Long term effect of monosodium glutamate in liver of albino mice after neo-natal exposure

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ABSTRACT

Mono Sodium Glutamate (MSG) is a naturally occurring excitatory neurotransmitter. It is extensively used as a food additive and flavoring agent for its UMAMI taste. Simaltaneously it is being implicated for varied pathological condition like obesity, gonadal dysfunction, learning difficulty etc. It produces oxygen derived free radicals and metabolized in liver. Neonate mice are sensitive and suffer from adverse effects. Present work was undertaken to study the long term effects on histology of liver following MSG injection in neonates. The changes in the liver parenchyma of 75 days old mice showed variable changes. Areas around central vein were most affected. The liver cords were disrupted, dialated sinusoids, prominent Kupffer cells with accumulation of particulate matter.There were inflammatory cells around central vein. The hepatocyte cell membrane were disrupted, cytoplasm vacuolated, nucleus were pyknotic. Even the normal looking cells showed depletion of PAS +ve material in the cytoplasm.The long term effect on histology showed moderate and patchy hepatocellular damage.

Keywords: Monosodium glutamate, umami taste, free radicals, neurotransmitter, hepatocellular disruption.

INTRODUCTION

Mono Sodium Glutamate (MSG) is one of the most popular flavouringagent of modern time. It increases the perception of sweetness and saltiness and diminishes the sourness and bitterness of food. It is widely used in many commercial packed food (Maggi Noddles, Knorr Soup etc), restaurant and household cooking. The unique flavour and taste of this compound has been categorized and established as a separate taste sensation UMAMI taste.[1]

MSG is a naturally present excitatory neurotransmitter in brain, mediating fast synaptic transmission in one third of all CNS synapses. It is metabolized in liver. Kidney plays an important role in its elimination.[2] The daily turnover is 48 gm whereas daily intake is 24gms. Glutamic acid is transformed into alanine in intestinal mucosa and lactate in liver.[3] 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.[4] Subsequently it was documented that MSG produces oxygen derived free radicals.[5] It is reported that MSG causes disturbances of central endocrine axis

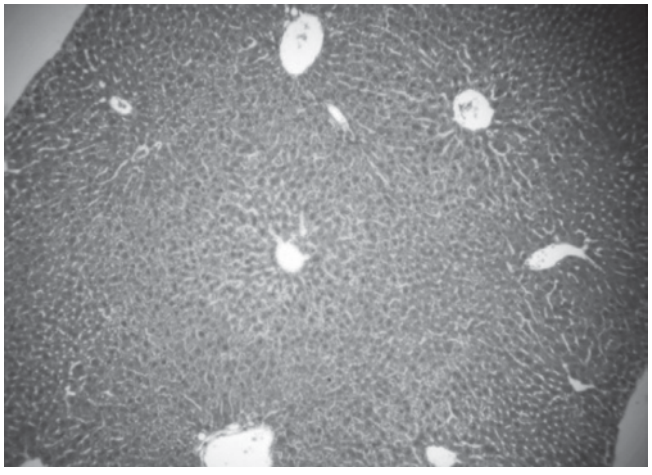


Fig. 1. Photomicrograph of a section of liver of control animal showing radiating cords of hepatocytes, anastomosing network of sinusoid in between them (H/E, X100)

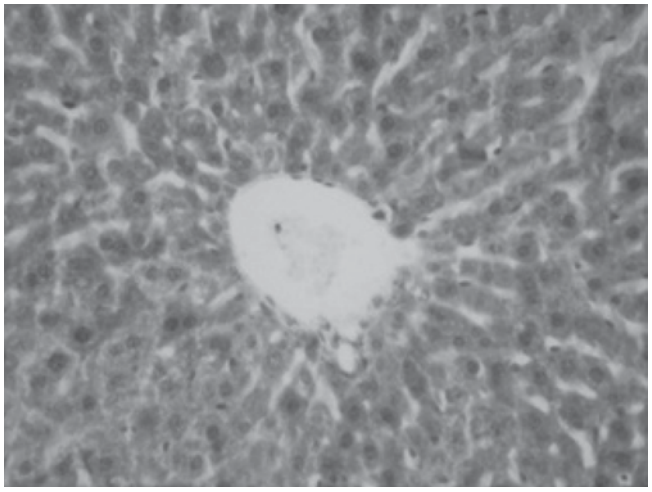


Fig. 2. Photomicrograph of a section of liver of control animal showing uniform distribution of PAS positive hepatocytes around central vein (PAS, X 400).

affecting wide areas of body causing learning difficulty,[6] obesity[7] and gonadal dysfunction.[8] Its neurotoxicity was established by many workers.[9] Whileother researchers using MSG experimentally could not establish the triads of Chinese restaurant Syndrome.[10]

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 reported that Glutamate was safe in adults as well as in infants when consumed with food.[11]

Aims and objectives

Since there were a large number of documents available about toxic effects of MSG particularly in children, yet few observations had been recorded on the changes occurring in liver and kidney following MSG administration. Hence, present study was undertaken to see the long term effects on histology of liver in albino mice when MSG was administered during neonatal period.

MATERIALS AND METHODS

Permission for the study was obtained from institutional ethical committee. Five adult male and 15 adult female mice were procured from institutional animal house. They were kept in standard mice cages in a ratio of 1:3::M:F. with water and food adlibitum. When pregnancy was confirmed the female mice was isolated. Litter derived from these pairings were grouped in two (A and B) of 25 pups in each group irrespective of sexes. They were left with their mothers for first 28 days after that they were separated and kept in separate cages. Group A was taken as control while group B was taken as experimental animal to whom MSG was administered subcutaneously. The pups were weighed on 3rd,5th,7th,9th

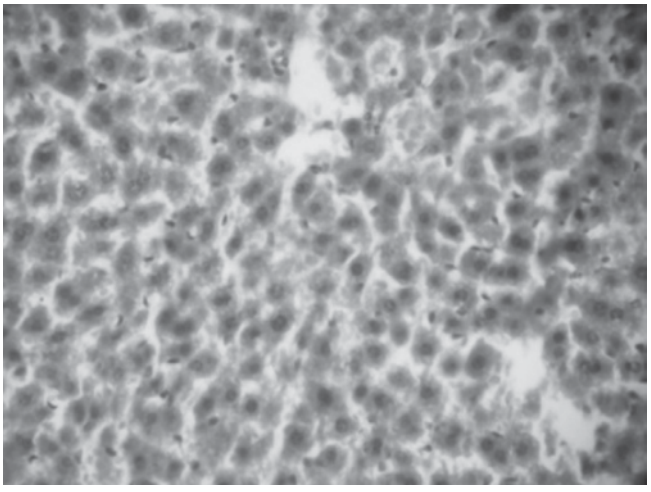


Fig. 3. Photomicrograph of a section of liver of experimental animal showing disruption of cords and sinusoidal network with degenerative changes in the form of atrophy of cells and disintegration of nuclei(H/E, X400).

Table-1: Growth Records of control animal

Ser.No.	Sexof Animal	Wt in gmOn day 28	Wt in gOn day 75
1	M	12.5	26.5
2	M	12.5	26
3	F	11.2	26.5
4	F	11.2	25
5	F	11.2	24.5
6	M	11	29
7	M	13.8	27
8	M	13	27
9	M	12.5	26
10	F	12	25.5
11	M	12	29
12	M	13.8	28.5
13	M	13.5	28
14	M	13.4	27
15	F	12	26
16	M	11	25.5
17	M	11	27
18	M	12	26.5
19	M	12	26.8
20	F	11	23.5
21	F	10.5	23.5
22	F	10.5	23.5
23	F	12.5	26
24	F	12.5	26
25	M	12.4	28

Mean weight of pups on 28 days: 12.04 gm., Mean weight of pups on 75 days: 26.47 gm.

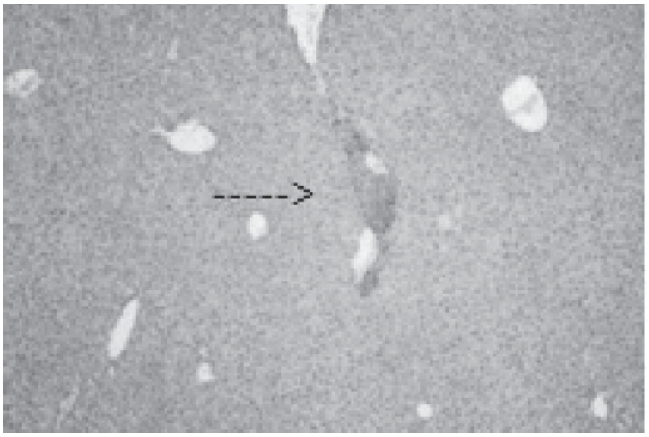


Fig. 4. Photomicrograph of a section of liver of experimental animal showing infiltration of inflammatory (arrow cells in portal area(H/E, X100)

Table-2: Growth records of experimental animal

Ser.No.	Sex of Animal	Wt in gmOn day 28	Wt in gOn day 75
1	M	14	29
2	M	13.5	28.7
3	F	13.5	27
4	F	12.5	27
5	F	13.5	26.1
6	M	14	29
7	M	13	28
8	M	15	29
9	M	15	28
10	M	15	28
11	M	14	27.5
12	M	15	24
13	M	15	28
14	M	12	26
15	M	14	26.5
16	M	14	26.5
17	M	13.5	26.5
18	F	13.5	24
19	F	15	23
20	F	15	26
21	F	14	26
22	F	14	28

Mean weight of pups on 28 days: 13.36 gm., Mean weight of pups on 75 days: 26.9 gm.

and 11th day and MSG were injected as 2mg/gm of b.w on those days in experimental group while the control group of pups received same volume of distilled water only.

Preparation of solution: Four gm MSG was dissolved in 100ml of distilled water so that.05 ml of water contains 2mg of MSG. This solution was injected according to wt of pups so that each of the pups will receive 2mg/gmb.w MSG.[12] Three pups died in experimental group whereas in control group there was no mortality.

Sacrifice of animals and collection of tissues: These grown up pups were aneasthetised on 75th day with a dose of Thiopentone Sodium [50 mg/kg of b.w intraperitoneal].[13] Liver tissues were collected after perfusion with normal saline during anaesthesia. Tissues were processed in a routine manner[14] after formalin fixation and paraffin embedding. Six micron thick sections were cut and stained with H/E and PAS stain.

Morphometric study: Calibration of ocular micrometer: The length of each division of linear ocular micrometer (graticle) was calibrated by first coinciding the ocular micrometer with a stage graticule under 100X objective lens. The length of each division of stage micrometer was 1 micron,30 divisions of stage micrometer coincided with 20 divisions of ocular micrometer, therefore 1 division of ocular micrometer measured 1.5 microns, when projected on stage.

Diameter of nuclei of hepatocytes ware measured with the help of linear ocular micrometer under oil immersion objective. Diameters of 200 nuclei of each animal were measured taking 10 cells randomly in every 5th section. Binucleate hepatocytes were not taken in account and care was taken to avoid repetition of the same cell.

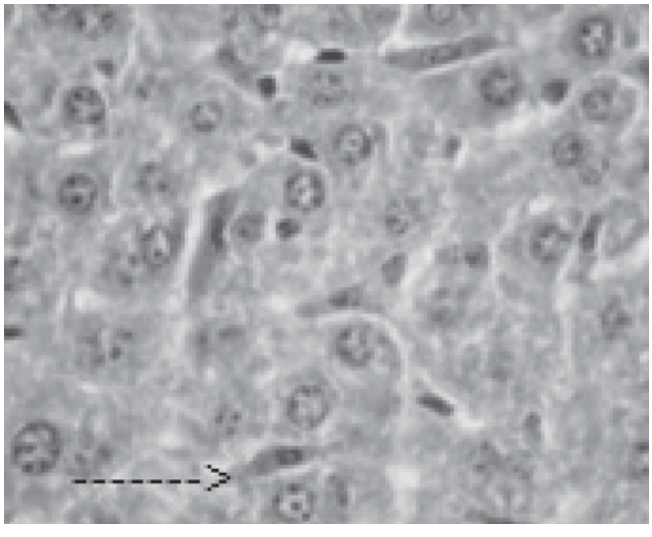


Fig. 5. High power photomicrograph of a section of liver of experimental animal showing prominent Kupffer cell (arrow) distinctly seen because of presence of brownish granules (H/E, X400)

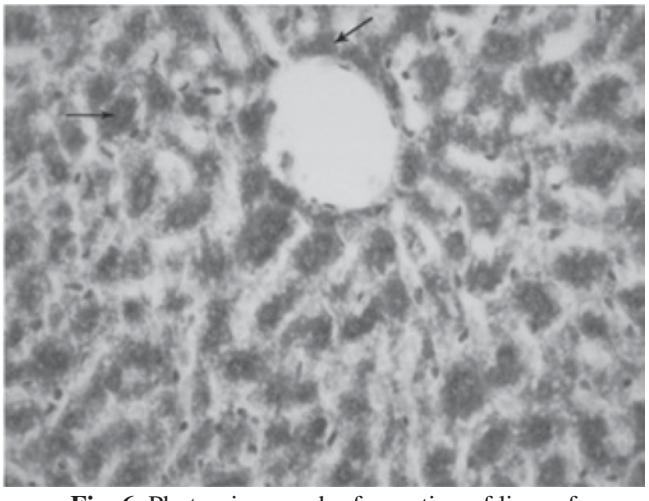


Fig. 6. Photomicrograph of a section of liver of experimental animal showing diminished PAS positivity in hepatocytes, PAS positivity is slightly more in hepatocytes around central vein (arrow) (PAS, X 400).

Table-3: Morphometry of the diameter of nuclei of hepatocytes of control animal

Sr. No.	4.5 µ	46	46	9 µ	> 9 µ		Sr. No.	4.5 µ	6 µ	7.5 µ	9 µ	> 9 µ
1	46	56	38	32	2		15	42	60	64	32	2
2	38	60	48	28	6		16	42	60	58	36	4
3	48	56	46	32	4		17	46	56	62	30	6
4	46	60	48	28	4		18	36	58	66	32	8
5	48	58	42	34	2		19	34	64	60	34	8
8	42	54	44	30	6		20	44	68	48	34	6
7	44	58	40	28	4		21	36	60	66	34	4
8	40	70	50	24	6		22	48	54	64	30	4
9	50	60	48	26	4		23	48	56	60	30	6
10	48	56	60	32	4		24	38	52	68	36	6
11	46	72	50	28	4		25	48	58	54	32	8
12	44	74	48	30	4		Total	1081	1518	1508	773	120
13	40	72	54	30	4		%	21.62	30.36	30.16	15.46	2.4
14	39	66	60	31	4		Total Nuclei studied (5000)					

Diameters were measured in two axes, longitudinal and transverse, at right angles to each other.

Observation:

Behavior and attitude of animals: Control group: During the study period no pups died in control group. Pups were very active, playful and explorative in nature. They maintained steady weight gain pattern; mean weight 28 days was 12.04gm and on 75 days 26.47gm (Table-1).

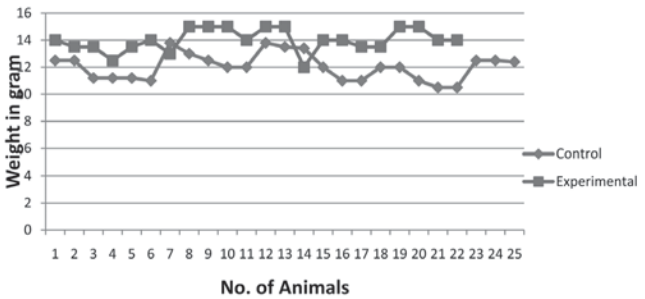
Experimental group: The three pups died in this group during this period. Pups were lazy, showed less limb movement. They were not inquisitive in nature and usually remained confined to a corner of the cage. Weight gain of these pups upto 28 days was rapid and more than that of the control group pups, but in subsequent days the gain in wt was not as marked. At 28 days the mean weight was 13.36gm and while it was 26.9gm on 75 days which were similar to that of the control group of pups (Table-2).

Histology

Control group: Histology of liver in control group of animals revealed normal vertebrate histology with cords of hepatocytes around central vein separated by sinusoids.[15] Classical hepatic lobules were not seen though portal triads were visible at peripheral part of lobule Fig. 1. Cords around one central vein were continuing into cords around other central vein without any connective tissue barrier. Cytoplasm of hepatocytes was eosinophilic and granular. Few vacuoles were seen under high power. With PAS staining, PAS positive material was seen in the cytoplasm. These were uniformly distributed in the cytoplasm of the hepatocytes (Fig. 2).

Experimental group: In MSG treated group, histological feature of hepatic parenchyma were variable in different parts. At places though radiating cords of hepatic cells were observed, the hepatocytes lost their histological features with disruption of cell membrane, small and pyknotic nuclei (Fig.3). Strangely, some of the hepatocytes nuclei were even larger than that of largest nuclei of control group (larger than 9 micron) [Table-4]. There were increased vacuolations in the cytoplasm (Fig. 3). These changes were not uniform. They were more pronounced around the central vein. Kupffer cell could be identified because of accumulation of pigment granules (Fig. 5). Sinusoids were more dilated and apparently more Kupffer cells were visible.An important feature of this is presence of inflammatory cells in majority animals around central vein

Graph. 1. Comparison of weight of control and experimental mice on 28th day



Graph. 2. Comparison of weight of control and experimental mice on 75th day

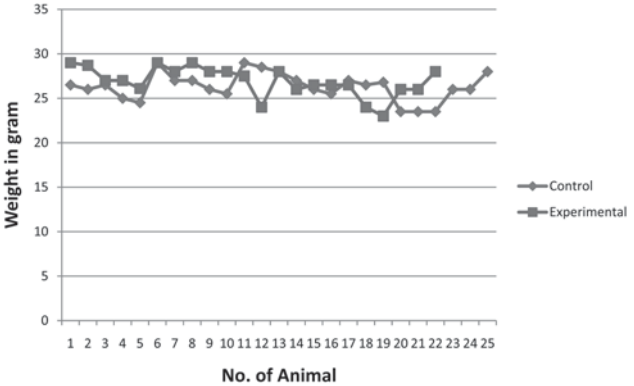


Table-4: Morphometry of the diameter of nuclei of hepatocytes of experimental animal

Sr. No.	4.5 µ	6 µ	7.5 µ	9 µ	> 9 µ		Sr. No.	4.5 µ	6 µ	7.5 µ	9 µ	> 9 µ
1	35	60	60	30	15		15	34	52	54	40	20
2	38	56	56	40	10		16	44	48	48	32	28
3	30	54	54	42	20		17	44	47	49	36	24
4	42	53	53	32	20		18	40	50	52	38	20
5	28	58	58	36	20		19	38	53	51	36	22
6	40	55	55	32	18		20	36	51	51	40	22
7	26	58	58	36	22		21	38	52	50	36	24
8	36	55	52	31	26		22	42	50	50	32	26
9	36	58	57	33	16		Total	829	1166	1167	772	466
10	38	52	55	38	17		%	18.84	26.5	26.5	17.54	10,59
11	46	50	50	36	18		Total Nuclei studied(4400)					
12	34	52	52	32	30							
13	44	52	52	30	22							
14	40	50	50	34	26							

(Fig. 4).Though some hepatocytes seemed normal under H/Estaining. When studied under PAS staining, there was a distinct loss of PAS +ve material (Fig. 6).

Quantitative Observation: While we measured the diameter of nuclei of hepatocytes in both control and experimental group we met with an interesting observation. Nuclei of hepatocytes in control group of animals were generally bigger with maximum number having size of 6 micron to 7.5 microns (30.36% and 30.16%), maximum being 9 micron (Table-3). Only 2.4 were larger than 9 micron.

In experimental group though maximum number of nuclei were between 6-7.5 micron, they were far less compare to that of control group, i.e. 26.5% and 26.5% respectively. Strangely the number of nuclei having more than 9 micron diameter was more in number, i.e.10.6% in this group (Table-4).

DISCUSSION

MSG gained popularity in first half of twentieth century as taste enhancer but at the same time doubts were raised about MSG as a causative agent of Chinese Restaurant Syndrome. Extensive research were carried out on different types of animals including human to clear the doubts. It was observed that infant mice on account of poorly developed blood brain barrier showed neurological lesion even when MSG was given in lower dose.[16]

Different workers used different routes of administration though subcutaneous and intraperitoneal injections were the most preferred routes.[17] A maximum intake of 60 mg/kg b.w is regarded safe for human consumption.[18] Some researcher reported that MSG taken with food showed no adverse effect.[19]

Easy handling, early sexual maturity, large litter size and high sensitivity to MSG etc made swiss albino mice preferred animal.[16] The experiment can be repeated easily hence we selected Swiss mice as the experimental animal.

We used subcutaneous route in a dose of 2mg/gm of b.w on alternate days of 5 doses starting from 3rd day. This dose schedule was much less than the lethal dose of 6.9gm/kg of b.w I.P route.[20] Others also administered 2mg/gm of b.w. (subcutaneous) for 5 days after birth, almost the same dose schedule as ours.[12] We made a dose schedule of alternate day to avoid acute toxic effect. The weight records of experimental animals showed more gain in weight up to 28thday (13.97gm in experimental group compared to that of control group i.e. 12.04gm (Graph-1) and indication of tendency to obesity. But by 75th day the weight of both groups of animals were almost same 26.9gm in experimental group and to 26.47gm in control group (Graph-2) possibly due to organ failure and shrinkage of organs in the experimental group. Similar observations were also recorded by others.[12]

In the present study the liver of experimental animal showed change in histological pattern in the form of disruption of hepatic cords, presence of inflammatory cells around central vein with uneven sizes of nucleus in hepatocytes. Not many workers have observed detailed histological features as a long term effect of MSG in liver. Eweka reported distruption of architecture of liver and evidence of hepatocyte degradation and hypertrophy as a response of oral MSG in adult wister rats.[21] They also noted presence of haemolysed RBCS in central vein and hemorrhagic necrosis in centrilobular are similar to us. Ortiz reported elevation of SGOT, SGPT with degenerative changes in hepatocyte after a single high dose intraperitoneal injection of MSG in rats.[22] Our observation of more damage of the liver architecture of zone 3 of acinar conceptwere reported by others, who used ethanol and nimuselide and found

more changes in zone 3.[23,24] In another article Mehrotra[25] described inflammatory cells around central vein as we have found. They also found large sized nucleus in hepatocytes like we have noticed. We could not found any reason for some unusually larger nuclei inthe liver of experimental group of animals. Our observation of reduced glycogen content of hepatocytes were also reported[26] other researcher after exposure of hepatotoxic agent.

The long term effect of MSGafter neonatal administration even with a low dose showed hepatocellular damage in albino mice.

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