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Mono Sodium Glutamate-induced Damage in Rabbit Retina: Electroretinographic and Histologic Studies

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Abstract: Mono Sodium Glutamate (MSG) is widely used as a flavor-enhancing food additive. It is being added more and more to fast foods, children foods, snacks, soups and many other canned foods all over the world for more than a century. The present work aimed to investigate the effects of high dietary intake of MSG on the rabbit retina. MSG was given to rabbits in drinking water in three different concentrations : 1,2 and 4 g/ kg b.wt. day, for 3 groups respectively in addition to a control group. The retina was investigated by the Electroretinogram (ERG) and light microscopy after 3, 6 and 12 months in each group. The adverse effects of MSG in a dose of 1g /kg/day were observed after three months by The ERG (significantly delayed reduced b-wave& delayed a-wave). Histological changes appeared after six months. The electroretinographic and histopathological changes were related to the dose and duration of MSG intake. The dose of 4g/kg MSG, showed extensive retinal damage and marked ERG attenuation. In conclusion, MSG has many unwanted effects on the rabbit retina when they are ingested in the examined doses and the use of it as a flavour enhancer should be minimized below 1g/kg/day. Further studies are required to evaluate the effects of MSG intake below this level.

Key words: Monosodium Glutamate · Electroretinogram · Retinal Toxicity · Experimental Animals

INTRODUCTION

Glutamic acid, which is also termed glutamate, is an acidic dicarboxylic α-amino acid ($C_5H_9O_4N$)), one of the nonessential amino acids found in proteins. Glutamate has many roles in body function, among these roles; it plays important roles in regulating gene expression, cell signaling, antioxidative responses and immunity. Additionally, glutamate is a major metabolic fuel for the small intestine and it, along with glycine, regulate neurological function [1].

All meats, poultry, fish, eggs, dairy products, tomato and some protein-rich plant foods are excellent sources of glutamic acid. Hydrolyzed protein such as yeast extract and many fermented or aged foods, including soy sauce, fermented bean paste also serve as sources. Ninety-five percent of the dietary glutamate is metabolized by intestinal cells in a first pass [2].

Glutamate is a main constituent of dietary protein, it also consumed in many prepared foods as an additive in the form of monosodium glutamate (MSG) the flavor contributions made by MSG was only scientifically identified early in the twentieth century. Brown crystals left behind after the evaporation of a large amount of Kombu broth was identified as glutamic acid. The flavor sensation of MSG is unlike that of any of the other four or five basic flavor sensations of sweet (sucrose), sour (lemon juice), salt (sodium chloride), bitter (quinine, or pungent mustard or chili peppers). The flavor sensation of MSG is often described as “meaty” and has been given the name “umami” [3].

The issue of adverse reactions to MSG was considered over the past 40 years Dietary MSG has been reputed to induce a variety of unwanted effects in humans, including sweating, muscle pain and fatigue, headache, skin reactions and asthma [4].

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Simultaneously it is being implicated for varied pathological condition like obesity, gonadal dysfunction, learning difficulty and production of free radicals in liver [5, 6].

While many researchers reported many unwanted side effects of MSG, other groups claimed that it is safe to be added to human foods even in unphysiologically high doses [7,8]. Therefore the purpose of the present study is to evaluate the effects of dietary MSG on the retina in albino rabbits.

MATERIALS AND METHODS

Chemicals and Drugs: MSG was purchased from The French Company for food industries, 6th of October City, The industrial zone, Cairo, Egypt. Ketamine hydrochloride vials (Ketamar®, Amoun, Egypt); Lignocaine hydrochloride vial (Xylocaine®, AstraZeneca, Sweden); Tropicamide 1% eye drops (Mydriacyl®, Alcon, Belgium);. Glutraldhyde (Boehringer-Ingelheim, germany).

Animals and Experimental Design: Thirty six male New Zeeland albino rabbits weighing 2.5-3 Kg were used in the study. They were housed individually in separate cages under veterinary supervision. They were used in accordance with institutional guidelines and with the statement for use of animals in Ophthalmic and Vision Research. The rabbits were fed with the standard diet and water and kept in 12 hours dark/light cycles under controlled temperature and humidity. Animals were divided into four groups each consisting of nine rabbits with a total of 18 eyes in each group. Group I, consists of 9 rabbits received tap water and served as controls. Group II, consists of 9 rabbits received MSG (dissolved in water) by stomach tube in a dose of 1g/kg b.wt./day in divided doses two times /day. Group III, consists of 9 rabbits received MSG in a dose of 2g/kg b.wt./day. Group IV, consists of 9 rabbits received MSG in a dose of 4g/kg b.wt./day.

The duration of the experimental work was about one year. The animals were subjected to electrophysiological examinations after 3, 6 and 12 months. Each period (3,6,&12 months) three rabbits from each group were sacrificed for histological examination. The animals of group IV were examined only after three months because of the high mortality rate, as many rabbits died after three months.

Electrophysiological Tests: Electroretinogram (ERG) using the Italian EREV 99 system (for recording and analysis by averaging) was performed before the study to establish baseline standards. The animals were anesthetized intravenously using lignocaine hydrochloride (5 mg/kg) and ketamine hydrochloride (50 mg/kg). The rabbits were then dark adapted for at least 30 min after pupillary dilatation. The active electrode was placed near the margin of the lower eyelid; the reference electrode was placed on the forehead and the earth electrode was clipped to the earlobe. Recording of combined response was carried out using white flash stimulus having frequency of 1 flash/second, energy of 2 joules and no background intensity. Amplitudes were measured from baseline to the lowest point of the negative peak for the a-wave and from the latter (or baseline, if absent) to the positive peak for the b-wave. The ERG plots the changes in the electric signal attained from the recording electrodes in microvolt (µv) on the y-axis, against the time of recording in milliseconds (ms) on the x-axis. The used Electroretinogram (*EREV 99*) can only print the recorded data using a dot matrix printer. The printouts are then optically scanned and transferred to the computer.

Histological Examination: After ERG testing, the animals were sacrificed and the eyes were enucleated, then, the retinas were dissected and fixed in 2.5% glutraldehyde. The specimens were postfixed in 1.33% osmium tetroxide, dehydrated in graded alcohols and embedded in epon semi-thin sections (1-2 µm) were cut and stained with toludrine blue for examination by light microscope.

RESULTS

Electrophysiological Tests: The ERG parameters of group I (Controls) showed normal values which were not significantly changed all through the duration of the experiment. The ERG and Histological results for group IV were obtained within the first 3 months. No data could be attained after that time because of the death of all animals of group IV.

After three months of MSG intake in a dose of 1g/kg/day (group II) (Fig. 1, Table 1), the ERG recordings showed a significant delay in the a-wave and the b-wave peak latency and a significant reduction in the b-wave amplitude with no change in the a-wave amplitude when compared to the control group.

Table 1: Mean values (±SD) of ERG amplitudes (µv) and peak latencies (ms) after 3 months in group I (controls), group II (rabbits received MSG in a dose of 1g/kg/day), group III (rabbits received MSG in a dose of 2g/kg/day) and group IV (rabbits received MSG in a dose of 4g/kg/day).

Groups	Group l	Group II	Group III	Group IV
Mean ± SD of a-wave: amplitude.	9.00 ±2.82	8.47 ±2.89	3.65 ±1.34	0.88 ±0.78
& Peak latency	28.04 ±3.09	34.33 ±5.60	33.08 ±7.47	84.70 ±3.38
P_1		0.616	0.000*	0.002*
		0.001*	0.023*	0.000*
P_2			0.000*	0.004*
			0.568	0.000*
P_3				0.000*
				0.000*
Mean ± SD of b-wave: amplitude.	21.38 ±2.63	18.49 ±4.46	11.88 ±3.13	2.25 ±2.21
& Peak latency	45.63 ±6.63	65.39 ±8.87	63.17 ±5.89	114.78 ±6.71
P_1		0.029*	0.000*	0.000*
		0.000*	0.000*	0.000*
P_2			0.000*	0.000*
			0.438	0.000*
P_3				0.001*
				0.000*

Data expressed as mean ±SD, n=18, µv=microvolt; *significant difference at $P<0.05$; P_1 value: compared to group I; P_2 value: compared to group II; P_3 value: compared to group III.

Table 2: Mean values (±SD) of ERG amplitudes (µv) and peak latencies (ms) after 6 months in group I (control), group II (rabbits received MSG in a dose of 1g/kg/day), group III (rabbits received MSG in a dose of 2g/kg/day).

Groups	Group l	Group II	Group III
Mean ± SD of a-wave: amplitude.	9.03 ±2.62	3.93 ±2.29	2.12 ±0.75
& Peak latency	26.91 ±3.21	32.76 ±5.27	36.16 ±6.71
P_1		0.001*	0.000*
		0.005*	0.002*
P_2			0.040*
			0.090*
Mean ± SD of b-wave: amplitude.	21.52 ±2.80	12.05 ±1.98	9.91 ±.98
& Peak latency	47.21 ±9.62	55.83 ±11.69	67.42 ±5.94
P_1		0.000*	0.000*
		0.043*	0.000*
P_2			0.010*
			0.003*

Data expressed as mean ±SD, n=12, µv=microvolt; *significant difference at $P<0.05$; P_1 value: compared to group I; P_2 value: compared to group II.

Table 3: Mean values (±SD) of ERG amplitudes (µv) and peak latencies (ms) after 12 months in group I (controls), group II (rabbits received MSG in a dose of 1g/kg/day), group III (rabbits received MSG in a dose of 2g/kg/day).

Groups	Group l	Group II	Group III
Mean ± SD of a-wave: amplitude.	8.73 ±2.31	2.58 ±1.55	1.43 ±1.20
& Peak latency	26. 40 ±3.16	39.88 ±5.21	64.95 ±3.00
P_1		0.003*	0.000*
		0.003*	0.000*
P_2			0.030*
			0.001*
Mean ± SD of b-wave: amplitude.	20.72 ±2.06	5.63 ±2.05	2.08 ±0.81
& Peak latency	52.40 ±6.68	84.68 ±8.18	108.95±7.85
P_1		0.000*	0.000*
		0.000*	0.000*
P_2			0.008*
			0.004*

Data expressed as mean ±SD, n=6, µv=microvolt; *significant difference at $P<0.05$; P_1 value: compared to group I; P_2 value: compared to group II.

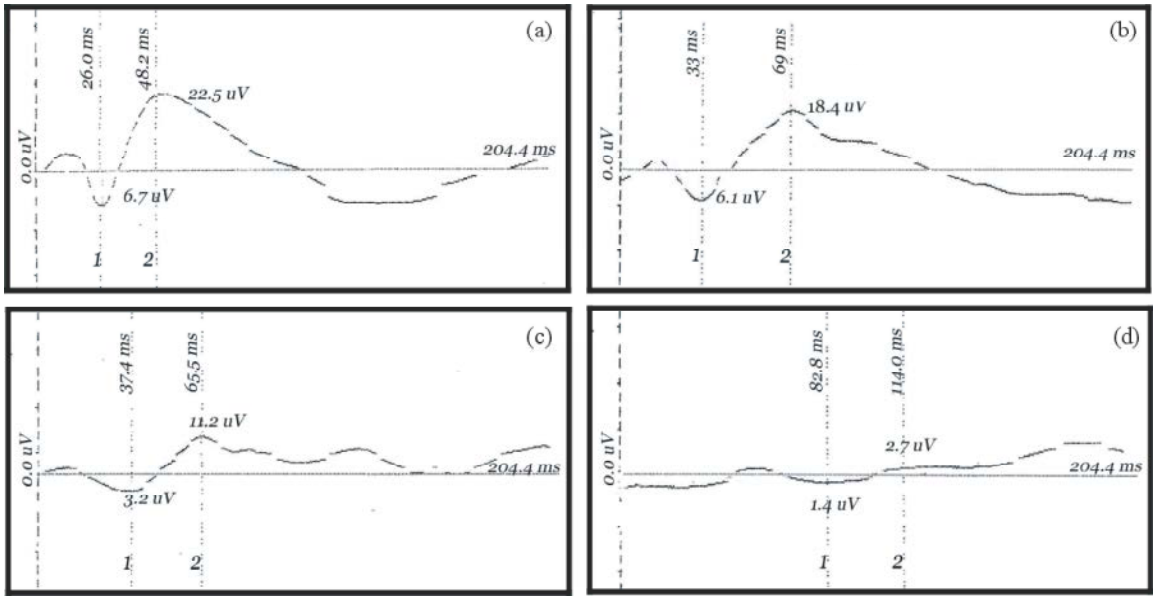

Fig. 1: ERG recordings after 3 months of selected rabbit eyes in: (a) group I (controls), (b) group II (animals received MSG in a dose of 1g/kg/day), (c) group III (rabbits received MSG in a dose of 2g/kg/day), and (d) group IV (rabbits received MSG in a dose of 4g/kg/day). Combined response demonstrated delayed a-wave and delayed subnormal b-wave in group II, reduced a-& b-wave amplitudes and delayed peak latencies in group III, and markedly delayed reduced response in group IV. Cursor 1and 2 points to: a-wave and b-wave respectively.

For a higher dose of MSG (2g/kg/day) in group III, the animals exhibited a significant reduction of both the a- and b-wave amplitudes and peak latencies when compared to the control group. In group IV (animals that received MSG in a dose of 4g/kg/day), the ERG parameters, (a- and b-waves) were severely delayed in their peak latencies and markedly reduced in amplitudes. The response was almost extinguished in 30% of eyes. At the same time, there were significant differences in ERG parameters between the treated groups which were "'dose- dependant"'. The amplitudes of the a-wave and b-wave were significantly lower in group III than in group II and the a and b-wave amplitudes and peak latencies were significantly much lower in group IV than groups II and III.

After six months of MSG intake, the animals exhibited more deterioration of ERG parameters (Table 2, Fig. 2). Also, within groups II and III, the a- and the b-wave amplitudes were significantly more reduced after six months than that measured after three months. Again, after12 months (Table 3, Fig. 3) the a- and b-wave amplitudes and peak latencies reach the lowest values and were significantly more reduced than that after six months in groups II and III. At the same time, significant differences in these parameters were still found among groups.

Histological Examination: Light microscopic examination of semi thin sections from the control retina revealed normal histological appearance of the nine layers of the sensory retina and the supportive pigment epithelium layer (Fig. 4).

Examinations of the retina of rabbits that received glutamate in a dose of 1 g/day (group II) for three months exhibited normal structure. Only mild changes were observed after six months (Fig. 5). After twelve months, the retinal layers were edematous. The cytoplasm of pigment epithelial cells contained numerous vacuoles. The damage extended to the nuclei in the outer and the inner nuclear layers (Fig. 6).

In group III (rabbits that received glutamate in a dose of 2g/kg/day), the retina showed histopathological changes in all layers. After three months, the pigment epithelium showed vaculation of the cytoplasm and numerous dense granules at the periphery of the cell. The photoreceptors showed detachment between outer and inner segments (Fig. 7 a). Many nuclei of the outer nuclear layer appeared diffused with lack of chromatin details. Inner nuclear and inner plexiform layer were less in the thickness. Some cells of the inner nuclear layer were pyknotic and appeared very dense; the ganglion cells were swollen (Fig. 7-b).

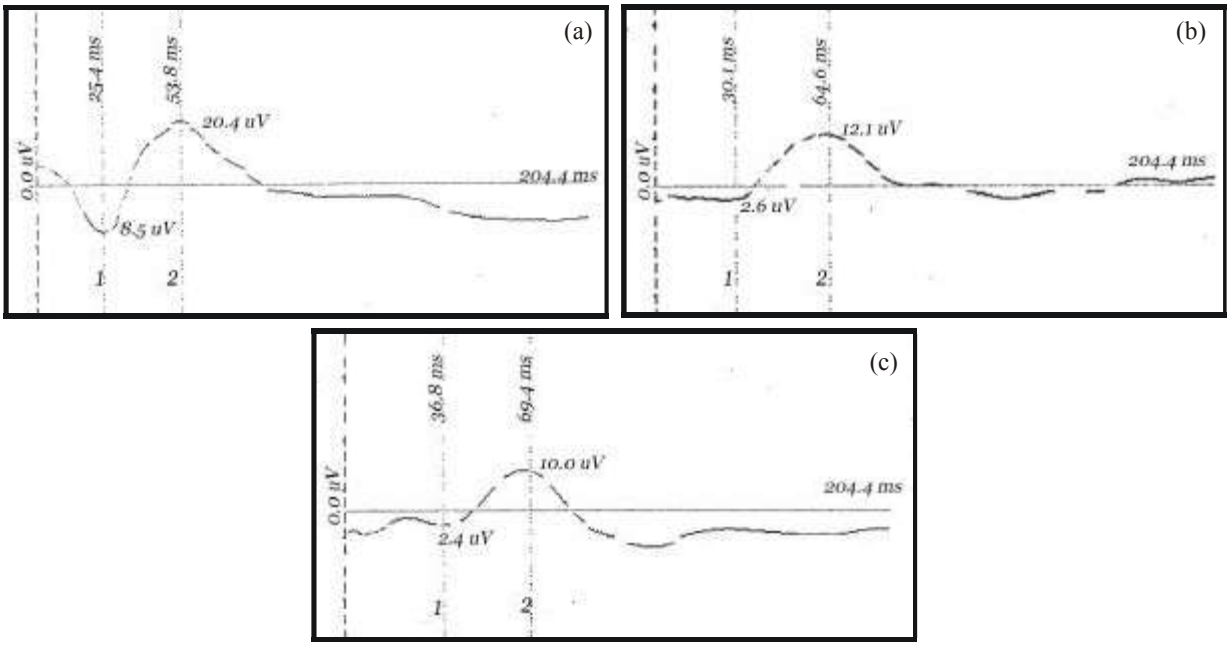

Fig. 2: ERG recordings after 6 months of selected rabbit eyes in: (a) group I (controls), (b) group II (animals received MSG in a dose of 1g/kg/day), and (c) group III (rabbits received MSG in a dose of 2g/kg/day). Combined response demonstrated delayed reduced a-& b-wave in group II, and the response was much more delayed and reduced in group III. Cursor 1and 2 points to: a-wave and b-wave respectively.

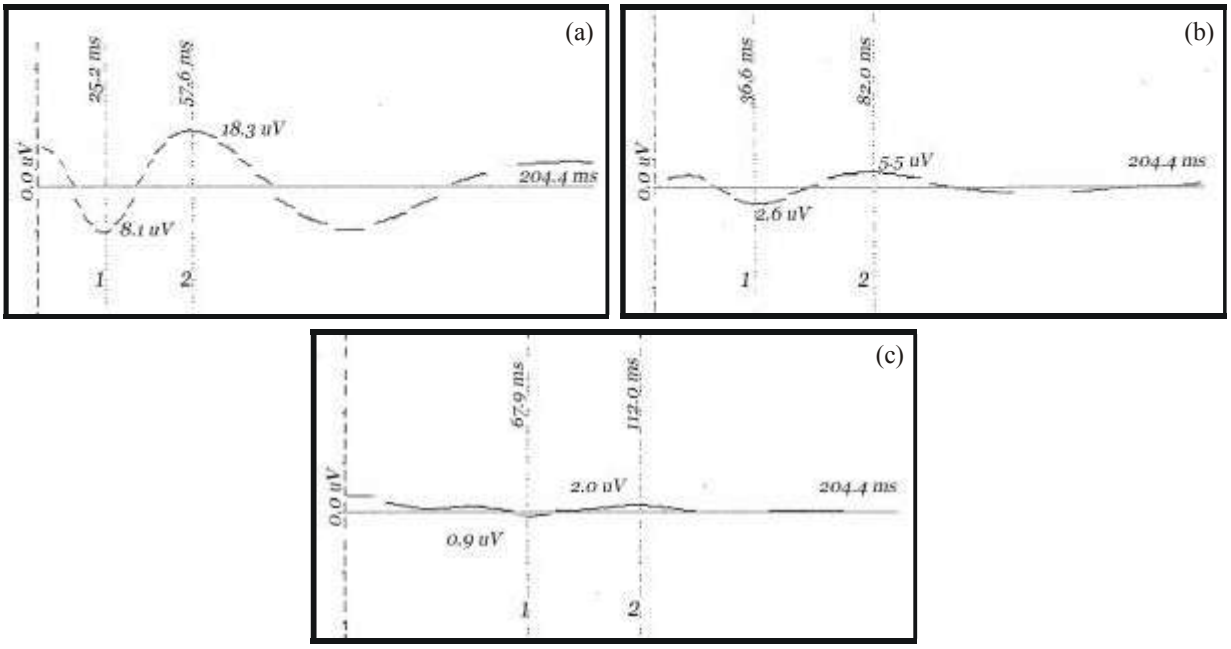

Fig. 3: ERG recordings after 12 months of selected rabbit eyes in: (a) group I (controls), (b) group II (animals received MSG in a dose of 1g/kg/day), and (c) group III (rabbits received MSG in a dose of 2g/kg/day). Combined response demonstrated markedly reduced and delayed a-& b-wave in group II, and minimal response in group III. Cursor 1and 2 points to: a-wave and b-wave respectively.

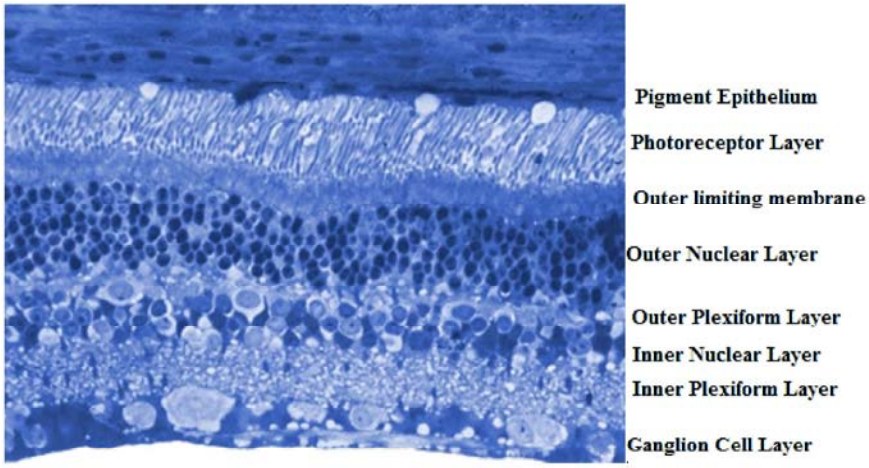

Fig. 4: Light micrograph of a retina representing the control group (taken at the same time with other samples), showing normal appearance of retinal layers [x500].

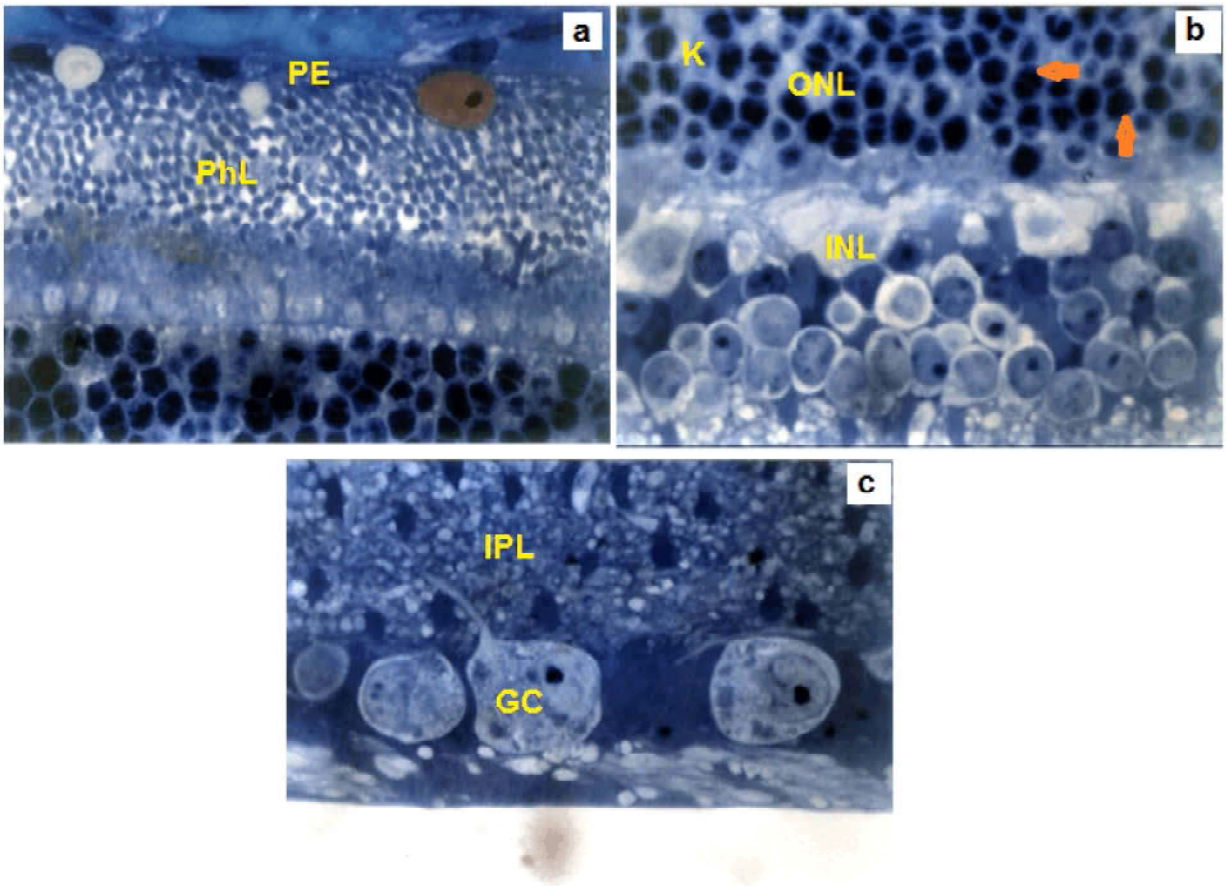

Fig. 5: Light micrograph of rabbit retina of group II after 6 months of treatment showing vacuolation of the pigment epithelium (PE) and slight fragmentation of the outer segments of photoreceptors (Phl) [ax1250]. Some nuclei of the outer nuclear layer(ONL) appear pyknotic (arrow), while others show signs of karyolysis(K). All nuclei of the inner nuclear layer (INL) appear swollen [bx1250]. Inner plexiform layer (IPL) contains many glial cells. Ganglion cells(GC) appear intact [cx1250].

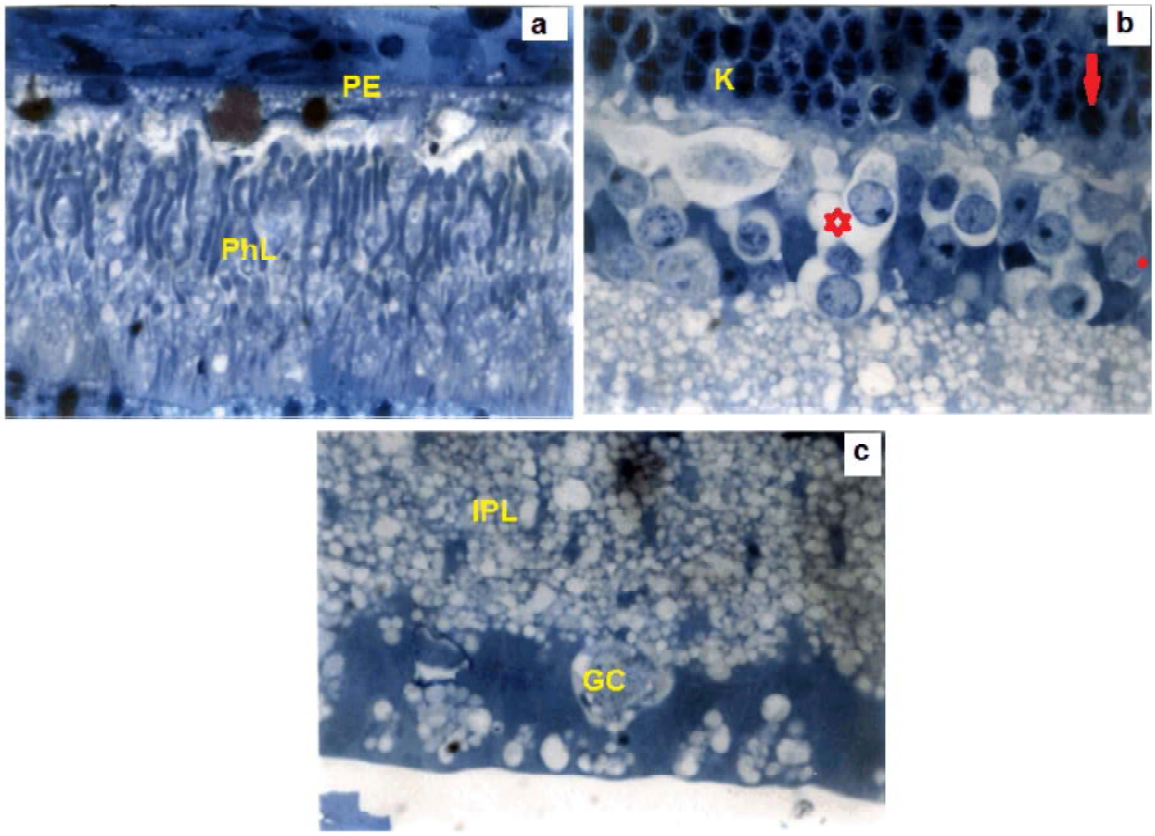

Fig. 6: Light micrograph of rabbit retina of group II(1gm/kg/day) after twelve months of treatment showing vacuolation of the cytoplasm of the pigment epithelium(PE) and damage of the outer and the inner segments of photoreceptors (PhL) [ax1250]. Some nuclei of the outer nuclear layer (ONL) appear pyknotic(arrow), while others show signs of karyolysis(K). All nuclei of the inner nuclear layer (INL) have a characteristic pattern of chromatin. Note, fusion between two or more of bipolar cells(*) [bx1250]. Inner plexiform layer (IPL) appears edematous. Ganglion cells (GC) appear pale [cx1250].

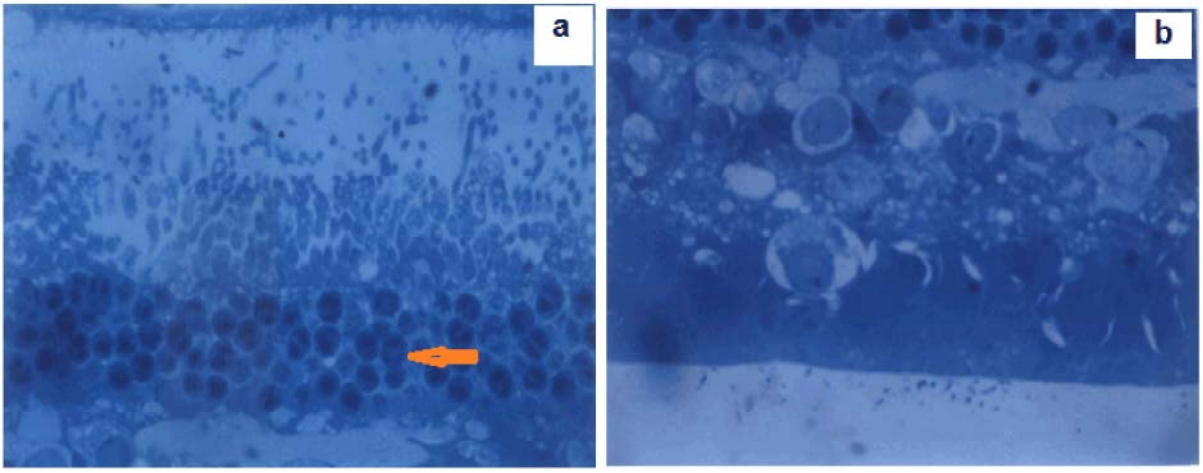

Fig. 7: light micrograph of rabbit retina of group III (2gm/kg/day) after three months of treatment showing extensive damage with severe fragmentation and disorganization of the outer and inner segments of the photoreceptors. Some nuclei of the outer nuclear layer appear pyknotic (arrow) [a x1250]. The inner nuclear and the inner plexiform layers are less in thickness [a x1250].

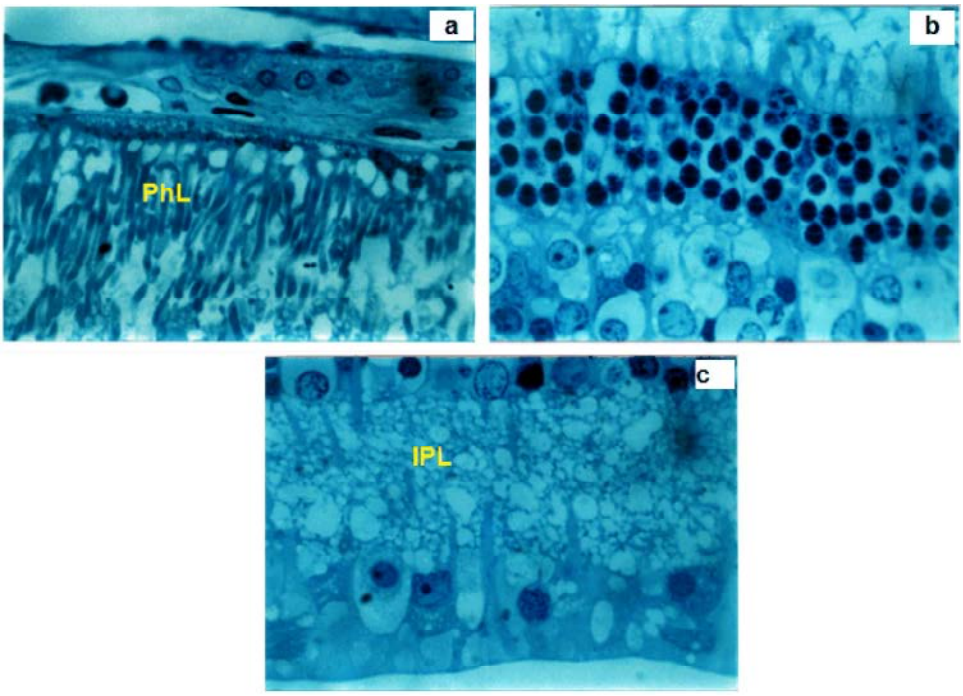

Fig. 8: Light micrograph of rabbit retina of group III (2gm/kg/day) after twelve months of treatment showing severe degenerative changes in the photoreceptors (PhL) [a x1250]. All nuclei of the inner nuclear layer have distinct patches of chromatin. Edema of the inner plexiform layer is seen (IPL) [b &c x1250]

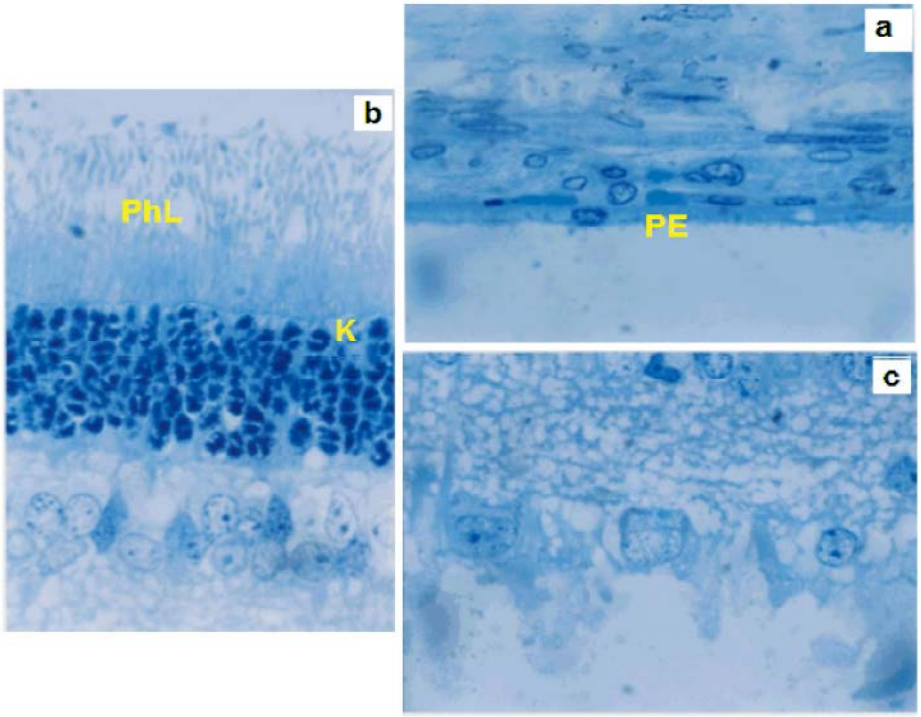

Fig. 9: Light micrograph of rabbit retina of group IV (4gm/kg/day) after three months of treatment showing extensive changes in the retina. There is a detachment between the pigment epithelium (PE) and the layers of the retina [a x1250]. Some nuclei of the outer nuclear layer show signs of karyolysis (K) [bx1250]. Nuclei of the inner nuclear and ganglion cell layers appear swollen and have a characteristic pattern of chromatin [b &c x1250].

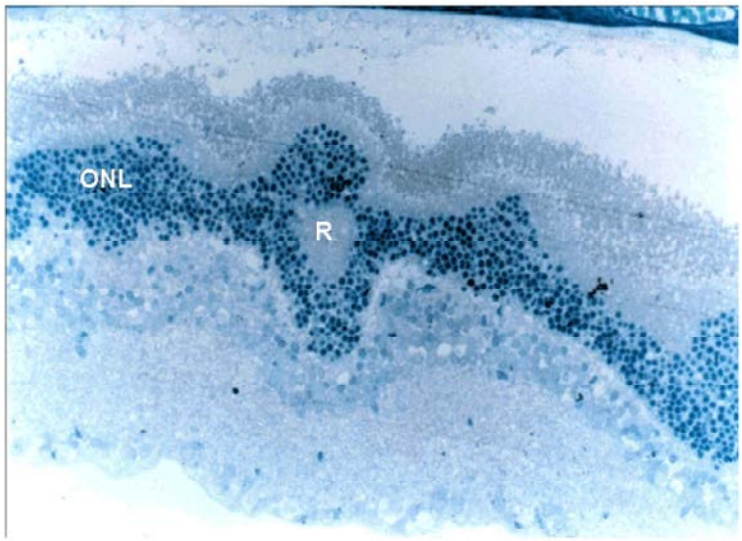

Fig. 10: Light micrograph representing group IV after 3 months showing detachment between the pigment epithelium and the layers of the retina .there is marked folding and bulging of all neuronal layers. The outer nuclear layers [ONL] is involuted and wound with variable thickness from one place to another. In some areas, the outer nuclear is branched with focal rosette formation [R]. The center of the rosette contains degenerated inner segments of photoreceptors [x 125]

Similar changes were observed after six months of treatment. However, after twelve months, the retina showed severe degenerative changes in all layers. The cytoplasm of the pigment epithelial cells contained numerous vacuoles of variable sizes and dense bodies. Nuclei appeared swollen. In addition, the cell membranes of the pigment epithelial cells were ruptured at some regions. The photoreceptors were ruptured and completely disorganized (Fig. 8-a).

Nuclei of the outer nuclear layer showed signs of Karyorrhexis or karyolysis. Nuclei of the inner nuclear layer had a characteristic pattern of chromatin. Some of which showed signs of karyolysis many nuclei were surrounded by a halo of clear spaces (Fig. 8-b). The outer and the inner plexiform layers were edematous. Some nuclei of the ganglion cell layer appeared small and rounded in shape (Fig. 8-c).

It was found that glutamate in a dose of 4g /kg /day (group IV) for three months produced extensive changes in the retina. There was a detachment between the pigment epithelium and the layers of the retina.Nuclei of the pigment epithelium showed condensation of chromatin into small parts. The cytoplasm contained numerous vacuoles of variable sizes (Fig. 9-a). The photoreceptor outer segments were disorganized while the inner segments appeared intact. Some nuclei of the outer nuclear layer showed signs of karyolysis. Nuclei of the inner nuclear layer and ganglion cell layer appeared swollen and had a characteristic pattern of chromatin [Fig.9 b&c]. Inner plexiform and nerve fiber layers were reduced in thickness. In addition inner limiting membrane was irregular in some regions [Fig. 9-c].

In few cases of this group, the retina showed marked changes in the form of marked folding and bulging of all neuronal layers resulting in alteration of the normal organization of the retinal layers. The outer nuclear layer was involuted and wound with variable thickness from one place to another. In some places, an increase in thickness as well as branching of this layer was evident. Moreover, focal rosette formation was observed in some parts of the outer nuclear layer. The center of the rosette contained degenerated inner segments of photoreceptors (Fig. 10).

DISCUSSION

MSG is widely used as a flavor enhancer in many of the processed foods and drinks [9]. At the time of discovery, MSG was thought to be safe since it was a natural substance (an amino acid). Recently considerable attention has been focused on its unusual neurological effects [6,10].

In the present study, the adverse effects of MSG on the retinal function have been revealed after dietary intake for three months. Oral administration of MSG in a dose of 1g/kg/day for three months caused significant electrophysiological changes, while there was no appreciable effect on retinal histology. The b-wave which related mainly to bipolar cell currents and to a less extent to Muller cells showed a significant reduction in its amplitude and appeared with more peak latency. The a-wave which reflects the photoreceptor activity was significantly delayed. Such changes indicate that MSG exerts an early diffuse effect on the retina that begins particularly in the inner retinal layers. As the dose increased to 2g/kg/day for 3 months, retinal toxicity was manifested in histological examination in the form of decreased thickness of the inner nuclear layer, disturbed photoreceptor layer and vacuolated retinal pigment epithelium. These were accompanied with profound decline in a- and b- waves amplitudes and delay in their peak latencies, suggesting the markedly affected inner nuclear layers as well as photoreceptors layer. The histopathological and functional changes increased in severity and became more aggravated after 12 months; the retina showed severe degenerative changes in all layers as shown in the light microscopic images. The light-microscopic findings were correlated with the minimal ERG response in that group.

The neurotoxic effects of MSG were the severest when the dose increased to 4g/kg/day for only 3 months. The ERG parameters were markedly attenuated, reaching extinguished response. By the histological examination, there was retinal detachment, marked folding and bulging of all neuronal layers. These data suggest that prolonged and high level of MSG intake in the diet has accumulative toxic effects on the retina.

The ERG and histological results in this study were in agreement with previous studies demonstrating MSG neurotoxicity. Ohguro and his colleagues [11] found a significant decreased ERG response and a significant thinning of the retinal inner layers in rats receiving excess MSG in diet. In addition, it was reported that subcutaneous injection of MSG into newborn rats caused severe retinal damage especially to the inner layers which correlates with in-vitro MSG cytotoxicity in those cells [12, 13]. Moreover, neurotoxic and gliotoxic effects of glutamate were studied in isolated chick embryo retinas of various ages. It was found that initial cellular changes in retinas were localized to glial Müller cells. However, in the older retinas, an additional lesion was consistently found in the photoreceptor cells [14, 15]. Regarding MSG toxicity in other areas of the brain, neurodegenerative changes have been identified in arcuate nucleus of the rodent hypothalamus [16]. Other neurological disorders have been identified in many experimental animals [17,18].

Glutamate is the neurotransmitter released by neurons in the vertical pathway. It is also the neurotransmitter used by all vertebrate photoreceptor [19]. In addition, bipolar cells make chemical synapses on amacrine cells and ganglion cells through glutamatergic receptors [20] primarily are the KA, AMPA and NMDA receptors [21]. Although glutamate is required for normal brain function the presence of excessive glutamate can lead to neuronal death [22]. The glutamate receptors, particularly the NMDA, could be claimed to be responsible for the toxic effects of MSG on the retina. Extracellular excessive glutamate has been found to stimulate glutamate receptors [23, 24]. Stimulation of NMDA or AMPA/KA receptor channels allow cellular influx of Ca^{2+}, which (in excess) can activate a variety of potentially destructive processes [25]. Excessively high intracellular Ca^{2+} damages the mitochondria. Also, Glu/Ca^{2+} mediates promotion of transcription factors for the pro-apoptotic genes (that are responsible for cell death), or down regulation of transcription factors for anti-apoptotic genes.

Additionally, it has been postulated that both local depletion of Na^{+} and K^{+} (due to the high intracellular Ca^{2+}) as well as small but significant elevation of extracellular Zn^{2+} are factors that can activate both necrotic and pro-apoptotic cascades leading to glutamate induced neuronal death [26, 27]. MSG was also reported to induce oxidative stress. This process is the production of reactive compounds such as hydrogen peroxide and oxyradicals. These reactive species can lead to DNA damage, peroxidation of membrane lipids and neuronal death [28].

In the present study the dietary intake of MSG in a dose of 1,2,4 g/kg/day in rabbits seems to induce glutamate cytotoxicity in the eye. Although immature animals were found to be much more vulnerable to the toxic effects of MSG than older animals, in the present experiment the negative effects of dietary MSG on the eye were largely detected in adult rabbits. The present study suggests that high dietary intake of MSG over a long period of time may lead to accumulation of glutamate in the eye and increases the possibility of its cytotoxicity.

In conclusion MSG has many unwanted effects when it is added to food, the toxic effects on the retina manifested with a dose of 1g/kg/day and the use of it as a flavor enhancer should be minimized below this dose level.

REFERENCES

1. Wu, G., 2010. Functional amino acids in growth, reproduction and health.Adv Nutr (Bethesda), 1: 31-37.
2. Burrin, D.G. and B. Stoll, 2009. Metabolic fate and function of dietary glutamate in the gut. Am. J. Clin. Nutr, 90: 850S-856S.
3. Berkes, E.A. and K.M. Wossner, 2003. Monosodium glutamate. In Food allergy: Adverse reactions to food and food additives, Eds. Metcafe, D.D. H.A. Sampson and R.A. Simon. pp: 343-350.
4. Geha, R.S., A. Beiser, C. Ren, R. Patterson, P.A. Greenberger, L.C. Grammar, A.M. Ditto, K.E. Harris, M.A. Shaughnessy, P.R. Yarnold, J. Corren and A. Saxon, 2000. Multicenter double blind placebo controlled, multiple challenge evaluation of reported reaction to monosodium glutamate. J Allergy Clin. Imunol, 106: 973-980.
5. Bhattacharya, T., A. Bhakta and S.K. Ghosh, 2011. Long term effect of monosodium glutamate in liver of albino mice after neo-natal exposure. Nepal. Med. Coll J., 13: 11-16.
6. Reis, H.J., C. Guatimosim, M. Paquet, M. Santos, F.M. Ribeiro, A. Kummer, G. Schenatto, J.V. Salgado, L.B. Vieira, A.L. Teixeira and A. Palotás, 2009. Neuro-transmitters in the central nervous system & their implication in learning and memory processes. Curr. Med. Chem, 16: 796-840.
7. Fernstrom, J.D., J.L. Cameron, M.H. Fernstrom, C. McConaha, T.E. Weltzin and W.H. Kaye, 1996. Short term neuroendocrine effect of a large oral dose of monosodium glutamate in fasting mail subjects. J Clin. Endocrinol. Metab., 112: 420-424.
8. Beyreuther, K., H.K. Biesalski, J.D. Fernstrom, P. Grimm, W.P. Hammes, U. Heinemann, O. Kempski, P. Stehle, H. Steinhart and R. Walker, 2007. Consensus meeting: monosodium glutamate - an update. Eur. J. Clin. Nutr., 61: 304-313.
9. Rundlett, K.L. and D.W. Armstrong, 1994. Evaluation of free D-glutamate in processed foods. Chirality, 6: 277-282.
10. Beas-Zarate C. M.I. Perez-Vega and I. Gonzalez-Burgos, 2002. Neonatal exposure to monosodium L-glutamate induce loss of neurons and cytoarchitectural alternations in hippocampal CA1 pyramidal neurons of adult rats. Brain Res., 952: 275-281.
11. Ohguro, H., H. Katsushima, I. Maruyama, T. Maeda, S. Yanagihashial, T. Metoki and M. Nakazawa, 2002. A high dietary intake of sodium glutamate as a flavoring (aginomoto) causes gross changes in retinal morphology and function Exp. Eye. Res., 75: 307-315.
12. Hyndman, A.G., 1984. The effects of glutamate and kainate on cell proliferation in retinal cultures. Investigative Ophthalmology & Visual Science, 25: 558-563.
13. Dénes, V., M. Lakk, N. Czotter and R.A. Gábriel, 2011. Precise temporal dissection of monosodium glutamate-induced apoptotic events in newborn rat retina *in vivo*. Neurochem Res., 36: 1464-1474.
14. Casper, D.S., R.L. Trelstad and L. Reif-Lehrer, 1982. Age-dependent effects of glutamate on photoreceptor cells of the isolated chick embryo retina. J. Neuropathol. Exp. Neurol., 41(5): 522-35.
15. Varga, B., K. Szabadfi, P. Kiss, E. Fabian, A. Tamas, M. Griecs, R. Gabriel, D. Reglodi, A. Kemeny-Beke, Z. Pamer, Z. Biro, A. Tosaki, T. Atlasz and B. Juhasz, 2011. PACAP improves functional outcome in excitotoxic retinal lesion: an electroretinographic study J. Mol. Neurosci., 43: 44-50.
16. Krajewski, S.J., M.C. Burke, M.J. Anderson, N.T. McMullen and N.E. Rance, 2010. Forebrain projections of arcuate neurokinin B neurons demonstrated by anterograde tract-tracing and monosodium glutamate lesions in the rat. Neuroscience. 166: 680-697.
17. Brustovetsky, T., A. Bolshakov and N. Brustovetsky, 2010. Calpain activation and Na+/Ca2+ exchanger degradation occur downstream of calcium deregulation in hippocampal neurons exposed to excitotoxic glutamate. J. Neurosci. Res., 88: 1317-1328.
18. Lupinsky, D., L. Moquin and A. Gratton, 2010. Interhemispheric regulation of the medial prefrontal cortical glutamate stress response in rats. J. Neurosci., 30: 7624-33.
19. De-Vries, S.H., 2000. Bipolar cells use kainate and AMPA receptors to filter visual information into separate channels. Neuron, 28: 847-857.
20. Wu, S.M., F. Gao and B.R. Maple, 2000. Functional architecture of synapses in the inner retina: segregation of visual signals by stratification of bipolar cell axon terminals. Neurosci., 20: 4462-4470.
21. Hensley, S.H., X.L. Yang and S.M. Wu, 1993. Identification of glutamate receptor subtypes mediating inputs to bipolar cells and ganglion cells in the tiger salamander retina. J. Neurophysiol., 69: 2099.

22. Lipton, S.A. and P.A. Rosenberg, 1994. Excitatory amino acids as a final common pathway for neurologic disorders. N. Engl. J. Med., 330: 613-622.
23. Xi, Z., D.A. Baker, H. Shen, D.S. Carson and P.W. Kalivas, 2002. Group II metabotropic glutamate receptors modulate extracellular glutamate in the nucleus accumbens. Journal of Pharmacology and Experimental Therapeutics. 300: 162-171.
24. Augustin, H., Y. Grosjean, K. Chen, Q. Sheng and D.E. Featherstone, 2007. Nonvesicular release of glutamate by glial xCT transporters suppresses glutamate receptor clustering in vivo. Journal of Neuroscience, 27: 111-123.
25. Verleye, M., R. Steinschneider, F.X. Bernard and J.M. Gillardin, 2007. Moclobemide attenuates anoxia and glutamate-induced neuronal damage in vitro independently of interaction with glutamate receptor subtypes. Brain Res., 23: 30-38.
26. Gillessen, T., S.L. Budd and S.A. Lipton, 2002. Excitatory amino acid neurotoxicity. Adv. Exp. Med. Biol., 513: 3-40.
27. Frandsen, A. and A. Schousboe, 1992. Mobilization of dantrolene-sensitive intracellular calcium pools is involved in the cytotoxicity induced by quisqualate and N-methyl-D-aspartate but not by 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propionate and kainate in cultured cerebral cortical neurons. Proc Natl. Acad. Sci. U.S.A. 89: 2590-4.
28. Ahlu, W.P., K. Tewari and P. Choudhary, 1996. Studies on the effects of MSG on the oxidative stress in the erythrocytes of adult mail mice. Toxicol. Lett., 84: 161-165.