HISTOLOGICAL & HISTOCHMICAL CHANGES IN LIVER OF ADULT RATS TREATED WITH MONOSODIUM GLUTAMATE: A LIGHT MICROSCOPIC STUDY

Vandana Kumbhare, Ujwal Gajbe, Brij Raj Singh, Anil Kumar Reddy, Samarth Shukla

1Department of Anatomy, N S C B Medical College, Jabalpur, Madhya Pradesh, India.
2, 3Department of Anatomy, J.N.M.C, Sawangi (Meghe), Wardha, Maharashtra, India.
4Department of Anatomy, ANIIMS, Port Blair, Andaman and Nicobar Islands, India.
5Department of Pathology, J.N.M.C, Sawangi (Meghe), Wardha, Maharashtra, India.

ABSTRACT

Introduction: Monosodium Glutamate, also known as MSG,[1-3] Monosodium glutamate is a controversial additive. It is metabolized in liver and kidney plays an important role in its elimination.[4] In 1968 Schaumburg H.H and R. Byck[5] were the first people to draw attention towards Chinese restaurant syndrome characterized by headache, chest discomfort and facial flushing.[6, 7, 8] The diversity in manifestation of toxic effects and susceptibility of different species of animals to MSG was such that till date no specific dietary limitations have been recommended. The current research work is carried out to Study the morphology and histology of adult rat liver treated with Monosodium Glutamate (3mg and 6mg/gm body weight). Materials & Methods:

The study was started after permission from Institutional Ethical committee which has duly authorized CPCSEA approval for animal experiment. In all 51 adult Wistar rats were assigned into three study groups randomly. All three group experimental animal were sacrificed according to CPCSEA guideline, and Liver tissue sample were taken for morphological and histological examination. Conclusion: The findings revealed that there were deleterious changes in the liver tissue such as dilated and congested central vein, inflammatory cell infiltration, cytoplasmic vaculation and pyknotic nucleus, cyto architecture
disturbance, and degenerative changes more marked in the peripheral lobular areas of the liver tissue.

KEYWORDS: Monosodium Glutamate, CPCSEA guideline, Schaumburg H.H and R. Byck.

INTRODUCTION
Monosodium glutamate is a controversial additive. It is a major component of many proteins such as meat, fish, milk and some vegetables and it is available commercially by the fermentation of molasses, but exists in many products made from fermented proteins, such as soy sauce and hydrolyzed vegetable protein.\textsuperscript{[1, 2, 3]}

Monosodium Glutamate is a naturally present excitatory neurotransmitter in brain, mediating fast synaptic transmission in one third of all CNS synapses. Glutamic acid is absorbed from gut by active transport system specific for amino acids.\textsuperscript{[4]} Glutamic acid is transformed into alanine in intestinal mucosa and lactate in liver.\textsuperscript{[5]} It is metabolized in liver. Kidney plays an important role in its elimination.\textsuperscript{[6]}

Monosodium Glutamate is responsible for Chinese restaurant syndrome characterized by headache, chest discomfort and facial flushing following the ingestion of Chinese meal.\textsuperscript{[7, 8]} subsequently it was documented that Monosodium Glutamate produces oxygen-derived free radicals.\textsuperscript{[9]} It is also reported that Monosodium Glutamate causes disturbances of central endocrine axis affecting wide areas of body causing learning difficulty,\textsuperscript{[10]} obesity and gonadal dysfunction.\textsuperscript{[11]} Its neurotoxicity was established by many workers.\textsuperscript{[12]} While other researchers using Monosodium Glutamate experimentally could not establish the triads of Chinese restaurant syndrome.\textsuperscript{[13]} The diversity in manifestation of toxic effects and susceptibility of different species of animals to MSG was such that till date no specific dietary limitations have been recommended. On the contrary, U.S. Food and Drug Administration FDA lists it as a GRAS (generally recognize as safe) and limits its use only in baby food.\textsuperscript{[8]}

Despite its taste stimulation and improved appetite enhancement, reports indicated that Monosodium Glutamate is toxic to human and experimental animals. Monosodium Glutamate may have some deleterious effect on the Liver of adult rats at higher dose and it may affect the functions of liver.\textsuperscript{[14]} The present study was conducted to assess the effects of MSG on liver in albino rats.
MATERIALS & METHOD

The present animal interventional study was conducted in Animal House of Jawaharlal Nehru Medical College, Sawangi (M) Wardha. The study was started after permission from Institutional Ethical committee which has duly authorized CPCSEA approval for animal experiment. In all 51 adult Wistar rats were assigned into three study groups randomly. Before conducting the study, the experimental rats were kept in the department research laboratory for one week in normal environment (24 ± 2°C) and supplemented by a standard diet and water. The histological studies conducted after the sacrifice and corroborate the result of previous researchers.

Grouping of Animals

The rats were divided into three groups 17 rats in each group and treated orally as follows;

**Group A:** The control group in which rats were only administrated distilled water along with regular diet, daily for 45 days.

**Group B:** The experimental group in which rats were administrated orally the therapeutic dose of monosodium glutamate (3 mg/gm body weight) daily for 45 days along with regular diet.

**Group C:** The experimental group in which rats were administrated orally the therapeutic dose of monosodium glutamate (6 mg/gm body weight) daily for 45 days along with regular diet.

All three group experimental animal were sacrificed according to CPCSEA guideline, and Liver tissue sample were taken for morphological and histological examination by light microscopy.

Individual study subject histological finding were recorded and entered in Microsoft Excel sheet data were analyzed with statistical software SPSS version 16. The obtained results were statistically analyzed and the test of significance was used (P<0.05).
RESULTS

Table 1: Showing the mean values of animal’s weight on the day of commencement of experiment (1st day) for group A (the control group), B and C (the Study group) respectively was 182.47 ± 20.5, 183.76 ± 22.50, and 184.5 ± 23.42. The Mean values of animals weight at the end of experiment (46th day) respectively was 239.75 ± 23.60, 278.71 ± 23.42 and 334.35 ± 29.57.

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight in gm on day 0</th>
<th>Weight in gm on 46th day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>MIN</td>
<td>164</td>
<td>156</td>
</tr>
<tr>
<td>MAX</td>
<td>212</td>
<td>210</td>
</tr>
<tr>
<td>MEAN</td>
<td>182.47</td>
<td>183.76</td>
</tr>
<tr>
<td>SD</td>
<td>20.75</td>
<td>22.50</td>
</tr>
</tbody>
</table>

*P value

| P<0.05 considered as Significance

<table>
<thead>
<tr>
<th>Weight in gm on 46th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
</tbody>
</table>

*P values compared with their respective group 0 day values

HISTOPATHOLOGICAL OBSERVATIONS

Group A: Control sections of liver revealed normal histological features with hepatic lobules. Each hepatic lobule consists of cords that are regularly arranged hepatocyte enclosing the sinusoidal network and central vein that located in the center of the lobule. The portal area contained branches from hepatic artery, portal vein and bile duct. The hepatocyte are polygonal in shape and have clear round to oval nuclei. The blood sinusoids and non parenchyma cells includes Kupffer cells and endothelial cells. (Fig. 1)

Group B: Examination of liver sections prepared after administration of daily dose of Monosodium Glutamate (3mg/g body weight) for 45 days revealed a mild disturbance of liver architecture, the enlarge and congested central vein with disturbed endothelial lining. There is a marked variation in the nucleoli in most of the hepatic cells. The section showed the existence of obvious inflammatory leucocytes infiltration in the portal area composed mainly of lymphocytes (rounded cells). Showing mild disturbance of liver architecture, enlarge congested central vein and (IF) infiltration of inflammatory cells in portal area (Fig. 2).

Group C: Examination of Liver sections prepared after administration of daily dose of Monosodium Glutamate (6mg/g body weight) for 45 days, show more injury than the above
recorded ones was detected in the tissues examined after treatment. The endothelial lining of the central vein as well as other blood sinusoids suffered from erosion. The cytoplasm contained large vacuoles (vacuolar degeneration) and small tiny vacuoles (hydropic degeneration), these vacuoles have indefinite borders. In some sections, we also observed there was focal necrosis of the hepatic cells, loss of hexagonal shape of hepatocytes and vacuolations in their cytoplasm with pyknosis of nuclei. In these degenerated cells, only slight cytoplasmic mass was left in these cells forming a narrow peripheral rim. There were marked loss of the uniformity and regularity of the hepatic cords. (Fig. 3)

Photomicrograph of liver section shows dilated central veins filled with stagnant blood cells were noticed and sinusoids were also widened. (Fig. 4)

The infiltrative leucocytes were observed in large numbers and distributed haphazard all over the tissue, lymphocytes (rounded inflammatory cells) were mostly observed. Hepatocytes were displaying marked hydropic degeneration, characterized by extensive vacuolization of the cytoplasm. Hepatocytes were highly damaged and they lost their characteristic architectural appearance too. Blood hemorrhage was noticed among liver parenchyma. Aggregations of R.B.Cs were noticed indicating clear signs of hemorrhage. The cell membranes were mostly unrecognized and if exist, they were ill defined and noticeably ruptured at several places. Some nuclei showed fragmentation of the condensed chromatin with breakdown of the nuclear membrane, which indicates a clear signs of karyohexis.(Fig. 5)

Fig. 1: Photomicrograph of a section of liver of control animal showing radiating cords of hepatocytes (H) , anastomosing network of sinusoids (S) and central vein (CV) and portal triad (PT) H/E, 100X)
Fig. 2: Photomicrograph of a section of liver of Group B, showing mild disturbance of liver Architecture, (CG) enlarge congested central vein and (IF) infiltration of inflammatory cells in portal area. (H/E 100 X)

Fig. 3: Photomicrograph of group C liver section showing loss of normal architecture, degenerating hepatocytes (H) with numerous vacuolations (Vc), nuclei pyknosis (Py) (H/E 100 X)

Fig. 4: Photomicrograph of group C liver section showing dilated central vein (CV) with blood cell, disturbed hepatic cord arrangements (Lysed).
HISTOCHEMICAL OBSERVATIONS:

**Group A:** Polysaccharide inclusions were illustrated in Periodic Acid Schiff’s reagent (PAS) preparation of the liver cells of adult normal rats in the form of numerous tiny sized and magenta-colored aggregates in the cytoplasm of hepatocytes. The nuclei were negatively stained. (Fig. 6)

**Group B:** Liver treated with 3 mg/gm body weight of MSG day after day for 45 days has apparently induced a slight loss of the carbohydrate material in the hepatocytes occupying the peripheral zonal lobular areas, which manifested a weak PAS reactivity than the normal conditions.

**Group C:** The PAS preparation of the liver the of rats given dose 6mg/gm of monosodium glutaminate for 45 days, indicated depletion of the carbohydrate materials of the constituent hepatocytes. This was marked especially in those cells lying in the peripheral zonal lobules. (Fig.No.7)
Fig. 6: Photomicrograph of a section of liver of control animal showing uniform distribution of PAS positive hepatocytes (PAS, X 400).

Fig. 7: Photomicrograph of a section of liver of control animal showing uniform distribution of PAS negative hepatocytes (PAS, X 400).

DISCUSSION

The findings on body weight, liver weight and histological changes in liver are mostly in conformity of findings in earlier studies by others. Albino rats are the commonest laboratory animals to be used for experimental work. They have greater sensitivity to most of the drugs.
They are the most standardized (pure and uniform strain) of all laboratory animals. Since they are small in size, they are easy to handle. They do not have their vomiting centre, so they cannot vomit and albino rats withstands long period of experimentation also.

It would therefore be worthwhile to examine the effects of Monosodium glutamate on the liver of adult Albino rat. The histological changes are compared under following categories of changes.

1. Dilated congested central vein
The histological study of Group B animals who received treatment of 3mg/kg body weight Monosodium Glutamate for 45 days, liver sections revealed congested and enlarged central veins. Group C animals showed more injury than the group B. Dilated central vein with ruptured endothelial lining and distended portal veins filled with stagnant blood cells were found in Group B animals. Eweka et al. (2008), Singh I (2009), and others also reported similar findings in their study.

2. Inflammatory cell infiltration
In the present study, the results of experimental animal showed the existence of an obvious inflammatory leucocytic infiltration in the portal area mainly composed of lymphocytes (rounded cells). The migration of leucocytes towards the area of inflammation is known as chemotaxis. The abundance of leucocytes in general and lymphocyte in particular is a prominent response of body tissue facing any injurious impacts.

These findings are in concurrence with the findings by Egbuonu et al. (2010) who reported rats treated with MSG showed moderate to severe infiltration of mononuclear leucocytes into the portal area. Nakanishi et al. (2008) also reported neutrophil aggregation in the lobules, and portal inflammation was also frequently present in liver parenchyma of rats treated with monosodium glutamate.

3. Cytoplasmic Vacuolation and pyknotic nucleus
The most marked signs of tissue impairment in our study carried out with the dose of 3mg/gm body weight; were the cytoplasmic vacuolation along with pyknotic nucleus detected in the vast majority of the hepatocyte. These vacuoles contained fat droplets. The presence of fat droplets represents an injurious response of the hepatocyte to the ingestion of monosodium glutamate. Nakanishi et al. (2008), found moderate to severe microvesicular fatty changes
throughout the liver parenchyma. Similar observations were reported by Mosaibih\cite{18} (2013) in their histological study of liver sections showed vacuolar degeneration of hepatocytes cords, nuclei pyknosis and congestion of blood vessels. Contini et al\cite{19} (2012) also reported that although in the liver lobular structures, trabecular and portals are preserved, but there is steatosis characterized by cytoplasmic vacuoles fat throughout the liver lobule in the animals treated with different doses of monosodium glutamate.

4. Cyto architecture disturbance

In our study, the liver of rat treated with 6 mg /gm body weight Monosodium Glutamate showed marked disturbance of radiating arrangement of hepatocyte cords, along with dilated congested portal vein. Blood haemorrhage was noticed among liver parenchyma. Similar finding have also been documented by Bhattacharya et al\cite{6} (2011) they observed in MSG treated mice, histological feature of hepatic parenchyma were variable in different parts. Onaolapo et al\cite{20} (2013) found liver histology that indicated a loss of normal liver architecture with varying degrees of disorganization and apoptotic cell death. Our study findings are also supported by Waer H. F. & Edress S\cite{21} (2006), Eweka et al\cite{22} (2008), Mosaibih\cite{18} (2013).

5. Degenerative changes

In the present study degenerative changes are more marked in the peripheral lobular areas of the liver tissue. The nuclei were hyperchromatic with distinct basophilia displaying some features of pyknosis; while some nuclei were partially fragmented showing karyorhexis. Also there were focal areas of necrosis, cellular degeneration that has resulted in cell death.

The cytoplasm contained large vacuoles (vacuolar degeneration) and small tiny vacuoles (hydropic degeneration). These vacuoles have no definite borders. Ibrahim et al\cite{23} (2010) reported the histopathological structure of the experimental group showing hepatic and local hemorrhage as well as necrosis of hepatocytes with pyknotic nuclei after 4 weeks of the treatment with monosodium glutamate. Mosaibih\cite{18} (2013) found similar histological changes such as vacuolar degeneration of hepatocyte cords, nuclei pyknosis and congestion of blood vessels in addition to some hepatocyte lysis and blood vessels fibrosis. Waer H. F. & Edress S\cite{21} (2006) observed that the deleterious effects of Monosodium Glutamate were dose related and cumulative. That varied considerably from moderate structural changes to cytoplasmic lysis and the signs of degeneration of cellular organelles. Ortiz GG et al\cite{24} (2006) also reported similar findings of degenerative changes in liver parenchyma.
6. Dilated sinusoids
In our study, varying degree of sinusoidal dilatation in group B tissue was mild, but in group A there were moderately dilated sinusoidal spaces which contained blood cells. The endothelial lining of blood sinusoids suffered from erosion. This result is in accordance with that by Bhattacharya et al\(^6\) (2011). They found that areas around central vein were mostly affected. The hepatic cords were disrupted, dilated sinusoids; prominent Kupffer cells with accumulation of particulate matter. Qudsi et al\(^{25}\) reported that liver seemed to have less cell density and a dilation of venous canals and blood sinusoid. Fibrosis, hemorrhage and congestion were seen in the central and portal veins.

7. Collagen fiber around vessels
In our study increased collagen fibers around vessels were observed in rat liver of group C. Waer H. F. & Edress S\(^{21}\) (2006) found moderate increase in connective tissue around the portal area after giving a daily dose of Monosodium Glutamate (60mg/1000g) for one month. Mosaibih\(^8\) (2013) observed blood vessels fibrosis. Nakanishi et al\(^{17}\) (2008) also reported that there were mild to moderate necrosis of the hepatocytes in the periportal areas and mild fibroplasias.

8. Carbohydrate content
In histochemical examination of general carbohydrate contents of the hepatic tissue indicate the occurrence of a gradual decrease in carbohydrate (glycogen) content in hepatocyte. There is decrease PAS +ve substance in group B and absent in group C. In an earlier study on the long term effect of Monosodium Glutamate on histology of mice liver; Bhattacharya et al\(^6\) (2011), reported similar findings. Inuwa H M et al\(^{26}\) (2011) reported that there is marked reduction in carbohydrate content of tissue after the treatment with Monosodium Glutamate.

Body Weight of Animals
Our study revealed that at the different doses of Monosodium Glutamate, there was a dose dependent increase in body weight. A number of studies (Inuwa et al\(^{26}\) (2011), Rogers PJ et al\(^{27}\) (1990), Tawfik and Al-Badr\(^{28}\) (2012) have examined the potential link between Monosodium Glutamate and body weight. There have been speculations that people tend to eat more food with Monosodium Glutamate because it just tastes better than they would. Rogers PJ et al\(^{27}\) (1990) discovered a connection between Monosodium Glutamate and human appetite and suggested that monosodium Glutamate influences the appetite positively, and induces weight gain.
CONCLUSION

The observations and results revealed that after administration of Monosodium Glutamate in different doses orally for 45 days in group B and Group C animals their body weight show significant gain as compared to the body weights of animals in control group A.

The findings of the experimental study also revealed that the deleterious changes were severe with increased doses of the treatment. The cytoarchitecture of the liver tissue shows gross disturbances in form of the reduction in relative liver weight due to atrophy of liver tissue. The weight gain levels in study group shows that the intervention of the drug has significantly contributed to the weight gain in the study animals, higher the dose of treatment, more will be the weight gain.

REFERENCES


