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Predictive risk assessment of a common food additive monosodium glutamate : An in vivo biochemical, patho-physiological and molecular study

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Abstract

Monosodium glutamate (MSG) is a popular food additive commonly known as Aji-nomoto, which has a flavour enhancing effect on food. We investigated if the MSG has any potential to alter kidney and liver function and biochemical and molecular changes in mice. Healthy mice weighing between 20 and 25 grams were chosen for the biochemical, patho-physiological, and molecular studies. Mice were fed with MSG at the dose 2 mg / gm of b.w. There were reduced levels of blood glucose, total serum protein, serum albumin, urea, and BUN and increased level of creatinine, The present findings suggest that MSG has toxic effects on kidney and liver.

Keywords: Food additive, mice, MSG, SDS-PAGE.

Introduction

Monosodium glutamate (MSG) is a widely used food additive, which has a flavour enhancing effect on food. Multidimensional scaling experiments, which are used in sensory research, indicate that MSG falls outside the region occupied by the four classic tastes of sweet, sour, salty and bitter. This distinctive taste is known as 'umami' a word coined by the Japanese to describe the taste imparted by glutamate (Fuke and Shimizu, 1993; Yamaguchi, 1987) through its stimulation of the oro-sensory receptors and by improving the palatability of meals, MSG influences the appetite positively and induces weight gain. Despite its taste stimulation and improved appetite enhancement report indicate the MSG is toxic to human and experimental animal (Biodun and Biodun, 1993).

Glutamic acid is transformed into alanine in intestinal mucosa and lactate in liver (Garattiini, 2000). Glutamic acid is absorbed from gut by active transport system specific for amino acids. In 1968 Chinese Restaurant Syndrome characterized by headache, chest discomfort and facial flushing was first described (Schaumburg et al., 1969). Subsequently it was documented that MSG produces oxygen derived free radicals (Singh and Ahluwalia, 2003). It is also reported that MSG causes obesity (Nagasawa et al., 1974) and gonadal dysfunction (Pizzi et al., 1977).

MSG caused cellular hypertrophy of the theca folliculi, destruction of the basement membrane and stroma cells vacuolations in the ovaries. Degenerative and atrophic processes were observed at both doses with more pronounced changes in the group treated with higher dose (0.08 mg/kg) of MSG (Eweka and Om'iniabohs, 2011). In the present study molecular, biochemical and patho-physiological parameters were undertaken in mammalian system to investigate the adverse effect of monosodium glutamate.

Materials and Methods

Healthy inbred strain of Swiss albino mice (*Mus musculus*), reared and maintained in the animal house of the Department of Zoology (under the supervision of The Animal Welfare Committee), Maulana Azad College, served as materials. Mice were provided with food and water *ad libitum*. The food was generally made up of wheat, gram and powdered milk without any animal protein supplementation, unless mentioned otherwise. The experimental protocols were in accordance with the guidelines laid down by the Animal Welfare Committee, Maulana Azad College.

In the present study for the induction of toxicity, feeding method used by several workers (Olney, 1969; Bunyan et al., 1976) was adopted. The MSG stock solution was prepared and force feeding was done at the dose 2 mg / gm (Das and Ghosh, 2010) of b.w. MSG was procured from local market. Several biochemical and patho-physiological markers used in predictive risk factor assessment of toxicity like blood glucose level, total serum protein, serum albumin, serum globulin, serum creatinine, serum total bilirubin, serum urea and BUN (blood urea nitrogen) were also extensively studied at all the fixation intervals namely 14D and 21D, maintaining suitable controls.

Healthy mice weighing between 20 and 25 grams (about two months old) were chosen for the biochemical, patho-physiological, and molecular studies. Mice were fed with MSG. 5 mice each were used in each series for each fixation intervals. For biochemical and patho-physiological studies serum isolated from the blood was used. For molecular study liver was considered.

Biochemical and Patho-Physiological Parameters

Blood was drawn from retro orbital plexus of etherized (approximately 2.0 ml from each mouse) mice by the routine procedure using sterile disposable syringe and needle. Blood was collected in vials without EDTA. Serum was obtained by centrifugation for use in determination of biochemical and patho-physiological markers like blood glucose level, total serum protein, serum albumin, serum creatinine, serum total bilirubin, serum urea and BUN (blood urea nitrogen). Blood glucose content, serum albumin level, serum creatinine content and serum urea were assayed by the kit procured from Autospan India. Total serum protein content was determined by a standard protocol (Lowry et al., 1951). Serum Total Bilirubin level was assayed by the kit procured from AccuREX Biomedical Pvt. Ltd.

The concentration of BUN (Blood Urea Nitrogen) was determined as a derived data using the following formula:

BUN concentration (g/dl) = 0.467 X Urea Concentration (g/dl).

Scoring and statistical analysis of the data

The levels of blood glucose, total serum protein, serum albumin, serum creatinine, serum bilirubin, serum urea, BUN of both the control and treated series were analyzed and the mean and standard errors determined. Statistical analysis of the data in different

series was done by Students t-test (Fisher and Yates, 1953).

During patho-physiological estimation of the different parameters the 'observer' was kept 'blinded' of the animal belonged to different group in order to remove any 'bias' in observation and thereby uniformity was maintained in scoring data of both treated and control sets of mice.

Molecular Parameter

Changes at molecular level were studied by SDS-Poly Acrylamide Gel Electrophoretic study following the standard protocol (Sambrook and Russell, 2001).

Scoring of data

Analysis of the banding pattern

The protein bands were analyzed by Gel Doc XR+ (BIO RAD) SERIAL NO-721BRO3178 and Molecular Analyst Software, version 2.0.1 (BIO RAD).

Results

Biochemical and Patho-Physiological Markers

Blood Glucose

The mean normal blood glucose level was found to be 132.907 mg/dL. In the MSG fed mice the blood glucose level was decreased rapidly at first being 69.133 mg/dL at 14D and then 112.377 mg/dL at 21 D. The summarized data of blood glucose level in mice of different groups have been presented in histogram (Fig. 1.). The statistical significances have also been denoted in Fig.1.

Serum Protein Level (Quantitative)

The mean serum protein level in normal healthy mice was 885.00 µg/ml, which was found to be considerably reduced in MSG fed series being 858.00 µg/ml at 21D but at 14D there was no significant changes occur. The summarized data of serum protein level in mice of different groups have been presented in histogram (Fig. 2.). The statistical significances have also been denoted in Fig. 2.

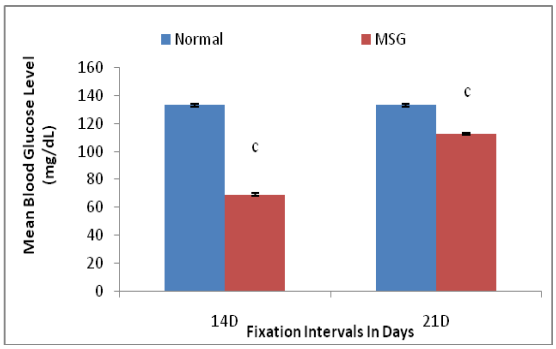

Fig. 1. Mean Blood Glucose level in different series of mice at 14D and 21D fixation intervals.

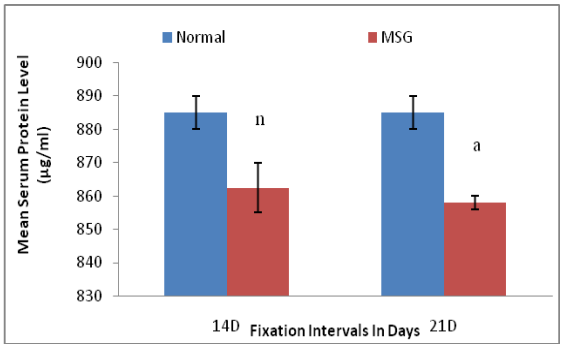

Fig. 2. Mean Serum Protein level in different series of mice at 14D and 21D fixation intervals.

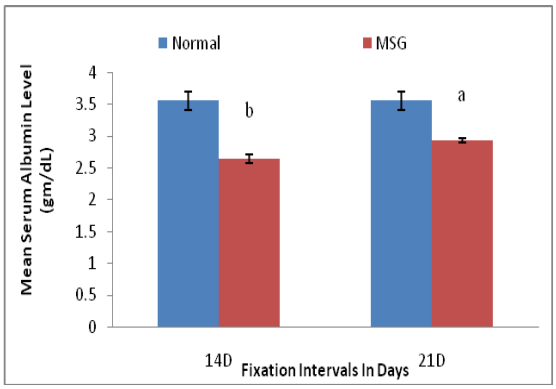

Fig. 3. Mean Serum Albumin level in different series of mice at 14D and 21D fixation intervals.

Serum Albumin level

The mean serum Albumin level in normal mice was estimated to be 3.556 gm/dL. In MSG fed series the level of serum albumin was considerably reduced to 2.637 gm/dL at 14 D and slightly increases at 21D than 14D

being 2.929 gm/dL at 21D. The summarized data of Mean Serum Albumin level in mice of different groups have been presented in histogram (Fig. 3.). The statistical significances have also been denoted in Fig. 3.

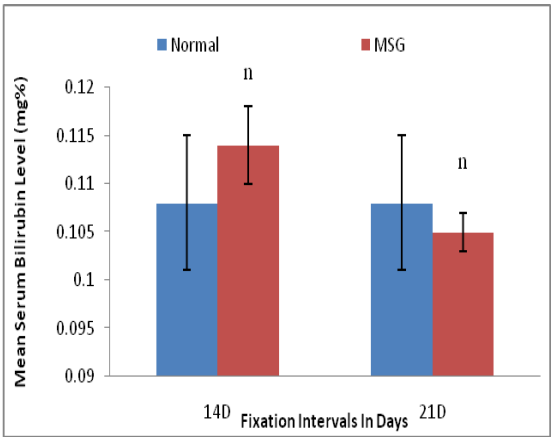

Fig. 4. Mean Serum Bilirubin level in different series of mice at 14D and 21D fixation intervals.

Serum Bilirubin

The mean serum total Bilirubin level in normal mice was found to be 0.108 mg %. In MSG fed mice there was no significant changes in total Bilirubin level. The summarized data of Mean Serum Bilirubin level in mice of different groups have been presented in histogram (Fig. 4.). The statistical significances have also been denoted in Fig. 4.

Serum Urea and BUN

The mean Urea level in normal mice was estimated at 23.333 mg/dL. In MSG fed mice the urea level was considerably decreased being 18.250 mg/dL at 14D and 15.333 mg/dL at 21D. The summarized data of Mean Serum Urea level in mice of different groups have been presented in histogram (Fig. 5.). The statistical significances have also been denoted in Fig. 5.

Since Blood Urea Nitrogen (BUN) level is also often used as a parameter to denote the toxicity level (higher value indicative of higher toxicity), data calculated on the basis of findings on serum Urea levels have been converted into blood urea nitrogen level which would corroborate the results obtained as Urea level. The summarized data of Mean BUN level in mice of different groups have been presented in histogram (Fig. 6.). The statistical significances have also been denoted in Fig. 6.

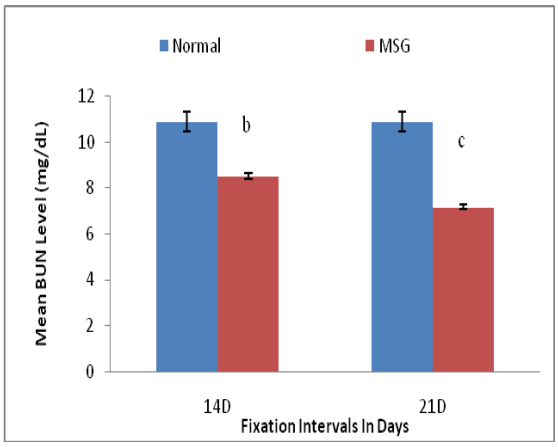

Fig. 5. Mean Serum Urea level in different series of mice at 14D and 21D fixation intervals.

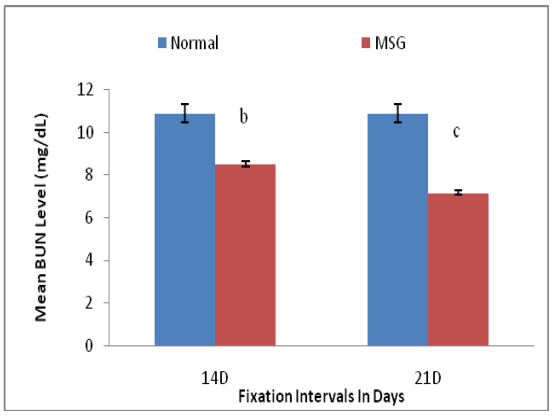

Fig. 6. Mean BUN level in different series of mice at 14D and 21D fixation intervals.

Serum Creatinine

The mean Creatinine content in normal mice was 1.090 mg/dL, which was considerably increased in MSG fed mice being 2.007 mg/dL at 14D and 2.027 mg/dL at 21D. The summarized data of Mean Serum Creatinine level in mice of different groups have been presented in figure (Fig. 7.).

Table 1. Total protein contents (µg/ml) and detailed data on the densitometric scanning of liver protein band profiles of mice of treated and control series at different fixation intervals (14D and 21D).

Fixation Interval	14D				21D			
Band (Peak) number	Normal Amount of protein = 1000.00 Number of bands = 07		MSG Amount of protein = 1000.00 Number of bands = 02		Normal Amount of protein = 1000.00 Number of bands = 07		MSG Amount of protein = 920.00 Number of bands = 03	
	MW (kD)	Band %	MW (kD)	Band %	MW (kD)	Band %	MW (kD)	Band %
1	302.6	0.6	277.1	23.0	302.6	0.6	288.3	13.8
2	280.8	4.3	12.8	77.0	280.8	4.3	211.9	19.9
3	203.7	6.2			203.7	6.2	12.7	66.2
4	46.5	36.0			46.5	36.0		
5	40.7	14.9			40.7	14.9		
6	13.8	22.3			13.8	22.3		
7	12.3	15.6			12.3	15.6		
MW = Molecular Weight.								

The statistical significances have also been denoted in Fig. 7.

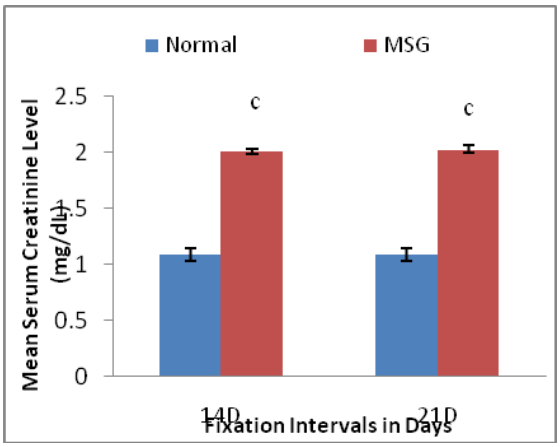

Fig. 7. Mean Serum Creatinine level in different series of mice at 14D and 21D fixation intervals.

Molecular Studies (SDS-PAGE)

The quantitative estimation of total protein contents (µg/ml) as well as the band characteristics of protein profiles in liver of normal healthy mice (negative control) and different treated series at 14D, 21D is summarized in table 1. It would be revealed from the data that the normal average protein content in liver was 1000.00 µg/ml and the total number of gel electrophoretic protein bands as analyzed by Gel Doc XR+ (BIO RAD) SERIAL NO-721BRO3178 and Molecular Analyst Software, version 2.0.1 (BIO RAD). A critical analysis of the data would reveal that there were decrease in protein amount and alteration of band characters in MSG fed mice at 14D (No. of bands = 02) and at 21D (No. of bands = 03) as compared to normal controls. The comparative data of protein content and protein bands as obtained from the present investigation are summarized in the table 1.

Discussion

The observations of the present investigation have shown that MSG at the dose of 2 mg /gm of body weight is capable to alter the liver and kidney function. Similar conclusions can also be arrived at when the data of the non-enzymatic patho-physiological parameters are taken into consideration. In the chronically MSG fed mice there were

reduced levels of total serum protein, serum albumin, urea, and BUN and increase in the level of creatinine, the trend which was also found by other workers (Tawfik and Al-Badr, 2012).

Among the patho-physiological parameters studied, creatinine is a fairly reliable indicator of kidney function. Blood urea nitrogen (BUN) level is another indicator of kidney function. Urea is also a metabolic byproduct which can build up if kidney function is impaired. But in our present finding it showed a decreasing trend at all the fixation intervals, cause of which is not clearly understood.

As the kidneys become impaired for any reason, the creatinine level in the blood will rise due to poor clearance of creatinine by the kidneys. A rise in creatinine level would signify impaired function of the kidney. Abnormally high levels of creatinine thus warn of possible malfunction or failure of the kidneys.

Significant decrease in blood glucose may be due to hyper-secretion of insulin. Increasing amount of insulin can also increase the protein synthesis and storage in the liver. Analysis of the protein gel of liver reveals the reduction in number of protein band and also in the band intensity. This may be caused due to the alteration in the expression of proteins as a result of MSG treatment. Thus our present findings may be accepted as an warning for not to use MSG as a food additive to enhance the taste.

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