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RESEARCH

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Histopathological and immunohistochemical studies on the influence of orally administration monosodium glutamate, a food additive dependent on time in vivo

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Abstract

Background: Monosodium glutamate is a widely used flavor enhancer/food additive in meat, fish, milk and cheese or vegetable origins. Our present study aimed to assess the effect of utilization monosodium glutamate on the cardiac, splenic, hepatic and renal organs in mice. Thirty animals were divided into three groups: control group; glutamate 10 treated groups and glutamate 15 treated group. The experimental period was two successive weeks.

Results: Oral administrations of monosodium glutamate for 10 days induced moderate histopathological changes in cardiac, splenic, hepatic and renal tissues and also resulted in significant increase of the nuclear factor-kB expression depend on time of administration as compared to the control group, while treatment with monosodium glutamate for 14 days resulted in severe histopathological changes as well as highly significant increase of the nuclear factor-kB expression as compared to the control group.

Conclusions: It could be concluded that oral administration of monosodium glutamate induced histopathological alterations, and cytotoxicity in cardiac, splenic, hepatic and renal tissues dependent on time.

Keywords: Monosodium glutamate, Histopathological, Immunohistochemistry, Male mice

Background

Monosodium glutamate, the salt of glutamic acid which is a common food additive, is widely used as a preservative or palatability (Ali et al., 2014). It consists of the sodium salt of glutamic acid (Eweka, 2007), of glutamic acid (78%), sodium and water (22%) (Samuels, 1999) and is metabolized in the liver (Garattiini, 2000). In nature, glutamate (Glutamic acid) is considered as one of the most common amino acids and is the main component of many proteins and peptides of most tissues (Aisha, 2014; Wu, 2010). In the human body, glutamate has an essential role in metabolism (Aisha, 2014), cell signaling, anti-oxidative responses and immunity (Wu, 2010). The main sources of glutamic acid are all meats, poultry, fish, eggs, dairy products, tomato and some protein-rich plant foods, hydrolyzed protein such as yeast extract and many fermented or aged foods, including soy sauce and fermented bean paste (Burrin & Stoll, 2009). Many prepared foods use glutamate in the form of monosodium glutamate as an additive (Berkes & Wossner, 2003). Monosodium glutamate is recorded on food labels as a "Flavoring" and has the ability to ameliorate the meals palatability and induce positive appetite and gain of weight. It is usually used in many food products such as noodles, flavored potato chips, many food snacks, soups, frozen foods and stuffed chicken (Eweka, 2007). In addition, it is mostly used in the home, fast foods, restaurant and food industries.

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It is known that consumption of dietary monosodium glutamate resulted in different unwanted effects such as sweating, muscle pain, fatigue, headache, neuropathy, ventricular arrhythmia, abdominal discomfort, skin reactions and asthma in human (Geha et al., 2000). Similarly, it also induces pathologies in the liver tissues (Eweka et al., 2011), testis of rat (Igwebuike et al., 2011) and ovaries of female mice (Das & Ghosh, 2011; Mustafa et al., 2015) and female rats (Bojanic et al., 2009). In addition to, monosodium glutamate, induced histopathological alteration on the retina of rabbit as recorded by Ali et al. (2012).

The U.S. Food and Drug Administration considers MSG safe, while the European Union's Food Safety Authority determines an intake of 30 mg/kg body weight per day as safe (Peng et al., 2018). However, administration of monosodium glutamate at a low concentration of 5 mg/kg of body weight for 28 days caused hepatotoxic (Egbuonu et al., 2009) and renal toxicity at dose of 4 mg/kg of body weight (Ragab, 2018) as evidences by histological, immunohistochemical and ultrastructure studies. In addition, Ebaid and Tag (2012) also found that rat administrated with 4 mg/kg of monosodium glutamate for 14 days showed severe spleen damage. Likewise, Hassan et al. (2014) reported toxicity of monosodium glutamate when administration to rats at a dose 3 g/kg body weight (1/5 LD50) daily for 8 weeks in thymic and splenic tissues after prolonged consumption. Diab and Hamza (2016) also recorded the toxic influences of monosodium glutamate in the function of liver tissue in rats. They reported that administration of monosodium glutamate at a dose of 60 mg/kg for 4 weeks resulted in the elevation of hepatic enzymes and lipid profile such as cholesterol, triglycerides. Likewise, Kumbhare et al. (2015) stated that monosodium glutamate at the dose 3 mg/kg body weight induced conspicuous pathological lesions in the liver tissue when it was administrated orally for 45 successive days. Neurotoxicity (hazards to the structure of cerebellar cortex, Purkinje cell layer with necrotic widely spaced cells) of monosodium glutamate was also evaluated by Aidaros et al. (2019). Therefore, the aim of our paper was designed to study the influence of monosodium glutamate administration on cardiac, splenic, hepatic, and renal tissues of male Swiss albino mice using histopathological and immunohistochemical studies.

Methods

Experimental design

Thirty animals were divided into three groups: The first group served as control and were treated orally with distilled water (1 ml/kg.bw), the second group (Glutamate 10 treated group) were orally administrated with monosodium glutamate at dose 4 mg/kg body weight for 10 days according to Ragab (2018), and the third group (Glutamate 14 treated group) were orally administrated with monosodium glutamate at dose 4 mg/kg body weight for 14 days according to Ragab (2018). At the end of the experimental, the animals were euthanized after 24 h of the last dose by decapitation by administering an intraperitoneal injection of sodium pentobarbital (50 mg /kg) and dissection and cardiac, splenic, hepatic and renal tissues were freshly collected and immediately fixed into 10% formalin for histopathological and immunohistochemistry examination.

Material

Chemicals

Monosodium glutamate was obtained from Shanghai Bio Life Science & Technology Co., Ltd. (China) while others chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Animals and ethics

Animals were obtained from the animal house of the National Organization for Drug Control and Research (NODCAR) in Giza, Egypt. In this study, thirty male Swiss albino mice aged 9–12 weeks and weighing 25–30 g were used. Animals were provided with a standard marketable diet pellets and water that Ad-labium reserved in cages of plastic for 7 days to be acclimatized to laboratory condition before treatment. All animals were grouped and housed according to the guidelines of the institutional animal's ethics committee of NODCAR. All male Swiss albino mice were grouped and housed according to the guidelines of the institutional animal's ethics committee of NODCAR. All experimental procedures were conducted in accordance with the ethical standards and were approved by the Institutional Animal Care and Use Committee (IACUC) at National Organization for Drug Control and Research (NODCAR), (approval no. NODCAR/III/41/2019).

Experimental procedures

Histopathological investigations

After 48 h of fixation in 10% formalin, the cardiac, splenic, hepatic and renal were washed, dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. Five-micron-thick paraffin sections were prepared, mounted on clean slides and stained with Ehrlich's hematoxylin–eosin for histological study (Bancroft & Gamble, 2002).

Immunohistochemical analysis

NF-κB (nuclear factor-kB) immunostaining was carried out on the heart, spleen, liver and kidney tissues with PBS containing 113 0.05 M EDTA followed by 4%

paraformaldehyde. 5-µm sections were incubated with blocking reagent, primary antibody anti-NF-κB (17) in the presence of 10% rabbit serum overnight at 4 °C, followed by biotin-conjugated goat anti-rabbit Ig, avidin-linked HRP complex and 3,3'-diaminobenzidine as substrate. For quantification measurement, slides were counterstained with hematoxylin, followed by dehydration, examination under a light microscope. The intensity of brown color indicator for cell immunopositivity was observed (Soria-Valles et al., 2015). Ten readings were obtained in each specimen using Leica Qwin 500 image analyzer the computer system (England) in the faculty of medicine, Cairo University.

Statistical analysis

The experimental data were analyzed using analysis of variance (ANOVA) with GraphPad Prism (version5.00 for Windows), GraphPad Software (CA, USA). $P<0.01$ is considered to be significant. All calculated data are expressed as mean ± standard deviation (SD).

Results

Histopathological examination of cardiac tissues

Microscopically examinations of cardiac tissues from control mice revealed normal arrangement of longitudinal cardiac myocytes with a centrally arranged nucleus. On the other hand, histopathological examination of cardiac tissues in male Swiss albino mice treated with monosodium glutamate for 10 and 14 days, respectively, revealed variable degrees of alterations in response to the time of the treatment and compared to control as follows: Cardiac sections from mice treated with monosodium glutamate for 10 days showed moderate pathological lesions, areas of cardiomyocyte display homogenous cytoplasm and pyknotic nuclei as well as marked congested dilated blood vessels and mild inflammatory cells aggregations in interstitial tissues and perivascular. Other areas revealed degenerated splitting fragmentation cardiac muscular fibers with widening interstitial tissues compared with control treated group. Animals treated with monosodium glutamate for 14 days showed severe degenerated changes. Severe degenerated atrophied fiber with pyknotic nuclei, edema, marked congested dilated blood vessels with ulcerated wall; widening interstitial tissues together with inflammatory cells aggregations in interstitial tissues and perivascular were seen (Fig. 1).

Histopathological examination of splenic tissue

Microscopic examination of splenic sections from normal mice revealed normal splenic architecture with normal appearance of white pulp and red pulp. White pulp is formed of a large number of lymphocytes surrounding the central arteries and red pulp is formed of reticular cells, red blood cells, lymphocytes, macrophage, and some plasma cells. On contrast, splenic sections from animal orally administrated with monosodium glutamate showed variable degrees of alterations dependent on time. Ten-day treatment revealed ill-defined spleen architecture, degenerated white pulp and red pulp together with lost and loosely cells accompanied with scattered giant cells compared to the control group. While severe degenerative changes in white pulp and red pulp as ill-defined lymphoid follicle with large necrotic foci by darkly stained cells accompanied with edema, diffused degenerated lymphoid cells were shown in splenic sections from mice treated with monosodium glutamate for 14 days (Fig. 2).

Histopathological examination of hepatic tissue

Examination of H & E hepatic sections from control mice showed normal appearing of hepatocyte that radiated from central vein and separated by sinusoids. However,

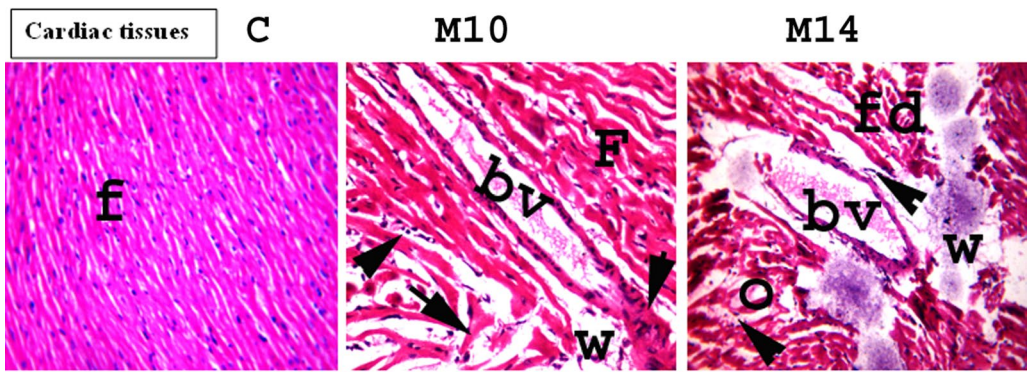

Fig. 1 A photomicrograph of cardiac section. (C) Control group; (M10) monosodium glutamate treated mice for 10 days and (M14) monosodium glutamate treated mice for 14 days. Cardiac muscular fibers (f); congested dilated blood vessels (bv); edema (o) and aggregation of inflammatory cells (arrow head); degenerated cardiac muscular fibers (fd); degenerated splitting fragmentation cardiac muscular fibers (arrow). H &E.X200

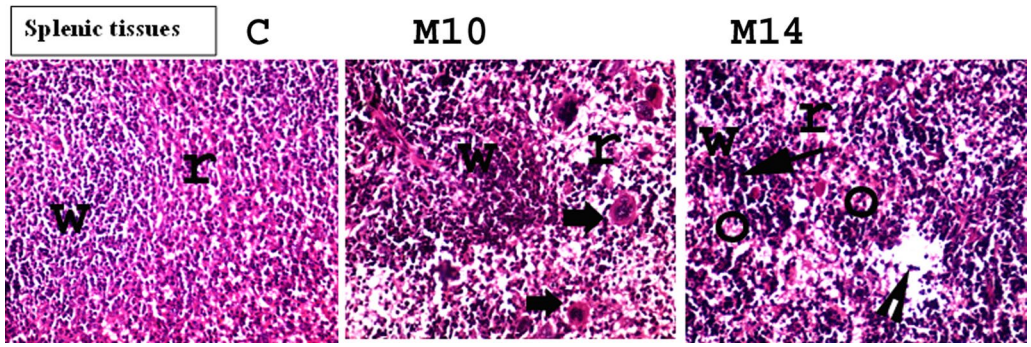

Fig. 2 A photomicrograph of splenic section. (C) Control group; (M10) monosodium glutamate treated mice for 10 days and (M14) monosodium glutamate treated mice for 14 days. White pulp (W) and red pulp (r); giant cells (thick arrow); diffused degenerated lymphoid cells (arrow); edema (o); necrotic foci filled by darkly stained cell (arrow head). H &E.X200

liver sections from mice administrated monosodium glutamate for 10 days showed moderate pathological changes as moderate dilated, central vein, and vacuolar degenerative hepatocyte together with mild to moderate inflammatory cells aggregation in perivascular, in sinusoids compared to control group, while treatment with monosodium glutamate for 14 days showed severe degenerative changes compared to control group. Prominent areas of marked vacuolar degenerative hepatocyte with pyknotic nuclei, severe dilated, congested portal veins andmoderate dilated bile ducts together with dense inflammatory cells aggregation in hepatic tissues and in perivascular (Fig. 3).

Histopathological examination of renal tissue

Histopathological examination of renal sections in non-treated mice revealed normal histological architecture with normal glomerular tuft, Bowman's space and renal tubules. On the other hand, light microscopically examination of renal sections treated with monosodium glutamate for 10 and 14 days showed revealed variable degrees of alterations in response to the time of the treatment and compared to control. Treatment with monosodium glutamate for 10 days resulted in mild to moderate shrunken in glomerular tuft, narrow Bowman's space and degenerated of some renal tubules with pyknotic nuclei together with mild to moderate aggregation of inflammatory cells and interstitial hemorrhage compared to control group, while loss of normal architecture, severe atrophied vacuolated glomerular tuft, with wide narrow Bowman's space, and scattered marked to moderate aggregation of inflammatory cells were seen in renal sections from animals treated with monosodium glutamate for 14 days. Most renal tubules revealed severe degenerative change in their epithelial cell lining with pyknotic nuclei, and other remaining tubules revealed hyaline casts in their lumen (Fig. 4c).

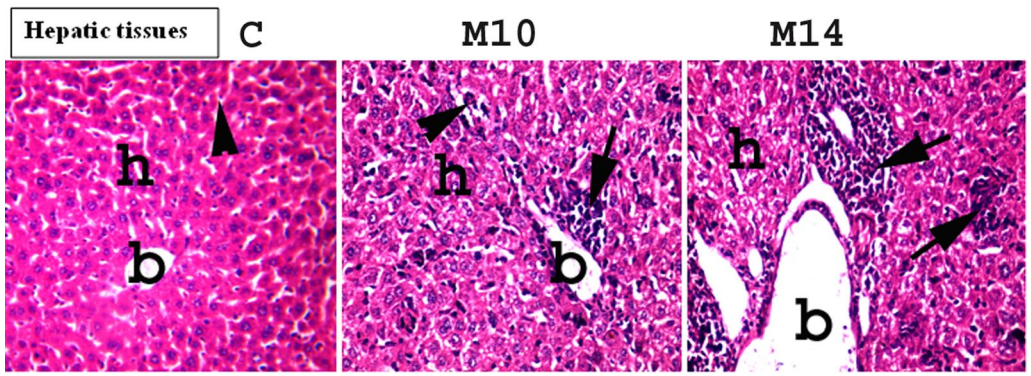

Fig. 3 A photomicrograph of hepatic section. (C) Control group; (M10) monosodium glutamate treated mice for 10 days and (M14) monosodium glutamate treated mice for 14 days. Hepatocyte (h); blood vessels (b); aggregation inflammatory cells (arrow); sinusoids (arrow head). H &E.X200

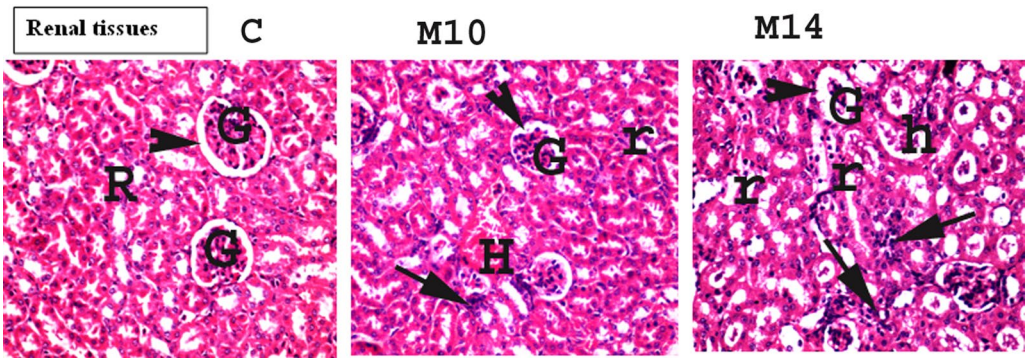

Fig. 4 A photomicrograph of hepatic section. (C) Control group; (M10) monosodium glutamate treated mice for 10 days and (M14) monosodium glutamate treated mice for 14 days. glomerular tuft (G); Bowman's space(arrow); renal tubules (R); hyaline casts in lumen of tubules (h); severe degenerative change in epithelial cell lining with pyknotic of renal tubules (r); aggregation of inflammatory cells infiltrations (arrow). H &E.X200

Immunohistochemical analysis (Nuclear factor κB)

Cardiac, splenic, hepatic and renal section staining with Nuclear factor κB staining showed moderate increase in the NF κB level in animals treated with monosodium glutamate for 10 days compared to control group, while the treatment with monosodium glutamate for 14 days induced marked and highly significant raising in Nuclear factor κB level as evidenced by appearance of brown color compared to control group (Fig. 5) (Table 1).

Discussion

Our investigation aimed to evaluate the effect of oral administration of monosodium glutamate on cardiac, splenic, hepatic and renal of male Swiss albino mice dependent on time using histopathological and immunohistochemistry studies. The experiment period extended for 14 successive days our study revealed that oral administration of monosodium glutamate resulted in histopathological alterations, and increased activity of NF κB, in all examined tissues, dependent on time of administration these were in agreement with (Ebaid & Tag, 2012; Tawfik and Al-Badr, 2012; AL-Mosaibih, 2013). These observed alterations in all examined tissues of animals treated with monosodium glutamate could be attributed to the presence of glutamate receptors on tissues. Glutamate is considered as the predominant excitatory neurotransmitters in in peripheral neural and non-neural tissues (Gill & Pulido, 2005). Likewise, Kalariti et al. (2005), Gill et al., (2007, 2008) stated that glutamate receptors have an important role in the pathophysiology of different organ systems and pathophysiology of syndromes and diseases such as epilepsy, stroke, schizophrenia, addiction, depression, anxiety, Alzheimer's, Huntington's, Parkinson's, brain injury and heart diseases.

Another reason for tissues damage observed in our study could be attributed to elevation of free radical species, oxidative stress, lipid peroxidation, malondialdehyde and to reduction antioxidant enzymes as superoxide dismutase, catalase and glutathione peroxidase activities (Mohamed et al., 2022; Bhattacharya et al., 2011; Kumar & Bhandari, 2013; Hamza and AL-Harbi 2014; Mustafa et al., 2015; Diab & Hamza, 2016). It is well known that oxidative stress leads to activation of nuclear factor-jB (NF-jB) signaling pathway which is crucial for the regulation of many genes involved in inflammatory responses, such as tumor necrosis factor-a (TNF-a), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and caspase family of proteases leading eventually to cell death (Chen et al., 2012; Tugcu et al., 2010).

El-Shenawy and Al-Eisa (2010) and Diab and Hamza (2016), who found that administration of monosodium glutamate resulted in an increased superoxide radical production and other reactive oxygen species thereby induce oxidative stress in the hepatic tissues (Inuwa et al. 2011; Diab & Hamza, 2016). Increase levels of triglycerides, low-density lipoprotein cholesterol and volatile low-density lipoprotein cholesterol and decreasing high-density lipoprotein cholesterol was proposed as the cause of coronary heart disease (Kumar & Bhandari, 2013; Al-Harbi et al., 2014; Diab & Hamza, 2016). Diab et al. (2016) and El-Shenawy et al. (2010) stated that elevated levels of aspartate transaminase, alanine transaminase and lactate dehydrogenase enzyme activities and lipid profile as cholesterol, triglycerides levels could be the reason of the hepatocellular damage induced by monosodium glutamate.

Moreover, our result supported and explained by work done by Mohamed et al. (2022), who stated that administration of monosodium glutamate for 30 days

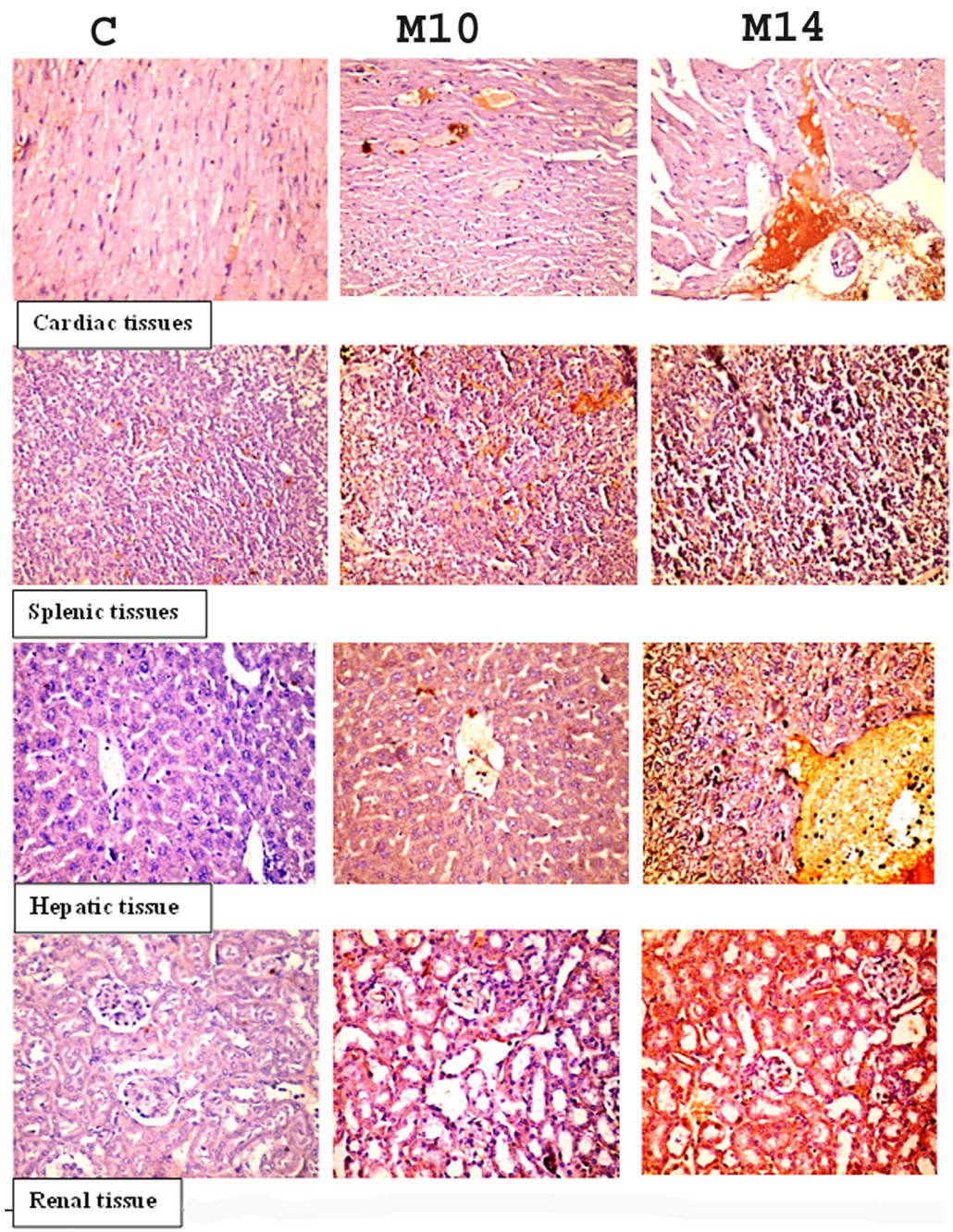

Fig. 5 A photomicrograph of sections from cardiac, splenic, hepatic and renal staining with Immunohistochemistry staining of NF κB, X200: (C) Control group; (M10) monosodium glutamate treated mice for 10 days and (M14) monosodium glutamate treated mice for 14 days. Brown color: positive expression of NF κB

and 60 days instigated a conspicuous expansion in AST and ALT and noticed a reduction in both all-out protein and albumin substance, these could be credited to the creation of oxidative stress that damage liver cells (El-Gharabawy et al., 2019).

Conclusions

Our present study indicated that oral administration of monosodium glutamate resulted in pathological lesions in the cardiac, splenic, hepatic and renal tissues as indicated by the microscopically examination. It also caused

Table 1 Areas % of NF κB expression on cardiac, splenic, hepatic and renal of animals treated with monosodium glutamate dependent on time

	Level of NF κB Cardiac (Mm2) (Mean ± S.D.)	Level of NF κB splenic (Mm2) (Mean ± S.D.)	Level of NF κB Hepatic (Mm2) (Mean ± S.D.)	Level of NF κB Renal (Mm2) (Mean ± S.D.)
Control group	9 ± 3	16 ± 2.2	7 ± 4.1	5 ± 1.2
Glutamate 10 treated group	35 ± 9.5*	44.2 ± 15 *	55.3 ± 8*	39 ± 12*
Glutamate 14 treated group	129 ± 9.16**	173.2 ± 35.5**	93.3 ± 10**	67 ± 13**

($P < 0.05$) ** is significant and ($P < 0.01$) ** is highly significant. Ten animals were used in each group. Data are represented as mean ± SD

increase in the expression of level of NF κB in examined tissues.

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Author contributions
SR conceptualized the study, performed the experiments, analysis date and wrote the draft. The author read and approved the final manuscript.

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Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
All experimental procedures were conducted in accordance with the ethical standards and were approved by the Institutional Animal Care and Use Committee (IACUC) at National Organization for Drug Control and Research (NODCAR) (approval no. NODCAR/III/41/2019).

Consent for publication
Not applicable.

Competing interests
The author declares that he/she has no competing interests.

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