ORIGINAL ARTICLE

Effects of Monosodium Glutamate on Liver Tissue of Wistar Albino Rats - A Histological And Biochemical Study

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ABSTRACT

Background and Objectives: Monosodium glutamate (MSG), chemically known as AJI-NO-MOTO (at the origin of flavor) is the sodium salt of glutamic acid, one of the most abundant naturally occurring non-essential amino acids. MSG is entering our bodies with absolutely no limits in hundreds of food items daily. The reports have indicated that MSG is toxic to human and experimental animals. Therefore, MSG has become a controversial food additive, and the scientific reality still remains obscure. The objectives of this study are to assess the histological and biochemical effects of MSG on liver tissue of Wistar Albino Rats.

Materials and Methods: In the present study, 24 rats were divided into four groups. Each group contained 6 rats. Group I was control and received standard diet with 2 ml distilled water orally by gavage, Group II were received 0.6 mg/g body weight MSG dissolved in 2 ml distilled water orally by gavage, Group III will received 1.6 mg/g body weight MSG dissolved in 2 ml distilled water orally by gavage, and Group IV will received Chaudhary group Wai Wai noodles mixed with standard rodent diet and 2 ml distilled water orally by gavage. All groups were treated over a period of 28 consecutive days. On 30 days, all rats were sacrificed by cervical dislocation and liver was taken for histology, while blood was collected for biochemical study.

Results: In MSG-treated rats, the examined sections showed the altered liver architecture, congestion in central vein, dilated sinusoids, and decreased size of hepatocyte nuclei diameter. Examination of liver histology of rats from Group IV showed normal histological features with hepatic lobules; however, mild disturbance of liver architecture was seen. The liver enzyme alanine aminotransferase, aspartate aminotransferase, and gamma-glutamyltransferase significantly increased in the serum, on MSG administration group compared to control.

Conclusions: The results showed that MSG-treated rats affected the histology of liver and affected the liver function.

Key words: Albino rats, liver architecture, liver enzymes, monosodium glutamate

INTRODUCTION

Monosodium glutamate (MSG), chemically known as AJI-NO-MOTO (at the origin of flavor) is the sodium salt of glutamic acid, one of the most abundant naturally occurring non-essential amino acids.[1] It contains 78% of glutamic acid, 22% of sodium and water.[2] Glutamic acid is a major component of many proteins such as meat, fish, milk, and some vegetables and plays an essential role in human metabolism.[3] For thousands of years, kombu and other seaweeds have been added to foods in Japan to enhance flavor. In 1908, a Japanese scientist Ikeda discovered that the active ingredient in kombu is glutamic acid, and then the use of its sodium salt, MSG is a flavor enhancer began in Japan.[4] Thereafter, the worldwide use of processed free glutamic acid began to explode. Modern commercial is produced by fermentation of starch, sugar, beet sugarcane, or molasses.[5]

Since free glutamic acid is cheap, and its nerve stimulation enhances the flavor of basically bland and tasteless foods so wonderfully, manufacturers are eager to go on using it and do not want the public realize any of the problems. In 1958 the U.S., Food and Drug Administration (FDA) designated MSG as a generally recognized as a safe ingredient. MSG is thus permitted not to be dangerous food additive. As a consequence, it requires no specified daily intake, or an upper limit intake requirement. Almost every bottled, bagged, frozen, or canned, processed food on supermarket shelves contains free glutamate in various forms and names.

Although glutamate is a naturally occurring amino acid found in varying concentrations in many foods, it should be acknowledged that it is the free glutamate molecule that is toxic. Bound glutamate, found naturally in foods, is less dangerous because it is slowly broken down and

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absorbed by the gut so that it can be utilized by the tissues, especially muscle, before toxic concentrations can build up.[6] Glutamate additives are free glutamates, completely unattached to any other protein which are easily and quickly absorbed and cause a spike in blood levels of glutamate. Thus, bound glutamates in diet are not dangerous while free glutamate is dangerous. This is because the body does not have to break down the free form of glutamate.[7] Despite its taste stimulation and improved appetite enhancement, various types of harmful effects on various organs have been reported in experimental animals.[8-10]

MSG-induced alterations in metabolic rate of glucose utilization and decreased antioxidant defenses. Generation of reactive oxygen species in different body cell is known to induce damage to DNA, lipids and proteins, and lipid peroxidation in cellular membrane due to damage of the polyunsaturated fatty acids in the cell membranes, which may lead to cellular death by apoptosis.[11] The mechanisms of MSG-induced damage include the production of free radicals that alter mitochondrial activity and genetic information.[12] It is metabolized in liver and eliminated through the kidney.[13]

Use of MSG has increased enormously, and the consumers are eating MSG on a daily basis in hundreds of food items. Since 1948 the amount of MSG added to foods has doubled every decade. By 1972, 262,000 metric tons were being added to foods. In many cases, they are being added in disguised forms, such as natural flavoring, species, yeast extract, autolyzed yeast, hydrolyzed protein, whey protein, textured protein, and soy protein extract each of the substances contain a percentage of free glutamate, the harmful component of MSG.[6]

Thus, MSG has generated much controversy, globally about its safe usage. Since the liver is one of the organs involved in detoxification, liver of adult Wistar albino rats will be taken to see the effect of MSG.

MATERIALS AND METHODS

Animals

A total of 24 healthy Wistar albino rats of either sex weighing 150–200 g were randomly selected for the study. All procedures were approved by the IRC, BPKIHS. Regarding Animal Care was performed in accordance with the guidelines of the "Principles of Laboratory Animal Care" (NIH publication No. 80-23, revised 1996). Rats were housed in standard plastic cages under room temperature (22–24°C) with a 12-h light/dark cycle (lights on 6:00 a.m.). Unless otherwise stated standard laboratory food and water were available throughout the experiments. Animals were allowed to acclimatize to the laboratory conditions for 7 days before the experimental procedures. All efforts were made to minimize the number of animals used and their suffering.

Drugs and treatment: MSG commercially available pack and Chaudhary group (CG) Wai Wai instant noodles pack were collected from the local market. MSG was dissolved in water and fed to the experimental group.

Group Division

After a quarantine period, 24 rats were randomly divided into four groups, each consisting of six animals. Group I was used as a control and received standard rodent diet and 2 ml of distilled water orally by gavage daily. Group II received 0.6 mg/g body weight MSG dissolved in 2 ml distilled water orally by gavage daily. Group III received 1.6 mg/g body weight MSG dissolved in 2 ml distilled water orally by gavage daily. Group IV received CG Wai Wai noodles mixed with standard rodent diet and 2 ml distilled water orally by gavage daily. All groups were treated over a period of 28 consecutive days. 24 h after the administration of last doses, on the 30th day, rats were sacrificed by cervical dislocation. Liver was taken out by abdominal dissection, weighed and was preserved in 10% buffered formaldehyde solution for light microscopic and histological examination.

Histopathological Examination

Histopathological evaluation was made in liver tissues. Preserved liver was dissected, and tissue samples were embedded in paraffin and then 5–6 µm sections were cut using rotary microtome. Thus, obtained tissue sections were stained with hematoxylin and eosin (H and E).

Biochemical Assays

At the end of treatment period, the animals were sacrificed 24 h following the last given dose. Blood samples were withdrawn and collected in glass tubes. Serum was separated by centrifugation at 3000 rpm for 10 min and stored at −80°C for the bending biochemical analysis. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and Y-GT were measured by IFCC recommended method.

RESULTS

The initial weight of experimental rat was 158.83 ± 11.03 g in Group I (control), 157.83 ± 6.49 g in Group II, 156.16 ± 7.22 g in Group III, and 155.16 ± 3.60 g in Group IV. The animal's weight at the end of the experiment (30th day) it was 188.83 ± 8.81 g in Group I, 196.50 ± 9.95 g in Group II, 204.167 ± 8.72 g in Group III, and 183.66 ± 5.57 g in Group IV, respectively.

The weight of rat was significantly increased ($P < 0.05$) at the end of the experiment as depicted in Table 1.

On comparison of the weight of rat of different groups, the increment in weight of Group III was statistically significant ($P < 0.05$) in comparison to Group I and Group IV [Table 2].

The average weight of liver was 3.34 ± 0.41 g in Group I (control), 3.66 ± 0.28 g in Group II (treated), 4.25 ± 0.36 g in Group III (treated), and 3.86 ± 0.32 g in Group IV [Table 3].

On comparison of the weight of liver of different groups, it was statistically significant ($P < 0.05$).

Tukey *post-hoc* comparison of Group III was highly significant ($P < 0.001$) in comparison to the Group I and significantly different ($P < 0.05$) in comparison to Group II [Table 4].

The mean diameter of hepatocytes significantly decreased in MSG-treated group as compared to the control. When Group IV compared with control there is also significant difference but when Group IV compared with MSG-treated group, there is no significant differences [Tables 5 and 6].

Biochemical Study

ALT, AST, and Y-GT were measured by IFCC recommended method. The final result was expressed as IU/L. The mean ALT, AST, and Y-GT value of different groups was shown in Tables 7 and 8.

On comparison of the ALT, AST, and Y-GT value in between different groups, the increase in its value was found to be significant ($P < 0.05$) in comparison to Group I.

Observational Analysis of Liver Histology of Rat

Examination of liver histology of rats from control group showed normal structure of central vein (CV), hepatocyte (H), and sinusoid (S) [Figure 1]. Examination of liver histology of rats from Group II showed congestion of CV, hepatocyte, and dilated sinusoids [Figure 2]. Examination of liver histology of rats from Group III showed congested and enlarged CV, ruptured endothelial lining of CV, decreased the size of hpatocyte, and nuclei [Figure 3]. Examination of liver histology of rats from Group IV showed sinusoids were not densely packed, hepatocytes binucleation [Figure 4].

DISCUSSION

MSG is frequently used as a flavor enhancer, the fact of which makes it one of the most applied food additives in

Table 1: Mean weight of rat before and after the experiment

Groups	Weight (g) day 0 (Mean±SD)	Weight (g) day 30 (Mean±SD)
I	158.83±11.03	188.83±8.81
II	157.83±6.49	196.50±9.95
III	156.16±7.22	204.167±8.72
IV	155.16±3.60	183.66±5.57
F-value	0.283	6.788
P	0.837	0.002

SD: Standard deviation

Table 2: Intergroup comparison of weight of rat on 30 days

Groups comparison	*P*
I versus II	0.414
I versus III	0.024
I versus IV	0.716
II versus III	0.414
II versus IV	0.069
III versus IV	0.002

Table 3 : Mean weight of liver after the experiment

Groups	Mean weight±SD (g)
I	3.34±0.41
II	3.66±0.28
III	4.25±0.36
IV	3.86±0.32
F-value	6.972
P	0.002

SD: Standard deviation

Table 4: Intergroup comparison liver control and experimental group

Groups compared	*P*
I versus II	0.401
I versus III	0.001
I versus IV	0.080
II versus III	0.044
II versus IV	0.773
III versus IV	0.258

Table 5: Mean diameter of hepatocytes of control and treated rats

Groups	Nuclei diameter of hepatocytes (micrometer) (Mean±SD)
I	6.25±0.993
II	5.62±1.03
III	4.56±0.73
IV	5.18±0.83
F-value	12.28
P	0.000

SD: Standard deviation

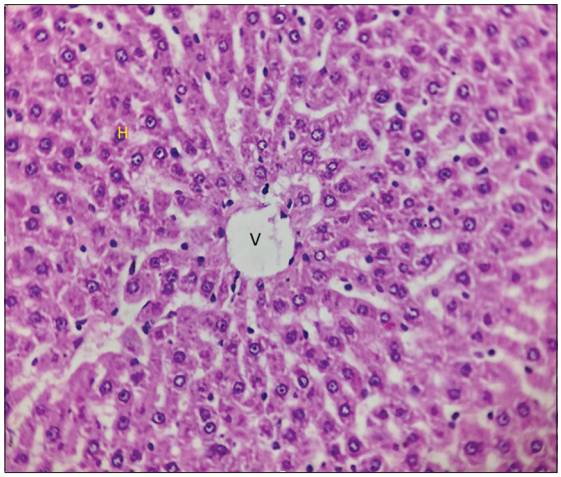

Figure 1: Photomicrograph of rat liver from control group, showing normal structure, central vein (V), normal arrangement of liver cords, hepatocytes normal (H), and sinusoids. H and E ×400

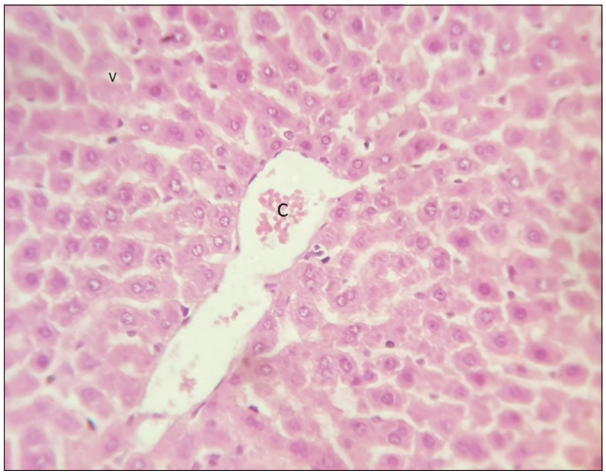

Figure 2: Photomicrograph of rat liver treated with 0.6 mg/g body weight monosodium glutamate showing congestion of central vein (C), vacuolation of hepatocytes (V), and dilated sinusoids. H and E. ×400

Table 6: Intergroup comparison of nuclei diameter of liver rat

Groups compared	*P*
I versus II	0.139
I versus III	0.001
I versus IV	0.002
II versus III	0.002
II versus IV	0.429
III versus IV	0.139

the modern nutrition all over the world. MSG is entering our bodies with absolutely no limits in hundreds of food items daily (fast foods, frozen meals, canned soups, potato chips, etc.). Commercially, processed foods and restaurant food items are increasing day by day because of various circumstances. The most of food contains MSG. Since it is cheap and easily available, people are using this flavor enhancer in household cooking also. In 1958 the U.S. though FDA designated MSG as a safe ingredient, various reports have indicated that MSG is toxic to human and experimental animals.[11,14]

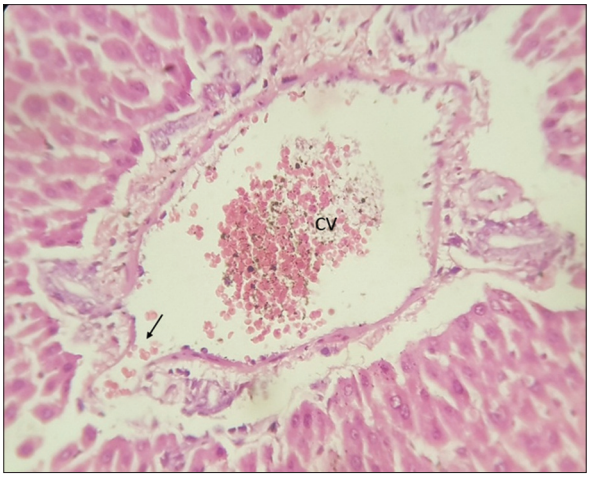

Figure 3: Photomicrograph of rat liver treated with 1.6 mg/g body weight monosodium glutamate showing congested and enlarged central vein with ruptured endothelial lining of central vein (arrow marked), vacuolation of hepatocytes and decreased size of hepatocyte and nuclei. H and E ×400

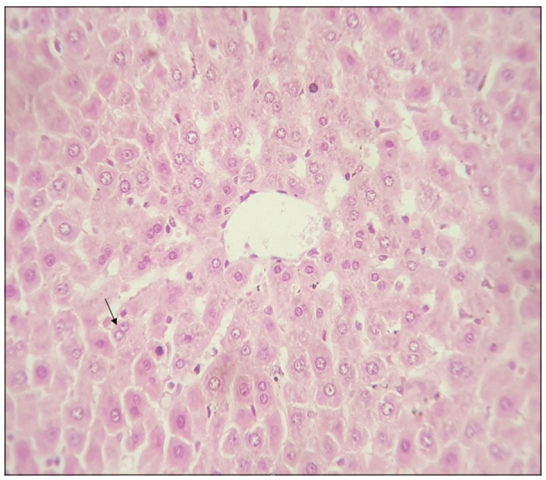

Figure 4: Photomicrograph of rat liver treated with Chaudhary group Wai Wai with standard rodent diet showing sinusoids were not densely packed, hepatocytes binucleation (arrow). H and E ×400

In the present study, we used oral route for administration of MSG in a dose of 0.6 and 1.6 mg/g body weight for 28 days. The weight records of experimental animals showed more gain in comparison to the control group. MSG intake could induce an increase in energy intake which could lead to obesity or alter the level of carbohydrate[5,11,15] which is similar to our study.

In our study, a significant increase in the liver weight of rats was observed after administration of 1.6 mg/g body weight in comparison to the control group ($P < 0.05$). The increase liver weight after administration of MSG might be an increase in activity of inflammatory agents in liver tissue.[16] However, 0.6 mg/g body weight MSG administration and CG Wai Wai treated groups rat livers showed more weight in comparison to the control group, but statistically, it was not significant.

In the present study, we also measured diameter of nuclei of hepatocytes from all the groups. The decrease

Table 7: AST, ALT, and GGT value in control and treated groups of rat

Groups	ALT value (IU/L) (Mean±SD)	AST value (IU/L) (Mean±SD)	Y-GT value (IU/L) (Mean±SD)
Group I	84.317±16.35	273.00±108.56	1.66±0.81
Group II	141.78±49.26	726.15±595.93	6.00±3.03
Group III	172.38±67.86	1394±989.66	9.67±3.92
Group IV	109.26±40.10	642.66±413.54	3.33±3.61
F-value	3.964	3.448	7.616
P	0.023	0.036	0.001

SD: Standard deviation, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma-glutamyltransferase

Table 8: Intergroup comparison of ALT, AST, and Y-GT value

Group	*P*		
	ALT value	AST value	Y-GT value
I versus II	0.797	0.729	0.105
I versus III	0.02	0.024	0.001
I versus IV	0.184	0.589	0.788
II versus III	0.128	0.183	0.204
II versus IV	0.680	0.269	0.461
III versus IV	0.638	0.995	0.010

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma-glutamyltransferase

of nuclei diameter of hepatocytes is in dose-dependent manner. The rats treated with 1.6 mg/g body weight MSG showed decrease of nuclei diameter was highly significant ($P < 0.001$) in comparison to the control group whereas rat treated with 0.6 mg/g body weight and CG Wai Wai treated showed just significant ($P < 0.05$) in comparison to control group. The dose-dependent changes in the liver of rat also reported by others.[17]

In this study, we measured liver enzyme marker (ALT, AST, and gamma-glutamyltransferase [GGT]). A significantly increases serum enzyme marker in the group treated with MSG in comparison to the control group. These enzymes are sensitive marker of liver damage. Therefore, the increase in the serum ALT, AST, and GGT activity might be indication of liver damage which is caused by the MSG-induced oxidative stress. A similar findings were observed by other researchers.[18,19]

In the histological examination, the MSG-treated animal showed the altered liver architecture, congestion in CV, dilated sinusoids, and decreased the size of hepatocyte nuclei diameter. Our findings are similar with the findings by Eweka *et al*. 2011. The 1.6 mg/g body weight treated animals showed more injury than the animals treated with 0.6 mg/g body weight animals. Examination of liver histology of rats from Group IV showed normal histological features with hepatic lobules; however, mild disturbance of liver architecture was seen in this group.

CONCLUSION

The findings obtained in this experimental study showed that the administration of MSG in the doses of 0.6 and 1.6 mg/g body weight to adult Wistar albino rats affected the histology of liver as well as its function. However, the animals treated with CG Wai Wai (Group IV) showed minimal change as compared to two other groups. This might be due to the actual content and quantity of CG Wai Wai consumed per day by each rat had not been ascertained since the CG Wai Wai was mixed with their standard feeds.

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