In the rat and mouse heart, NO increases the mitochondrial permeability thereby increasing cardiomyocyte apoptosis[58].

In the current study, neonatal MSG administration for 10 days led to a statistically significant increase of mean body weights of rats in contrast to control group. Similarly, animal studies revealed that neonatal MSG consumption could result in obesity later on[17,59-61]. The previous authors postulated that, insulin resistance, decreased glucose tolerance, disrupting the leptin-arbitrated hypothalamus signing cascade and alteration of orexigenic and anorexigenic molecules production are possible mechanisms concerned about development of obesity. Furthermore, MSG consumption could disturb energy balance and caloric storage by stimulating orosensory receptors thus increasing food palatability and intake.

In contradistinction to the current results, MSG fed mice revealed a significant reduction in the mean body weight relative to control group[62]. Meanwhile, Adebayo *et al*.[63] showed that MSG had non significant influence on the mean body weight of MSG treated rats. Abd El-Aziz *et al*.[64] mentioned that prolonged administration of MSG causes an initial increase in weight gain followed by late reduction, despite of increased food consumption. This might be due to a defect in digesting and absorbing foods. Mortensen *et al*.[65] revealed that, the reasons for these different observations might be attributed to the experimental animals' type, age and duration of administration and the concentration of MSG used.

In the current work, ventricular sections of rats administrated Vit C plus MSG in group (C1) showed great amelioration of the previous myocardial structural damage. Most cardiomyocytes exhibited a nearly normal architecture and narrower CT spaces. However, capillary congestion was still a finding in that group. In addition, statistically, the area percentage % of collagen fibers, vimentin and iNOS expression showed high significant decrease if compared to MSG group (B1) but was low significant increase relative to control group denoting great improvement. Similar findings were reported by Hassan *et al*[66] who stated that, Vit C administration corrected MSG induced disturbances in cardiac muscle structure, oxidative stress, physiological and functional changes in the heart. It also has been proved that Vit C could modify MSG induced cytotoxicity in rat testis[67], Kidney[68] and thymocytes[26]. It has been concluded that, Vit C is a strong reducing agent due to its electron donor property and it can reduce the oxidative stress via neutralizing hydroxyl and superoxide radicals and preventing the initiation and propagation of chain reaction[69,70]. Also, vitamin C inhibits lipid peroxidation by enhancing the endogenous antioxidative enzymes activity such as glutathione transferase and peroxidase[71]. Moreover, it is a free radical scavenger and its treatment maintains the activity and expression of Na+/K+-ATPase and Ca2+-ATPase enzymes being inactivated by MSG induced oxidative stress, ROS production , membrane lipid peroxidation and subsequent enzymatic failure[63]. Furthermore, vit C abolishes chromosomal damage that may arise from toxic molecules[72] and has an immunomodulatory effect proven in the liver and kidney[73].

In the present study, group (B2) revealed the same histopathological and immunohistochemical changes of group (B1) but with less degree. However, complete improvement was observed after cessation of treatment for 20 days following exposure to vitamin C plus MSG in group (C2). Moreover, the area percentage % of collagen fibers, vimentin and iNOS expression showed non-significant statistical difference in group (C2) relative to the control group denoting complete recovery. This means that MSG withdrawal only resulted in mild recovery and Vit C has the upper hand in reversibility of MSG toxicity. These results are in agreement with[14] who reported that MSG withdrawal for 28 days resulted in some degree of recovery by reducing the heart weight. Also, El-Helbawy *et al*.[7] said that, rats treated with MSG for 2 weeks and then were held without medication for another 2 weeks before sacrification showed a partial improvement in the histological and ultrastructural construction of the adrenal cortical zona fasciculata cells. Moreover, Nosseir *et al*.[74] reported that, in rat testis, MSG toxicity was mildly reversible when withdrawal occurred. In contrary to the current results, Qtaitat *et al*.[75] found no recovery in rat testis treated with MSG for one month then withdrawn for another one month. Even, Aloa *et al*.[76] mentioned that the withdrawal groups appeared with more histologically noticed degenerative alterations relative to the groups in which the MSG treated rats were immediately sacrificed.

CONCLUSION

The results of the present study emphasize that MSG induced myocardial toxicity in early postnatal period. Vitamin C greatly ameliorated histopathological and immunohistochemical alterations in cardiac tissue. MSG withdrawal for 20 days showed mild improvement but withdrawal after vitamin C administration was more effective in reversibility of MSG toxicity.

RECOMMENDATION

We encourage for the reduction of monosodium glutamate levels in processed foods especially that introduced in restaurants, homes, or sold in grocery stores and markets.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تأثير تناول جلوتامات الصوديوم الأحادي على تركيب القلب للجرذان البيضاء حديثة الولادة والدور الوقائي لفيتامين ج

حنان السيد لطفي مختار وأمل سليمان سويلم

قسم التشريح والأجنة- كلية الطب- جامعة الزقازيق

مقدمة: على الرغم من الاستخدام الواسع الإنتشار لجلوتامات الصوديوم الأحادي كمُحسِّن للنكهة، إلا أن استهلاكه كان بمثابة إنذار بسبب التعرض اليومي له بدون حد أقصى للسلامة. كما أن فيتامين ج (حمض الأسكوربيك) له نشاط قوي مضاد للأكسدة.

هدف البحث: لتوضيح تأثير إعطاء جلوتامات الصوديوم الأحادي عن طريق الفم على تركيب أنسجة القلب للجرذان البيضاء حديثة الولادة و لتقييم الدور الوقائي المحتمل لإعطاء فيتامين ج ضد سمية جلوتامات الصوديوم الأحادي وأيضا لتقييم إمكانية تراجع هذه التأثيرات عند سحب العلاج.

المواد والطرق المستخدمة: تم إستخدام ٤٠ جرذ أبيض من الذكور (عمر يوم واحد بعد الولادة) وتم تقسيمهم الى ثلاث مجموعات (أ-ب-ج) كما يلي: المجموعة الضابطة (أ) والمجموعة (ب) المعالجة بجلوتامات الصوديوم الأحادي فقط (٤ ملجم / جم من وزن الجسم/ يوم) والمجموعة (ج) المعالجة بجلوتامات الصوديوم الأحادي مع فيتامين ج حيث تلقت جرعة من جلوتامات الصوديوم الأحادي كما وصفت سابقا بالإضافة الى فيتامين ج (٥٠٠ ملجم / كجم من وزن الجسم / يوم) لمدة ١٠ أيام. توقف العلاج لمدة ٢٠ يومًا في جميع المجموعات المعالجة. ثم تم تقسيم المجموعات الثانية والثالثة (ب , ج) الى مجموعتين فرعتين وفقًا لعمر التضحية بالحيوان إلى (ب١, ج١) حيث تم التضحية بهما عند عمر ١٠ أيام بعد الولادة (بعد آخر جرعة من العلاج) و(ب٢ , ج٢) حيث تم التضحية بهما عند عمر ٣٠ يوم بعد الولادة (أي بعد ٢٠ يومًا من التوقف عن العلاج). في نهاية التجربة، تم أستخراج عينات القلب لمعالجة عينات البطين الأيسر ليتم فحصها من خلال الدراسات النسيجية الباثولوجية و الكيميائية المناعية والمورفومترية والإحصائية.

النتائج: قد أوضحت الدراسة النسيجية لتركيب أنسجة البطين الأيسر في المجموعة (ب١) تغيرات تنكسية هائلة بما في ذلك تفسخ وخلل لألياف العضلات بشكل ملحوظ ، وظهور فواصل بينهم مع زيادة عدد خلايا النسيج الضام والخلايا الليفية. بالإضافة الى ظهوربعض الشعيرات الدموية المتسعة و المحتقنة والنزيف المتسرب في الفواصل بين الألياف العضلية. أظهرت عضلات القلب تخثر للأنوية و تنكرز تجلطي للسيتوبلازم وظهورفجوات به. كانت التغييرات السابقة لا تزال موجودة في (ب٢) ولكن بدرجة أقل. أدى إعطاء جلوتامات الصوديوم الأحادي بالإضافة إلى فيتامين C إلى استعادة التركيب الطبيعي لعضلة القلب بشكل كبيرفي المجموعة (ج١) ولكن لوحظ الشفاء التام في المجموعة (ج٢). كما أظهرمتوسط قيم النسب المئوية من ألياف الكولاجين وتعبير وجود كل من الفيمنتين والإنزيم المصنع لاكسيد النيتريك المحفز زيادة عالية معتد بها إحصائيا في كل من المجموعتين (ب١, ب٢)، وارتفاع قليل معتد به إحصائيا في المجموعة (ج١) ولكن متوسط هذه القيم كان غير مختلف بشكل كبير في المجموعة (ج٢) إذا قورنت بالمجموعة الضابطة.

الخلاصة: تسبب جلوتامات الصوديوم الأحادي في سمية عضلة القلب في الفترة المبكرة ما بعد الولادة. ووجد أن فيتامين ج يحسن التغيرات النسيجية الباثولوجية والكيميائية المناعية لأنسجة القلب بشكل كبير. كما أظهر سحب العلاج بجلوتامات الصوديوم الأحادي لمدة ٢٠ يومًا إلى تحسنً طفيفً ولكن سحبه بعد تناول فيتامين ج كان أكثر فعالية في تراجع كامل لهذه التغيرات.