Egypt. Acad. J. biolog. Sci., 4(1): 1-8 (2012) **B. Zoology**
Email: egyptianacademic@yahoo.com **ISSN: 2090 – 0759**
Received: 21/1/2012 **www.eajbs.eg.net**

Monosodium Glutamate Toxic Effect on Spleen Structure and Potentiality of Recovery in Adult Albino Rats

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ABSTRACT

Histological effects of Monosodium glutamate (MSG) commonly used as food additive on the spleen of adult Wistar rats were carefully studied. The rats (n=30), average weight of 115 gm were randomly assigned into four treatments (n=6) and control (n=6) groups. The rats in the treatment groups received 4 mg/kg IP of MSG for fourteen days, while the control group receives the same volume of distilled water intraperitoneally. The rats were sacrificed after the last day of injection (group 1) and on 14 (group 2), 28 (group 3) and 42 day (group 4) after cessation of the treatment with MSG. The spleen was carefully dissected out and quickly fixed in 10% buffered formaldehyde for routine histological study after H&E method. The histological findings after H&E methods for group 1 indicated that the treated sections of the spleen showed atrophy of the while pulp, germinate centers were missing and red pulp of the spleen showed aplasia. The treatment (Group 2) showed vacuolation of some splenic cells with decreased cellularity, and sinusoidal spaces were large. Animals of group 3 showed slight atrophy in the white pulp and aplasia of red pulp where it began to retain normal structure. In the animals sacrificed at the end of the experimental period, the spleen retained its normal structure. These findings indicate that MSG consumption may have some deleterious effects on the spleen of adult Wistar rats which is reversible and does not lead to permanent damage but the normal structure of the spleen would need a long time to be regained. It is recommended that further studies aimed at corroborating these findings be carried out.

Key words: Monosodium glutamate, Rat, Spleen

INTRODUCTION

Most food additives act either as preservatives or enhancer of palatability, one of such food additives is monosodium glutamate (MSG), which generated much controversy locally and globally about its safety usage (Moore, 2003). Monosodium glutamate, which is chemically known as AJI-NO-MOTO, is one of the most common amino acids found in nature (Adrienne, 1999). Vinodini *et. al.* (2008) defined (MSG) as a sodium salt of naturally occurring non-essential L-form of glutamic acid, and as one of the main flavor enhancer used as an ingredient in various food products.

It is also produced in the body and plays an essential role in human metabolism. It is a major component of many proteins such as meat, fish, milk and some vegetables (IFIC, 1994). Despite its taste stimulation and improved appetite enhancement, reports indicated that MSG is toxic to human and experimental animals (Andrew, 2007). It contains exitoxins like most of the taste-enhancing additives, as mentioned by (Russel and Blaylock, 1994) who defined exitoxins as a group of excitatory amino acids, they also mentioned that although they are widely distributed in our food supply, we may not be able to depend on the food and drug administration (FDA) to protect us from these toxins because of the powerful food lobby known as

the glutamate association which counteract any negative reports or publicity of research showing the harmful effects of MSG.

Glutamate in high doses produce neuroendocrine abnormalities and neuronal degeneration (Moreno *et al.*, 2005), and oxidative damage in different organs (Farmobi and Onyemia, 2006; Pavlovic *et al.*, 2007).

The spleen, the largest secondary lymphoid organ, is considered the draining site for compounds that are administered intravenously, and is therefore considered an important organ to evaluate for treatment-related lesions. Due to the presence of B and T lymphocytes, the immunotoxic effects of xenobiotics or their metabolites on these cell populations may be reflected in the spleen. Therefore, it is one of the recommended organs to evaluate for enhanced histopathology of the immune system (Elmore, 2006).

Previous scientific investigations aimed at determining the effect of MSG on body organs (Nwaopara *et al.*, 2004; 2007a, b; 2008a, b). There are some reports of the toxic effects of MSG on the pancreas (Nwaopara *et al.*, 2004), liver (Nwaopara *et al.*, 2007b) and kidney (Nwaopara *et al.*, 2008 a). The aim of the present study was to go more into the toxic effects of MSG administration on the histopathological changes in spleen of albino rats and to determine possibility of reversibility of these effects.

MATERIAL AND METHODS

(a) Experimental animals

This study was performed on thirty young adult Wistar male rats (age 10 weeks), weighing about 100-130 gm. The animals were bred and maintained under standardized conditions away from any stressful conditions with 12∶12 light∶ dark cycle with free access to food and water in the animal house. They were acclimatized for one week prior to the experiment and caged six per cage in fully ventilated room at room temperature. All experimental procedures and animal maintenance were conducted in accordance with the accepted standards of animal care.

(b) Tested compound

Monosodium glutamate (MSG) was purchased from Roth Company, Germany. MSG was in the form of small pellets and before usage, it was dissolved in distilled water, 1gm of MSG in 1 ml of distilled water (Nayatara et al., 2008).

(c) Experimental design

The animals were divided randomly into 5 groups; each included 6 rats. The experimental design is to be seen in Table (1).

Table 1: Experimental design

Experimental group	Treatment	No. of animals	Dose	Duration
Control	distilled water	6	0.1 ml I.P	daily for 14 days
Group 1	MSG group	6	4 mg/Kg .b.w I.P	Administrated daily for 14 days and the animals were sacrificed after the last day of injection
Group 2	Recovery 1	6	4 mg/Kg .b.w I.P	Administrated daily for 14 days and the animals were sacrificed 2 weeks after stoppage of the treatment
Group 3	Recovery 2	6	4 mg/Kg .b.w I.P	Administrated daily for 14 days and the animals were sacrificed 4 weeks after stoppage of the treatment
Group 4	Recovery 3	6	4 mg/Kg .b.w I.P	Administrated daily for 14 days and the animals were sacrificed 6 weeks after stoppage of the treatment

(d) Histological procedures

At the end of the experimental period, the animals were sacrificed by cervical section. Pieces of spleen were excised, rinsed in physiological saline and fixed in formalin saline for 24 hours. The preserved organs were cut into smaller portions for processing, dehydrated with isopropyl alcohol, cleared with terpinole, infiltrated and embedded with paraffin wax. Paraffin wax blocks were sectioned at 5µ thick with a rotary microtome using a disposable blade. Sectioned slides were stained with Harris' haematoxylin and eosin (H&E) (Luna, 1968) and mounted with (DPX). Six sections of spleen from each animal were examined by light microscope.

RESULTS

The structure of control spleen was composed of white and red pulps surrounded by a capsule of dense connective tissue (Figs. A and B). The white pulp was composed of a central, T-cell rich zone, and a peri-arterial lymphoid sheath surrounded by B-cell-rich primary follicles. The white pulp was separated from the red pulp by the marginal zone lymphocytes.

Pathohistologically, the experimental animals sacrificed after the last day of injection (Group 1) displayed that the limit between white and red pulp started to disappear, besides depletion of lymphocytes within the white pulp and the absence of germinate centers (Fig. C). The animals which sacrificed 2 weeks after stoppage of the treatment (Group 2) showed vacuolation of some splenic cells with decreased cellularity, and sinusoidal spaces were large (Fig. D). Animals sacrificed 4 weeks after stoppage of the treatment (Group 3) showed slight atrophy in the white pulp and aplasia of red pulp where it began to retain normal structure (Fig. E). In the animals sacrificed at the end of the experimental period, the spleen retained its normal structure with where the marginal zone between white pulp and red pulp were well definite (Fig. F).

DISCUSSION

The safety of MSG usage has been generated much controversy locally and globally (Redding *et al.*, 1971 and Pizzi *et al.*, 1977). This study aims to investigate the effect of MSG on rat spleen and the possibility of recovery after cessation of the treatment. Till the time of starting the experiment, no studies were reported investigating the possibility of recovery after stopping the treatment with MSG.

MSG improves the palatability of meals and thus influences the appetite centre positively causing increase in body weight (Rogers*et al.*, 1990). Though MSG improves taste stimulation and enhances appetite, reports indicate that it is toxic to human and experimental animals (Belluardo *et al.*, 1990). MSG has a toxic effect on many body organs by altering ionic permeability of neural membrane and induces persistent depolarization (Robinson, 2006).

The results (H&E) reactions revealed that animals treated with monosodium glutamate showing cellular disruption and degeneration of the white pulp in spleen as compared to the control sections. But after cessation of the treatment with MSG, there was varying degrees of cellular recovery which improved with time. Our result is in agreement with Ćirić *et al*. (2005) that administration of MSG induced degenerative and atrophic changes in rat spleen.

Recent studies have shown that glutamate receptors play very important role in pathogenesis of disorders induced by MSG. Glutamate is predominant excitatory

neurotransmitter in the mammalians central nervous system (Schlett, 2006; Greenwood and Connolly, 2007; Liguz-Lecznar and Skangiel-Kramska, 2007). There are two basic types of glutamate receptors: ionotropic (NMDA, kainite and AMPA) and metabotropic (mGluR) (Smith *et al.*, 2001; Weston *et al.*, 2006; Gerber *et al.*, 2007).

Neurotoxicity of MSG is related with glutamate receptors activation (Gao *et al.*, 1994; Beas-Zarate *et al.*, 2001). Such excessive activation of glutamate receptors and overloading with intracellular calcium can induce neural death (Gil-Loyzaga *et al.*, 1993). Glutamate receptors are present in different tissues: hypothalamus, spleen, thymus, liver, kidneys, endocrine system, ovaries, etc. (Gill and Pulido, 2005; Gill *et al.*, 2008).

Our results may support that reported by Fallarino *et al.* (2010) who demonstrated that Glutamate might affect neuroinflammation via effects on immune cells. Knockout mice lacking metabotropic glutamate receptor-4 (mGluR4) were markedly vulnerable to experimental autoimmune encephalomyelitis (EAE, a mouse model of multiple sclerosis) and developed responses dominated by interleukin-17–producing T helper (T_H17) cells.

Based on the obtained results, the conclusion may be drawn that the administration of monosodium glutamate to animals leads to reversible atrophy of cells of both white and red pulp, which explains the immunosuppressive effects of glucocorticoids.

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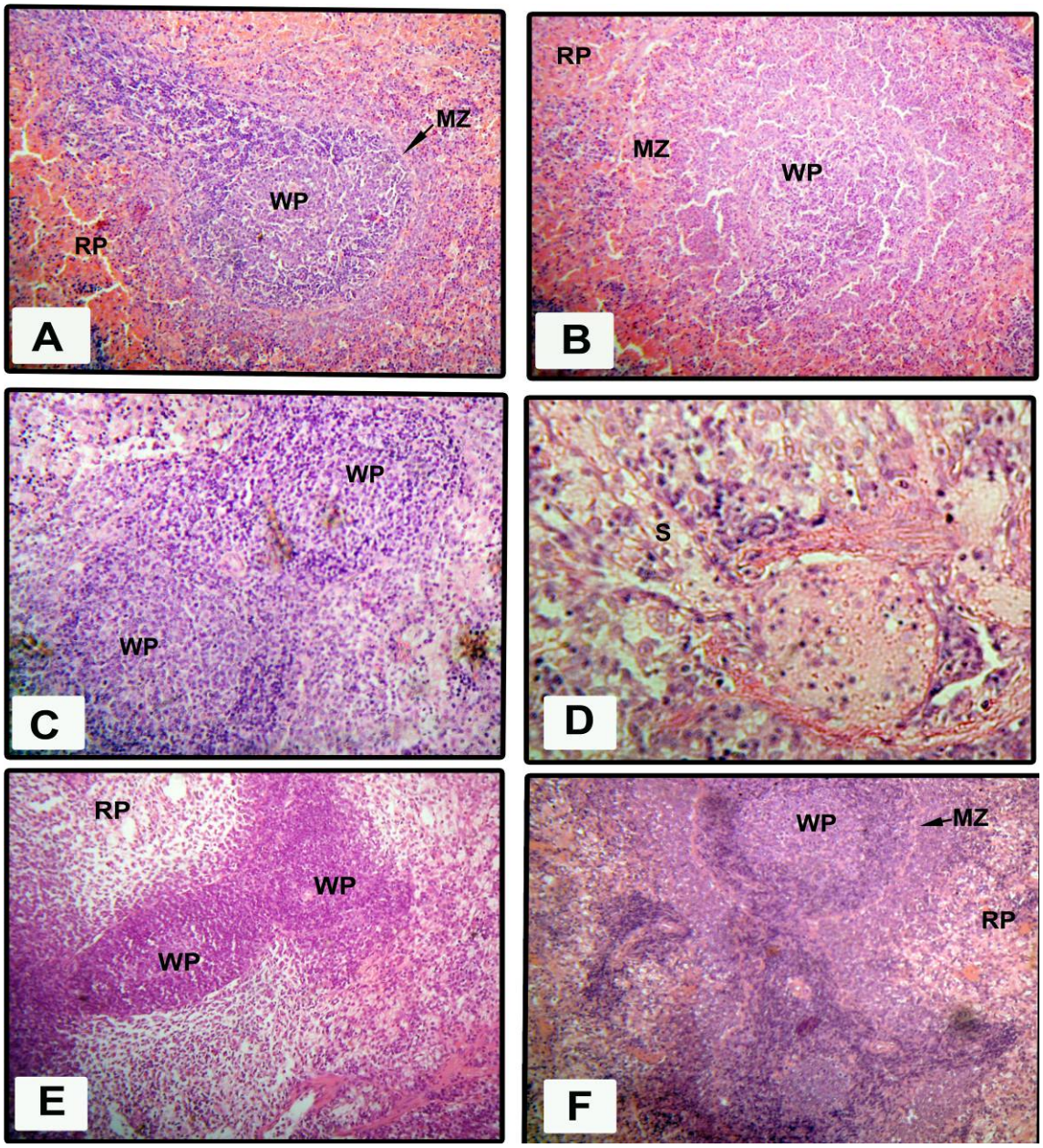

Figs. A, B: Normal spleen structure of control group, (WP) lymphatic follicles of the spleen, (RP) red spleen pulp and Marginal zone (MZ) (HE x20 and 40).
Fig. C: group 2 absence of germinate centers (HE x20).
Fig. D: group 3 showed vacuolation of some splenic cells and dilation of sinusoidal spaces (S) (HEx40).
Fig. E: group 3 atrophy in the white pulp (WP) and aplasia of red pulp (RP) (HE x20).
Fig. F: experimental group no. 4 splenic tissue retain normal structure (WP) lymphatic follicles of the spleen, (RP) red spleen pulp and Marginal zone (MZ) (HE x20).

ARABIC SUMMAEY

التأثير السام لملح أحادي جلوتاميت الصوديوم على الطحال واحتمالات الشفاء منه في الجرذان البيضاء

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تمت دراسة تأثير ملح أحادي جلوتاميت الصوديوم وهو من المواد واسعة الإنتشار من حيث الاستخدام فى الاضافات الغذائية كمكسبات للطعم ، علي التركيب النسيجي للطحال فى الجرذان. تم تقسيم الفئران (30) والتي يبلغ متوسط وزنها 115 جرام إلي أربع مجموعات تقسيماً عشوائياً بحيث تحتوي المجموعة الواحدة 6 فئران إلي جانب المجموعة الضابطة (6). تلقت الفئران في مجموعات العلاج جرعة من ملح أحادي جلوتاميت الصوديوم تبلغ 4 مجم بالحقن في التجويف البريتوني لمدة 40 يوماً ، بينما تم حقن المجموعة الضابطة بنفس الطريقة بالماء المقطر. تم ذبح الفئران بعد اليوم الأخير من الحقن (المجموعة الاولي) وبعد14 (المجموعة الثانية) و28 (المجموعة الثالثة) و 42 يوماً (المجموعة الرابعة) من وقف الحقن بملح أحادي جلوتاميت الصوديوم. تم تشريح الحيوانات واستخراج الطحال بعناية وثبت النسيج بسرعة في 10% فورمالديهايد متعادل لاجراء دراسة نسيجية روتينية باستخدام صبغة الهيماتوكسالين والإيوسين. وقد أوضحت الدراسة النسيجية للطحال في المجموعة المعالجة الاولي وجود ضمور في اللب الأبيض وفقدان مراكز الإنبات وعدم التنسج اللب الأحمر. بينما أوضحت المجموعة الثانية وجود فجوات في الخلايا وقلة في عدد الخلايا لنسيج الطحال. وأوضحت المجموعة الثالثة ضمور طفيف في اللب الابيض وبدأ اللب الأحمر في استعادة شكله الطبيعي. وأخيرا الحيوانات التي تم ذبحها في نهاية التجربة أظهرت استعادة نسيج الطحال لشكله الطبيعي. هذه النتائج تشير إلى أن استهلاك ملح أحادي جلوتاميت الصوديوم لا يؤدي إلى ضرر دائم لكن الهيكل الطبيعي للطحال سيحتاج وقتاً طويلاً ليستعيد شكله الطبيعي . يوصي بإجراء المزيد من الدراسات لتدعم هذه النتائج.