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Some Hematological and Histological Impact of sub-acute exposure to Mono Sodium Glutamate in Mice

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Summary

Mono Sodium Glutamate (MSG) is a food additive commonly consumed as a flavor enhancer. However, both animal models and human clinical reports have established its harmful effects. The present study aimed to investigate the effect of MSG consumption on some hematological parameters and histological sections of brain, small intestine, liver and testis tissues of Swiss Albino mice. Albino mice (n=15) of average weight 27.4gm were randomly assigned into three groups A, B and C in each group (n=5). Treatment groups (A & B) were given 3g and 6g per Kg of body weight of MSG (amount of MSG in a single sachet consumed by human is 3g) respectively. The mice were sacrificed on day fifteenth of the experiment. Brain, small intestine, liver and testis were carefully dissected out and immediately fixed in 10% formalin for routine histological procedure. Blood samples were analyzed for hematological parameters. Histological findings of small intestine in the treated groups showed evidence of cellular hypertrophy and increased number of goblet cells. Large nuclei with multi nucleoli were seen in liver sections, while prevascular and preneuronal edema were seen in brain sections and thickening of basement membrane of semineferons tubules. Vacuolation of spermatogonia were seen in testicular tissue sections. Blood analysis showed a significant increase at $P<0.01$ in lymphocytes count compared to the control which can be considered indication of a compromised immune status and poisoning in the treated animals. Packed Cell Volume (PCV) hemoglobin (Hb) and red blood cells count (RBCs) were all indicative of an anemic condition in the treated animals. Significant increase in body weight at $P<0.01$ was seen in both treatment groups (A & B). All above findings indicate that MSG has some deleterious effects on some hematological parameters and caused histological changes of examined organs as well as on body weight. It is recommended that further studies aimed at corroborating these findings to be carried out.

بعض التغيرات الدموية والنسيجية للتعرض شبه الحاد لكلوتاميت الصوديوم الأحادي (الملح الصيني) في الفئران المهقاء

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الخلاصة

كلوتاميت الصوديوم الأحادي المعروف بالملح الصيني مستخدم بصورة شائعة كمحسن نكهة.أشارت التقارير السريرية الى التأثيرات الضارة لهذه المادة على صحة الأنسان والحيوان على حد السواء.صممت هذه الدراسة للتقصي عن تأثير إستهلاك الملح الصيني على المعايير الدموية والمقاطع النسيجية لأدمغة,المعي الدقيق,أكباد وخصى الفئران المهقاء ولأتمام الدراسة تم إستخدام خمسة عشر فأر أمهق معدل أوزانها بلغ 27.4غم وزعت عشوائيا على ثلاث مجاميع (A,B and C) بواقع خمسة فئران لكل مجموعة.مجموعتي المعاملة Aو B عوملتا بجرعتين من الملح الصيني (3غم و 6غم) لكل كغم من وزن الجسم على التوالي (الكيس الواحد المستهلك من قبل الأنسان يحوي 3 غم من الملح) تم قتل الفئران في اليوم الخامس عشر من التجربة.أدمغة,المعي الدقيق,أكباد وخصى الفئران جمعت و حفظت بالفورمالين 10% لإجراء التقطيع النسيجي للمعي الدقيق أظهر زيادة قي نمو الخلايا وأعداد الخلايا الكأسية أما في الكبد فقد أزدات أعداد خلاياه مع إحتوائها نوى بحجم كبير مع إزدياد أعداد النويات.تم ملاحظة خزب حول الأوعية والأعصاب في المقاطع النسيجية للأدمغة أما في الخصى فقد تم ملاحظة تثخن الغشاء القاعدي للنبيبات الخصوية وتفجي في الخلايا الأولية للنطف.تحليل عينات الدم أظهر زيادة معنوية بمستوى $P\leq0.01$بأعداد الخلايا اللمفية مقارنة بأعدادها في مجموعة السيطرة حيث يمكن اعتباره مؤشرا على الحالة المناعية للفئران.قيم حجم الخلايا المرصوصة'الهيموغلوبين وأعداد كريات الدم الحمراء أعتبرت مؤشرا لحلة فقر الدم في الفئران.تم ملاحظة زيادة معنوية وبمستوى $P\leq0.01$في أوزان الفئران في كلتا مجموعتي المعاملة مقارنة

بمجموعة السيطرة.أظهرت نتائج الدراسة أعلاه تأثيرا مضرا على الصحة وعلى مستوى الدم,أنسجة ووزن الجسم مما يتطلب إجراء بحوث أوسع للتقصي عن مضار الملح الصيني

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Introduction

Mono Sodium Glutamate (MSG) is one of the common amino acids found in nature and the main component of many proteins and peptides of most tissues contains 78% of glutamic acid, 22% of sodium and water (1). Glutamate is also produced in the body and plays an essential role in human metabolism actually it is a major component of many protein-rich food products such as meat, fish, milk and some vegetables. In the late sixties the first publish report of an adverse reaction to MSG in the New England Journal of Medicine where it was reported that MSG is a neurotoxic, killing brain cells, causing retinal degeneration, endocrinal disorders besides being associated with number of pathological conditions such as stroke, epilepsy, brain trauma, schizophrenia, anxiety, Parkinson disease and Huntington's disease (2).

Materials and Methods

Blood samples were collected into heparinized bottles then were analyzed for hematological parameters such as packed cell volume (PCV) (3), hemoglobin concentration (Hb) (4), total red blood cells count (RBC's) and lymphocytes count (5). Tissues for histological examination were dehydrated in ethanol, cleared in xylene and embedded in paraffin. Several sections were obtained using a rotary microtome then stained with haematoxyline and eosin. Statistical analysis was done according to the Steel and Torries (6).

Fifteen Albino Swiss Mice of both genders with average weight 27.4g were randomly assigned into three groups A, B and C (n-5) in each group. A and B served as treatment groups, while group C served as a control group. Mice in treatment groups (A and B) were given MSG orally via stomach tube at doses of 3g and 6g per Kg of body weight respectively for fourteen days then mice were sacrificed on the fifteenth day of the experiment by cervical dislocation, the thoracic cavity was carefully cut open to gain access to the heart and blood samples were collected through heart puncture. Brains, livers, kidneys and intestines were preserved for the histological examination. Mice gained maximum acclimatization before actual commencement of the experiment.

Results

Animals' weights were taken before and after treatment with MSG. Results showed no significant increase in group C where mice served as a control group, while results showed a significant increase in weight at $p<0.01$% in group A and B where animals were given MSG at a dose of 3g and 6g respectively for fourteen days as shown in table 1.

Table (1) Body weight of Mice dosed with MSG at a dose of 3g and 6g per Kg of body weight (after being adjusted according to the body weight) for fourteen days

Groups	Before Treatment M±SE	After Treatment M±SE
Group C	**27.40 ±1.91 a**	**29.20 ±3.10 a**
Group A	**28.80 ±1.36 a**	**34.80 ±1.56 b**
Group B	**28.40 ±2.08 a**	**35.80 ±1.88 b**

Different small letters indicated significant differences between groups at level p<0.01

The present study showed that MSG had a significant effect on the hematological parameters. Hence, these findings support the fact that MSG is detrimental to health as been referred by (Eweka; 2007). Reduction effects of MSG were seen on the packed cell volume (PCV) in group B when it decreased into (24.40 ±1.54) after being (32.20 ±0.97) in the control group. Hemoglobin concentration (Hb) decreased into (10.20 ±0.46) in group A & B respectively after being (14.00 ±0.45) in the control group, while red blood cells count (RBC's) decreased into (1.84 ±0.37) in group B after being (3.50 ±0.19) in the control group. Eventually an elevated numbers of lymphocytes were seen in both treated groups (A & B) where numbers

reached into (30.40 ±1.17) and (37.60 ± 0.87) respectively after being (25.8 ±1.01) in the control group as shown in table (2) where MSG were given at a dose of 3g and 6g per Kg of body weight respectively.

Table (2) Lymphocyte, PCV, RBC's count and Hemoglobin of Mice dosed with MSG at a dose of 3g and 6g per Kg of body weight (after being adjusted according to the body weight) for fourteen days.

Blood Parameters	Control Group	A Group	B Group
Lymphocytes (×100/cu.mm)	25.80 ±1.01 c	30.40 ±1.17 b	37.60 ± 0.87 a
P.C.V (%)	32.20 ±0.97 a	29.40 ±1.12 a	24.40 ±1.54 b
R.B.C's ($\times 10^6$/cu.mm)	3.50 ±0.19 a	2.64 ±0.24 b	1.84 ±0.37 b
Hb (g %)	14.00 ±0.45 a	12.80 ±0.57 a	10.20 ±0.46 b

Different small letters denoted that significant differences between treated groups in the same column at the level p<0.01

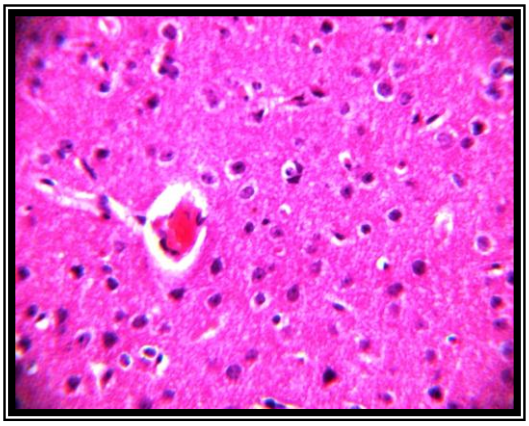

Fig. 1 (H&E 400 X)

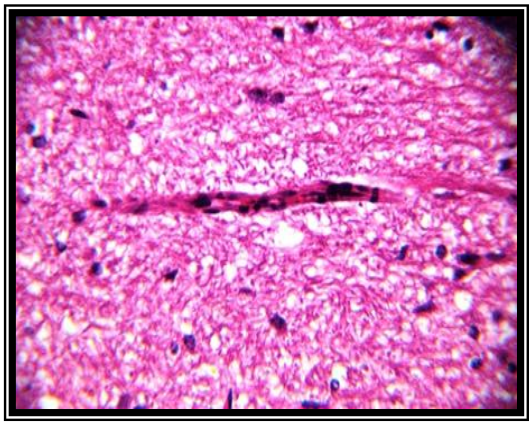

Fig. 2 (H&E 400 X)

Histological sections of mouse brain treated orally with MSG at a dose of 3g and 6g per Kg of body weight respectively showed the cerebrum with pre vascular and pre neuronal edema with highly congestive blood vessels also showed a proliferative endothelium and demylinative nerve fibers in both treated groups.

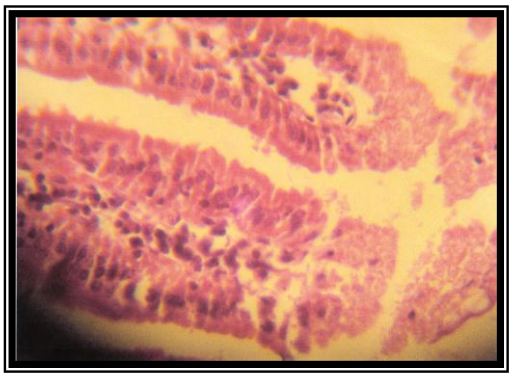

Fig. 3 (H&E 400 X)

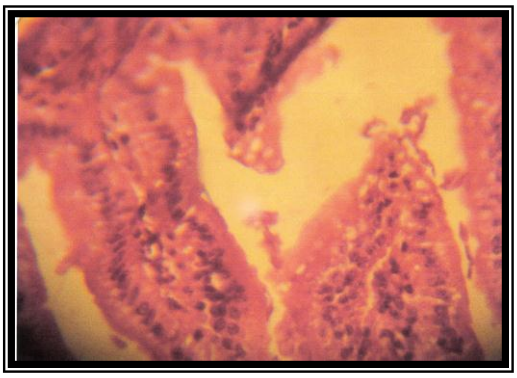

Fig. 4 (H&E 400 X)

Small intestine of a mouse treated orally with MSG at a dose of 3g and 6g per Kg of body weight showed an increased number of goblet cells as well as high mucosal secretion of intestinal lumen.

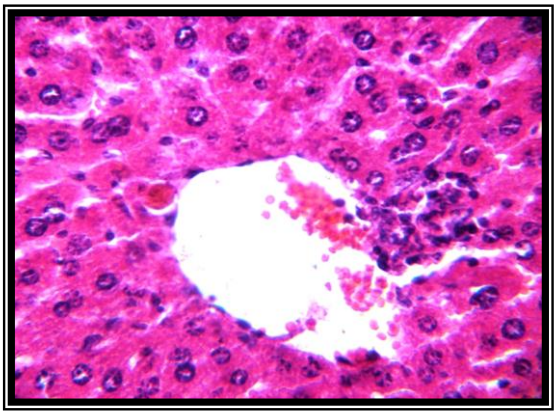

Fig. 5 (H&E 400 X): Histological sections of mouse liver treated orally with MSG at a dose of 3g per Kg of body weight showed large numbers of hepatocytes contain large nuclei with more nucleoli.

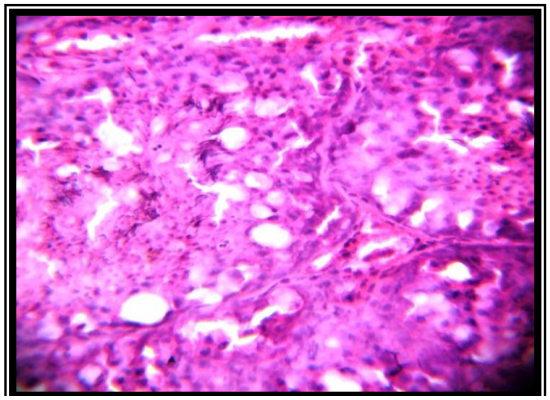

Fig. 6 (H&E 400 X): Testis histological sections of a mouse treated orally with MSG at a dose of 3 g per Kg of body weight showed thickening of the basement membranes of the semineferons tubules with vaculation of spermatogonia.

Discussion

Body weight gains in group (AandB) may be ascribed to the repeated ingestion of MSG which elevates levels of glucagon which may then caused increased insulin levels led to enhance appetite and increased feed consumption as been referred by Bell *et al*; (7) or it may be ascribed to ability of MSG in alteration of proteins, carbohydrates and lipids metabolism as been referred by (8).

The present study showed that MSG had a significant effect on the hematological parameters. Hence, these findings support the fact that MSG is detrimental to health as been referred (9). Decreased numbers of RBC's count, PCV and Hb values in the treated groups (A & B) might be mediated through a deleterious effect of MSG on the hemopoietic stem cells in the bone marrow. MSG might cause increased oxidative stress which induced the formation of micro nucleated polychromatic erythrocytes (10 and 11). Also the present study showed that MSG had a significant effect on lymphocytes count as been shown in table 2. Increased number of lymphocytes in group (A & B) been mentioned by (12), which could be a consequence of the interaction between MSG and gastrointestinal macrophages. Macrophages serve as antigen presenting cell and the antigenic products (polypeptides) to the helper T cells and the B lymphocytes bringing about their activation. Also macrophages secrete substances called interleukin-1/cytokines which brings about the activation, proliferation and increases lymphocytes count as been mentioned recently by (13).

MSG has the ability to cross the blood brain barrier to react with brain receptors causes brain destruction (14). Increased number of goblet cells as well as high mucosal secretion of intestinal lumen in small intestine histological sections in figure 3&4 may be resulted from MSG ingestion (9) to cause intestinal necrosis. Large numbers of hepatocytes in figure 5 may be attributed to the hepatotoxic effect of MSG (15) mentioned where taking MSG with diet may induce oxidative stress in the liver which may be a consequence to the liver damage. Thickening of the basement membranes of the semineferons tubules with vaculation of spermatogonia in figure 6 can be attributed to the MSG which causes significant oligospermia as referred by (16).

References

1- Samuels A (1999). The Toxicity/ Safety of MSG: A study in Suppression on Information. Accountability Res. 6(4): 259-310.

2- Mozes S., Sefcikova Z (2004). Obesity and changes of Alkaline Phosphotase Activity in the Small Intestine of 40 and 80-day old rats subjected to early postnatal overfeeding of monosodium glutamate. Physiol. Res. 53: 177-186.

3- Archer, R. K. (1985). Hematological techniques for use on animals. Oxford: Blackwell, Scientific Publication.
4- Varley, H., Gownlock, A. H. and Bell, M. (1980). Practical clinical biochemistry. 5th ed. William Heinemann. Medical books. Ltd. London.
5- Coles, E. H. (1974). Veterinary Clinical Histology. 2nd ed. W.B Sounders Company.
6- Steel, R. G. and Torries, J. H. (1980). Principles and Procedures of Statistics. Abiometrical approach, 2nd edition. McGraw-Hill Book Co. New York, USA.
7- Bell M.E, Bhatnagar S., Akana S.F (2000). Disruption of paraventricular nucleus connection changes body energy balance and response to acute stress. 20: 6707-6713
8- Diniz Y.S, Fernando A.A, Campos K.E, Mani E., Rebas B.D, Novelli E.L (2004). Toxicity of hyper caloric diet and monosodium glutamate oxidative stress and metabolic shifting in hepatic tissue. Food and Chem. Toxicol. 42: 319-325
9- Eweka A.O (2007). Histological studies of the effects of monosodium glutamate on the kidney of adult Wistar rats. Internet J. Health, 6(2). (www.ispub.com).
10- Farombi E.O, Onyema O.O (2006). Monosodium glutamate induced oxidative damage and genotoxicity in rat: Modulatory role Vitamin C, Vitamin E and quercetin. Human Exptl. Toxicol. 25: 251-259
11- Elphick L.M, Hawat M., Toms N.J., Meinander A., Mikhailov A., Eriksson J.E (2008). Department of Biochemistry and Pharmacy, Abo Akademi University, FIN-20521 Turku; Kass, George E. 2008-01-01
12- Sembulingham K (2005). Essentials of Medical Physiology 2nd Edition pp.46-56
13- Barrett K.E, Barman S.M, Boitano S., Brooks H.L (2010). Ganong's review of Medical Physiology, 23rd Edition, pp. 63-78.
14- Papa P.C, Vargas A.M, Tavares D (2002). Glutamate protein is differently modulated during development of obesity in monosodium glutamate treated mice. Life Science. 74: 1917-1928.
15- Onyema O.O, Farombi E.O, (2006). Effect of Vitamin E on Monosodium glutamate induced hepatotoxicity and oxidative stress in rats. Indian J. Biophys. 43: 20-24
16- Onakewhor JUE, Oforofuo IAO, Singh SP (1998).Chronic administration of monosodium glutamate induces oligozoospermia and glycogen accumulation in Wistar rat tests. Africa J. Reprod. Health. 2: 190-197