African Journal of Biotechnology Vol. 8 (13), pp. 3031-3035, 6 July, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Hepatotoxic effects of low dose oral administration of monosodium glutamate in male albino rats

A. C. C. Egbuonu[1]*, O. Obidoa[1], C. A. Ezeokonkwo[1], L. U. S. Ezeanyika[1] and P. M. Ejikeme[2]

[1]Nutrition and Toxicological Biochemistry Laboratory, Department of Biochemistry, University of Nigeria, Nsukka, Nigeria.
[2]Department of Pure and Industrial Chemistry, University of Nigeria, Nsukka, Nigeria.

Accepted 20 February, 2009

The present study is aimed at investigating the potentials of low concentration administration of monosodium glutamate in inducing hepatotoxic effects in male albino rats. Thus, monosodium glutamate at a dose of 5 mg/kg of body weight was administered to adult male albino rats by oral intubation. Treatment was daily for 28 days. The monosodium glutamate treatment significantly ($p<0.05$) decreased the serum alkaline phosphatase activity by 71.97% but increased ($p<0.05$) the serum enzyme activities of aspartate aminotransferase by 66.86% and alanine aminotransferase by 9.15%. The treatment also increased ($p<0.05$) the serum malondialdehyde concentration by 287.15% and the serum aspartate aminotransferase-to-alanine aminotransferase ratio by 56.59%. Thus, treating rats with monosodium glutamate at a low concentration (5 mg/kg of body weight) could be hepatotoxic without significant cholestasis or pathologies of the bone.

Key words: Monosodium glutamate, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, malondialdehyde, hepatotoxic effect.

INTRODUCTION

Monosodium glutamate (MSG), the sodium salt of amino acid glutamate, is a food additive, popularly used the world over as “flavour enhancer”. It is marketed under such trade names including A-One, Ajinomoto or Vedan and is a popular condiment in West African dishes (Obaseiki-Ebor et al., 2003). Additionally, MSG is abundant in yeast extracts and other food ingredients without otherwise appearing on the label (Healthnotes, 2005). Thus, it could be abused inadvertently.

The possible inadvertent abuse of MSG may have untoward results since the effects of MSG as reviewed in TILC (Anonymous, 2004a, b) include brain damaging potentials, stunted skeletal development, behavioral aberration, neuroendocrine disorder, possible learning deficits, seizures (epileptic fits), learned taste aversion and hyperglycemia. Added to these, MSG intake has been implicated in the Chinese restaurant syndrome manifested by migraine, diarrhoea, weakness, vomiting, stomach ache and tightness of the chest (Schaumberg et al, 1969; Anonymous, 2005). Also, MSG intake could induce an increase in energy intake (Bergen et al, 1998) which could lead to obesity (Mozes et al., 2004) or alter the levels of carbohydrates, lipids and proteins (Diniz et al., 2004) in rats. Furthermore, MSG is an excitotoxin, which may damage the brain especially by oral intake without food (Walker and Lupien, 2000) yet, it is used mostly as a condiment in meats, soups and vegetables without food protection like carbohydrate food (Obidoa, University of Nigeria, Nsukka, Personal Communication).

These reported adverse effects of MSG suggest that the use of this substance as a flavour enhancer over time may be hepatotoxic. Therefore, the present study was aimed at determining whether MSG intake at a low dose could be toxic on selected hepatocellular functions of male albino rats. We based our choice of MSG treatment dose on earlier reports (Olney, 1969; Goldberg, 1994; Onyema et al., 2006).

MATERIALS AND METHODS

Chemicals

Ajinomoto brand of MSG was purchased from a regular foodstuff

*Corresponding author. E-mail: tonycemalukegbuonu@yahoo.com.

market at Nsukka, south-east Nigeria. Other chemicals were of analytical grade unless otherwise stated.

Animals and treatment

The animals used in this work were adult male albino rats. They were procured from the animal house of the Faculty of Biological Sciences, University of Nigeria, Nsukka. All the animals received humane care in accordance with the guidelines of the National Institute of Health, USA for ethical treatment of laboratory animals.

Eight adult male albino rats with mean body weight (BW) of 93 ± 0.5 g were acclimatized for 1 week before they were randomly assigned to 2 groups of 4 rats each. Group 2 were fed MSG (5 mg/kg of BW) whereas Group 1 rats were given distilled water (1 ml/kg of BW). Treatment was by daily oral intubations and lasted for 28 days. The rats were housed in stainless steel cages at room temperature (25°C) and exposed to a normal daylight/dark cycle under humid tropical conditions. They were supplied with enough rat feed and drinking (tap) water *ad libitum* throughout the duration of the experiment.

Blood collection and preparation

The animals were sacrificed 24 h after the 28 days treatment. Their blood samples were collected individually with sterile capillary tubes into properly labeled polystyrene centrifuge tubes by ocular puncture technique of Scholm et al. (1975). The blood samples thus collected were allowed to clot and then centrifuged at 3000 rpm for 10 min. The resultant sera were collected individually into stoppered polystyrene tubes, stored in deep freezer for the determination of the serum activities of alkaline aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), and the serum malondialdehyde (MDA) concentration.

Assay of serum alkaline phosphatase (ALP) activity

The assay of alkaline phosphatase (ALP) activity was by the method of Walter and Schutt (1974). The method is based upon the principle that alkaline phosphatase hydrolyzes colorless phosphate esters of various alcohols and phenols to yield p-nitrophenol that occur as the yellow nitrophenolate ion in alkaline solution.

The buffer/substrate solution and serum samples were independently equilibrated to room temperature (25°C) before the start of the assay. To 1 ml of alkaline phosphatase buffer/substrate solution in test tubes were pipetted 0.01ml of serum samples, thoroughly mixed and allowed to stand for 1 min before measuring their absorbencies at 1 min interval 3 times at 405 nm. Then, the ALP activity (IU/L) was calculated by multiplying the change in extinction per minute with a given constant value.

Assay of serum aspartate amino transaminase (AST) activity

The assay of aspartate amino transaminase (AST) activity was carried out by the method of Reitman and Frankel (1957). The method is based on the principle that oxaloacetate (oxaloacetic acid) that is formed from the aspartate aminotransferase catalysed reaction between alpha ketoglutarate and aspartate is coupled with chromogen (2,4-dinitrophenyl hydrazine) in alkaline medium to form colored hydrazone. The concentration of the colored hydrazone is proportional to the aspartate aminotransferase activity and is measured with a colorimeter. To 0.05 ml of each serum sample in a test tube was added 0.25 ml of buffer/substrate solution. The content was incubated at 37°C for 60 min in a water bath followed by the addition of 0.25 ml of chromogen solution. The content was mixed and allowed to stand for 20 min at room temperature after which 2.5 ml of sodium hydroxide (0.4 N) was added and mixed. The absorbance was read after 5 min against blank at 540 nm. The blanks were treated as the samples but without the addition of chromogen solution used to stop all enzymatic reactions. AST activity (IU/L) was read off from the standard curve.

Determination of serum alkaline aminotransferase (ALT) activity

The serum alkaline aminotransferase (ALT) activity was assayed by the method of Reitman and Frankel (1957). The method is based on the principle that pyruvate (pyruvic acid) formed from the alanine aminotransferase catalysed reaction between α–ketoglutarate (oxoglutarate) and L-alanine is coupled with chromogen solution (2, 4-dinitrophenyl hydrazine) in an alkaline medium to form colored hydrazone, the concentration of which is proportional to the alanine aminotransferase activity as measured with a colorimeter.

To 0.05 ml of each serum sample in a test tube was added 0.25 ml of buffer/substrate solution. This was incubated at 37°C for 30 min in a water bath followed by the addition of 0.25 ml of chromogen solution. The content was mixed and allowed to stand for 20 min at room temperature. Then 2.5 ml of sodium hydroxide (0.4 N) was added and mixed. The absorbance was read after 5 min against the blank at 540 nm. The blanks were treated as the samples but without the addition of chromogen solution used to stop all the enzymatic reactions. ALT activity (IU/L) was read off from the standard curve.

Assay of serum lipid peroxidation product (malondialdehyde [MDA])

Serum malondialdehyde (MDA) concentration, one of the end products of lipid peroxidation, was determined by the method of Wallin et al. (1993) based upon the principle that thiobarbituric acid reacting substances (TBARS), in this case malondialdehyde (MDA), reacts with thiobarbituric acid (TBA) to give a red or pink colour which absorbs maximally at 532 nm.

To 0.10 ml of serum in test tube was added 0.45 ml of normal saline and thoroughly mixed before adding 0.5 ml of 25% trichloroacetic acid (TCA) (w/v) and 0.5 ml of 1% thiobarbituric acid. The blank tube contained the same volume of reagents but 0.10 ml of distilled water instead of serum. The mixture in the separate tubes was heated in a water bath at 95°C for 40 min. The content was allowed to cool before reading the absorbance of the clear supernatant against reagent blank at the wavelength of 532 nm and 600 nm. Thiobarbituric acid reacting substances (TBARS) were quantified as lipid peroxidation product by referring to a standard curve of malondialdehyde (MDA) concentration (that is, equivalent generated by acid hydrolysis of 1,1,3,3-tetraethoxypropane (TEP) prepared by serial dilution of a stock solution).

Calculation of serum aspartate aminotransferase to alanine aminotransferase (AST:ALT) ratio

The AST:ALT ratio was calculated from the results of the serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities obtained from the same experiment.

Statistical analyses

The data obtained from the present study were expressed as mean ± SEM. They were analyzed by one-way analysis of variance (ANOVA) based on the modified method of Scheffe (1952). Multiple comparisons of means were made using the least significant

Table 1. The effect of distilled water and monosodium glutamate (MSG) on the activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities and the serum AST:ALT ratio.

Measurement	Serum ALT activity (IU/L)		Serum AST activity (IU/L)		Serum AST:ALT Ratio	
	Water (I)	MSG (II)	Water (I)	MSG (II)	Water (I)	MSG (II)
Mean ± SEM	70.54 ± 0.41	77.00 ± 0.16*	13.40 ± 0.20	2.36 ± 0.15*	0.182±0.00	0.285 ± 0.00*
Percentage (%)	100	109.15	100	166.86	100	156.59
% difference		+ 9.15		+ 66.86		+ 56.59

The results show mean ± SEM; n = 4;* p<0.05; ANOVA followed by least significant difference (LSD) test; + = increased by.

Table 2. The effect of distilled water and monosodium glutamate (MSG) on serum alkaline phosphatase (ALP) activity and serum malondialdehyde (MDA) concentration.

Measurement	Serum ALP activity (IU/L)		Serum MDA concentration (mg/100 ml)	
	Water (I)	MSG (II)	Water (I)	MSG (II)
Mean ± SEM	87.37 ± 0.15	24.49 ± 0.14*	12.53 ± 0.13	48.51± 0.15*
Percentage (%)	100	28.03	100	387.15
% difference		- 71.97		+ 287.15

The results show mean ± SEM; n = 4; * p<0.05; ANOVA followed by least significant difference (LSD) test; +, increased by; - = decreased by.

difference (LSD) test with the statistical package for social sciences (SPSS) for Windows version 11.0 package. Differences were considered significant at p<0.05 level of significance.

RESULTS AND DISCUSSION

Significant (p<0.05) increases in the serum alanine aminotransferase and aspartate aminotransferase enzyme activities and the serum aspartate amino-transferase -to -alanine aminotransferase (AST:ALT) ratio were observed in the MSG-treated rats compared to the control rats (Table 1). The serum malondialdehyde (MDA) concentration increased (p<0.05) in the MSG-treated rats (48.51 ± 0.15 mg/100 ml) compared to the control rats (12.53 ± 0.13 mg/100 ml) (Table 2). However, the serum alkaline phosphatase (ALP) enzyme activity was significantly (p<0.05) decreased in the MSG-fed rats (24.49 ± 0.14 IU/L) compared with the control rats (87.37 ± 0.15 IU/L) (Table 2).

This study was necessitated by the observed indiscriminate use of monosodium glutamate (MSG) in Nigeria for seasoning and flavour enhancing purposes without minding the possible adverse effects from its use over time at low concentrations.

The ALT enzyme is a sensitive marker of liver damage (Al-Mamary et al., 2002). Therefore, the increase in the serum ALT activity by 9.15% observed in rats that were fed MSG at the tested dose may perhaps be an indication of liver damage. MSG could dissociate easily to release free glutamate. The deamination of GLU produces ammonium ion (NH_4^+) that could be toxic unless detoxified in the liver via the reactions of the urea cycle. Thus, the possible ammonium ion overload that may occur as a result of an increased level of glutamate following MSG intake could damage the liver, consequently releasing the ALT enzyme that may lead to its observed elevation. The result seemingly agrees with the reports of Farombi and Onyema (Farombi and Onyema, 2006) and Onyema et al. (2006) that the activity of serum ALT increased in male rats that were fed MSG probably due to MSG-induced oxidative stress in the liver. Thus, it could be concluded that MSG may be hepatotoxic at a low dose, hence should be avoided during the treatment of liver disorders. Furthermore, since ALT was a strong positive indicator of insulin resistance, diabetes mellitus and obesity (Chung et al., 2003) which are risk factors for coronary heart disease (Grundy, 1999; Haffner, 1998; Wilson et al., 1999), the use of MSG at a low dose should not be encouraged because of the possible untoward health implications.

An increase in serum AST activity by 66.86% was observed in rats that were fed MSG, probably predicting damage to the liver and other organs with high metabolic activity (including the brain, heart and lungs) as previously reported by Bain (2003). In addition, the observed increase in the serum AST activity may be indicative of myocardial infarction as suggested by Rodwell and Kennelly (2003). The observation agrees with the previous reports of Farombi and Onyema (2006) and Onyema et al. (2006) that the activity of serum AST increased in male rats that were fed MSG due to MSG-induced oxidative stress. In particular, damage to the brain may occur since MSG could spike blood plasma levels of glutamate, which is an excitotoxin (Stegink et al., 1985; Stegink et al., 1987; Himwhich and Peterson,

1954). High levels of excitotoxins have been shown in animal studies to cause damage to areas of the brain unprotected by the blood-brain barrier leading to a variety of chronic diseases (Meldrum, 1993; Nemeroff, 1980). Conceivably, therefore, such damage to the brain may lead to the observed increase in serum AST activity with MSG treatment. Thus, the result may imply that the oral intake of MSG at a low dose may be destructive to the liver and other organs with high metabolic activity which may have adverse health implications, hence has to be used with caution.

MSG-treated rats increased the serum AST-to-ALT ratio by 56.59%, indicating possible cirrhosis (hardening) of the major organs as reported previously by Johnston (1999). This appears to suggest the inherent risk in the use of MSG even at a low dose in rats. Therefore, caution should be exercised in the use of MSG over time till further studies are carried out to elucidate the effect in humans.

The decrease in the serum ALP activity by 71.97% observed in rats that were fed MSG at the tested dose may indicate possible absence of adverse effects of MSG intake on the pathologies of the bone since increased serum ALP activity has been associated with bone diseases (Bush, 1991). This finding however, neither agrees with previous reports in TILC (Anonymous, 2004a, b) that the oral intake of MSG caused stunted bone development in rats nor the report of Elefteriou et al. (2003) that MSG treatment caused hypogonadism, a condition inducing bone loss in mice. In addition, the significant reduction in serum ALP activity by MSG may perhaps indicate the absence of cholestasis (lack of bile flow) as previously reported by Kaneko (1989). Cholestasis may result from the blockage of the bile duct or from a disease that impairs bile formation in the liver itself (Stegink et al., 1985). Thus, the possible absence of cholestasis with MSG intake could not be explained by the observation in the present study of possible liver damage in rats fed MSG as indicated by increased serum ALT and AST activities. However, increases in the markers of liver damage without an increase in the marker of cholestasis have been reported in rats and interpreted as evidence of ongoing hepatocellular toxicity in the absence of significant cholestasis (Tolman, 1998; Futter et al., 2001).

Induction of oxidative stress as a consequence of MSG treatment has been reported previously (Onyema et al., 2006; Farombi and Onyema, 2006). Accordingly, the serum malondialdehyde (MDA) concentration, a marker of lipid peroxidation (LPO), increased in MSG-treated rats by over two-folds (287.15%) in the present study, probably due to the generation of reactive oxygen species as previously suggested by Tomita and Okuyama (1994). The observed increase in the serum marker of LPO by MSG appears to confirm an earlier report by Diniz et al. (2004) that the administration of MSG induced oxidative stress in experimental animals.

Further to this, the observation seemingly supports the suggestions of Farombi and Onyema (2006) and Onyema et al. (2006) that the activities of serum ALT and AST increased in male rats that were fed MSG due to MSG-induced oxidative stress. It appears, therefore, that the consumption of MSG even at a low concentration may promote the induction of oxidative stress in the liver, which may, as a consequence, lead to liver damage. This may further suggest that MSG could be an oxidant or may not be effective in scavenging the reactive oxygen species, hence should be avoided in the prevention and management of oxidative stress.

In conclusion, treating rats with MSG at a low concentration (5 mg/kg of BW) could be hepatotoxic without significant cholestasis or pathologies of the bone. The results, however, may have significant underlying clinical and public health implications hence warranting further investigation in humans.

REFERENCES

Al-Mamary M, Al-Habori M, Al-Aghbari AM, Baker MM (2002). Investigation into the toxicological effects of Catha edulis leaves: a short-term study in animals. Phytoether Res. 16: 127-132.

Bain PJ (2003). In: Latimer KS, Mahaffrey EA, Prasse KW, eds. Duncan and Prasse's Veterinary Laboratory Medicine: Clin. Pathol. Fourth edn. Lowa State Press. pp. 193-414.

Bergen HT, Mizuno TM, Taylor J (1998). Hyperphagia and weight gain after gold-thioglucose and monosodium glutamate: relation to hypothalamic neuropeptide. Y. Endocrin. 139: 4483-4488.

Bush BM (1991). Interpretation of laboratory results for small animal clinicians. Blackwel Scientific Publications. Oxford.

Chung RT, Casson DR, Murray G, Song S, Grinspoon S, Hadigan C (2003). Alanine aminotransferase levels predict insulin resistance in HIV lipodystrophy. J. Acquir. Immune Defic. Syndr. (JAIDS) 34 (5): 534-536.

Diniz YS, Fernando AA, Campos KE, Mani F, Ribas BD, Novelli EL (2004). Toxicity of hyper caloric diet and monosodium glutamate: oxidative stress and metabolic shifting in hepatic tissue. Food Chem. Toxicol. 42: 319-325.

Elefteriou F, Takeda S, Liu X, Armstrong D, Gerard K (2003). Monosodium glutamate-sensitive hypothalamic neurons contribute to the control of bone mass. Endocrin. 144 (9): 3842-3847.

Farombi EO, Onyema OO (2006). Monosodium glutamate induced oxidative damage and genotoxicity in rat: modulatory role of vitamin C, vitamin E and quercetin. Human Exptl. Toxicol. 25: 251-259.

Futter IE, Al-Swayeh OA, Moore PK (2001). A comparison of the effect of nitroparacetamol and paracetamol on liver injury. Br. J. Pharmacol. 132: 10-12.

Goldberg I (1994). Functional Foods: Designer Foods, Pharmafoods, Nutraceuticals. Chapman and Hall; Intl. Thomson Publishing, NY.

Grundy SM (1999). Hypertriaglyceridemia, insulin resistance, and the metabolic syndrome. Am. J. Cardiol. 83: 25F-29F.

Haffner SM (1998). Management of dyslipidemia in adults with diabetes (American Diabetes Association position statement) Diabetes Care 21: 160-178.

Healthnotes MSG (2005). Sensitivity, Available online at: www.healthnotes.com.

Himwhich WA, Peterson IM (1954). Ingested sodium glutamate and plasma levels of glutamic acid. J. Appl. Physiol. 7 (2): 196-199.

Johnston DE (1999). Special considerations in interpreting Liver function tests. Am. Acad. Family Phys. Vol. 59 No. 8: 2223-2232.

Kaneko JJ (1989). Clinical biochemistry of domestic animal. Academic press, San Diego.

Meldrum B (1993). Amino acids as dietary excitotoxins: a contribution to understanding of neurodegenerative disorders. Brain research. Brain research reviews 18 (3): 293-314.

Mozes S, Sefcikova Z, Lenharde L, Raeek L (2004). Obesity and changes of alkaline phosphatase activity in the small intestine of 40-80-day old subjects to early postnatal overfeeding of monosodium glutamate. Physiol. Res., 53: 177-186.
Nemeroff C (1980). Monosodium glutamate-induced neurotoxicity: Review of the literature and call for further research. Nutrition & Behavior edited by Sanford A. Miller (U.S. Food & Drug Administration): p. 177-211.
Obaseiki-Ebor EE, McGhee EM, Shankee DM (2003). Improved detection of the genotoxic and mutagenic potentials of a food condiment A-One (monosodium glutamate). Presented at the Fourth International Conference of the pan-African Environmental Mutagen Society (PAEMS) in Dar El Diafa- Ain Shams University, Cairo Egypt. 2-4th March: p. 63.
Olney JN (1969). Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. Sci. 164: 719-721.
Onyema OO, Farombi EO, Emerole GO, Ukoha AI, Onyeze GO (2006). Effect of vitamin E on monosodium glutamate induced hepatoxicity and oxidative stress in rats. Indian J. Biochem. Biophys. 43: 20-24.
Reitman S, Frankel S (1957). A colorimetric method for the determination of serum glutamate oxaloacetate and pyruvate transaminase. Am. J. Clin. Pathol., 28: 56-63.
Rodwell VW, Kennelly PJ (2003). Enzymes: Mechanism of action In: Murray, R.K., Granner, D.K., Mayes, P.A. and Rodwell, V.W. ed Harper's Illustrated Biochemistry 26th edn Lange Medical Books/McGraw-Hill, New York. chap 7, p. 57.
Schaumberg HH, Byck R, Gerst R, Mashman JH (1969). Monosodium L-glutamate, its pharmacology and role in the Chinese restaurant syndrome. Sci. 163: 826-828.
Scheffe H (1952). An analysis of variance for paired comparisons. J. Am. Statistics Assoc., 47: 38-400.
Scholm, O. W., Tain, N. C. and Corrall E. J. (1975). Collecting and handling blood for laboratory study In: Scholm Vet. Haematol., Lea and Febiger, Philadelphia. pp. 9-25.
Stegink LD, Filer Jr LJ, Baker GL (1985). Plasma glutamate concentrations in adult subjects ingesting monosodium L-glutamate in consomme. Am. J. Clin. Nutr. 42: 220-225.
Stegink LD, Filer Jr LJ, Baker GL (1987). Plasma amino acid concentrations in normal adults ingesting aspartame and monosodium L-glutamate as part of a soup/beverage meal Metabolism 36(11): 1073-1079.
TILC (Truth In Labeling Campaign)(a). (June, 2, 2004). Neuroendocrine disorders. Available online at: www.truthinlabeling.org
TILC (Truth In Labeling Campaign) (b). (July, 3, 2004). Learning and memory disorder. Available online at: www.truthinlabeing.org.
Tolman KG (1998. Hepatotoxicity of non-narcotic analgesics. Am. J. Med. 27: 13S-19S.
Tomita M, Okuyama T (1994). Effect of paraquat on the malondialdehyde level in rats liver microsome (*in vitro*). Arch. Toxicol. 68: 187-192.
Walker R, Lupien JR (2000). The safety evaluation of monosodium glutamate. J. Nutr. 130 (4S suppl): 1049-1052S.
Wallin B, Rosengren B, Shetzer HG, Cameja G (1993). Lipid oxidation and measurement of thiobarbituric acid reacting substances (TBARS) formation in a single microtitre plate: its use for evaluation of antioxidants. Anal. Biochem. 208: 10-15.
Walter K, Schutt C (1974). Alkaline Phosphatase in serum (Continuous Assay Method). In: Hans Ulrich Bergmeyer (Ed) Methods of Enzymatic Analysis. 2nd edn. Vol.2: 856-870.
Wilson PW, Kannel WB, Silbershatz H, D'Agostino RB (1999). Clustering of metabolic factors and coronary artery disease. Arch. Intern. Med. 159: 1104-1109.