Original article

Monosodium glutamate induced histomorphometric changes in thyroid gland of adult wistar rat

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Abstract

Monosodium Glutamate (MSG) is widely used as a flavor enhancer throughout the world. MSG contains glutamic acid, sodium and water. Glutamic acid serves as a neurotransmitter vital to the transmission of nerve impulses in many parts of the central nervous system, and in excess it may cause neurotoxicity leading to endocrinal disorders. The present study was conducted to evaluate histomorphometrically the effects of monosodium glutamate on the thyroid gland of adult albino rats. The experimental group was given 4mg/g body weight of monosodium glutamate intraperitoneally for seven days. Controls were maintained. After thirty days of the last dose, all the animals were sacrificed, their thyroid glands were dissected out, processed and sections stained with haematoxylin and eosin (H&E) and Periodic Acid Schiff (PAS) and examined for histomorphometry under Zeiss light microscope and Image Pro-Express Analyzer. The results of the present study showed a significant increase in the body weight of the MSG treated animals, although these animals consumed less food than the controls. A significant increase in the size of the follicles accompanied by an increase in the mean height and area of the follicular cells and decreased colloid in some of the follicles was observed, pointing towards an increase in thyroid gland activity.

Key words: Albino rats, histomorphometric changes, monosodium glutamate, thyroid

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Monosodium Glutamate (MSG) commonly known as Ajinomoto, is a sodium salt of glutamic acid, an amino acid found in nature¹. It was first identified as a flavor enhancer in 1907 by a Japanese professor². It is commercially produced in many countries by a fermentation process using starch, beet sugar, cane sugar or molasses³. MSG is added to the majority of packaged products, like chips, soups, salad dressings, gravies, cold cuts, and various foods served at fast food restaurants. The first report of MSG’s adverse reactions in humans was published in 1968 and was named as ‘Chinese Restaurant Syndrome’ by Dr. Kwok when he noticed burning of back, tightness of face and numbness of back radiating to arm after having meal containing MSG in Chinese restaurant⁴.

Brain lesions and obesity, in mice treated with MSG, was first reported by Olney⁵. Later, several studies have shown increased evidence of obesity, diabetes⁶-⁹, neurotoxic effects resulting in retinal degeneration⁰-¹³, brain cell damage¹⁴-¹⁶, and endocrine disorders¹⁶-²².
The present study was undertaken to investigate the histopathological changes produced in the thyroid gland of adult rats following administration of MSG, as the earlier studies conducted on rats or mice were targeted mainly on neonates.

Materials and methods

Inbred adult male Wistar albino rats weighing 150-200 grams were procured from the animal house of University College of Medical Sciences and GTB Hospital, Delhi after obtaining approval from the ethical committee. The experimental group consisting of ten animals in which 4mg/g body weight of monosodium glutamate dissolved in normal saline was injected intra-peritoneally for seven days. The control group (10 animals) was injected equal amount of normal saline intra-peritoneally for the same period.

All the animals were group housed (12 hour light/dark cycle) with ad libitum access to food and water. The animals were observed for thirty days after the last dose. The weight of both control and experimental animals was recorded on 1st, 7th and 37th day of the experiment and analyzed statistically. All the animals were sacrificed after thirty days of the last dose by perfusion with formal saline under ether anesthesia. The thyroid glands were dissected out and processed for paraffin sectioning. Seven micron thick paraffin sections were stained with haematoxylin and eosin (H&E) and Periodic Acid Schiff (PAS) and examined for digital measurements under Zeiss light microscope and Image pro express analyzer. The data obtained for the central and peripheral region of the thyroid gland was tabulated and analyzed for comparison between the control and MSG treated groups. The statistical analysis was done with independent sample “t” test using SPSS Software (SPSS Inc, Chicago, IL, USA) version 9.

Results

All the animals included in the study, survived well throughout the experiment. The rats did not show any signs of toxicity and no toxicity related deaths occurred among the animals. The animals treated with MSG developed reduced appetite and became slightly sluggish after fifteen days of the beginning of the experiment. At the end of the experiment they appeared more obese than the control animals.

The histological features of the thyroid gland in control rats showed oval or rounded sections of follicles lined by squamous to cuboidal cells resting on the basement membrane and the lumen of the follicle was filled with eosinophilic colloidal substance. Clear cells or parafollicular cells were seen, having round to oval nuclei and pale cytoplasm (Fig 1).

Fig 1. Photomicrograph of the thyroid gland in the control rat showing connective tissue septae dividing the thyroid parenchyma into lobules which are having aggregates of follicles. The peripheral follicles are larger than the central ones which are small to medium sized, containing eosinophilic colloid (Co) and lined by follicular cells. Haematoxylin and eosin stain (X4000).

Some of the follicles in MSG-treated group appeared distorted, were triangular, rectangular or semi lunar in shape (Figs. 2,3,4). The size of the follicles appeared larger in the experimental group as compared to the control group in both the central and the peripheral regions. Majority of the fol-
Follicles were lined by tall columnar cells having eosinophilic cytoplasm and moderately euchromatic elliptical or thick rod shaped purple nuclei (Fig 2). The apical part of cells appeared more eosinophilic. Few large clear cells having a pale cytoplasm and round nuclei were also seen within the basement membrane along with follicular cells. No changes were appreciated in the stroma as compared to control group except that the vascularity appeared to be increased (Fig 3). Some of the follicles were lined by squamous to cuboidal cells and were filled with darkly stained eosinophilic colloid in H&E and magenta in PAS stain. At places, the follicles were devoid of colloid and some follicles showed less staining reaction (Fig 4).

In PAS stained thyroid sections of the MSG treated animals, the follicles were lined by low columnar to tall columnar cells having purplish pink cytoplasm and moderately euchromatic elliptical or thick rod shaped nuclei. Magenta stained colloid was seen in the lumen of the follicle. The luminal surface of the follicular cells was dark purplish stained (Fig 4).

A statistically significant increase in the mean body weight was observed in the MSG treated rats than the control rats at the completion of the experiment. The mean average body weight of the rats on day one, day seven and day thirty seven was 186.50±18.75 g, 202±13.984 g and 268±14.944 g while in the MSG treated group it was 186.50±16.675 g, 207±13.984 g and 296.00±17.127 g respectively.

The average area of follicle was significantly increased (p<0.000) in the MSG treated group in both the central (1143.62±65.15 micron²) and peripheral regions (4278.80±419.40 micron²) of the thyroid gland (Table 1).

**Table 1: Comparison of mean area (µ²) of the follicles in the central and peripheral regions of the thyroid gland in control and MSG treated rats**

<table>
<thead>
<tr>
<th>Region</th>
<th>Group</th>
<th>Mean± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central</td>
<td>Control</td>
<td>677.58±38.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MSG treated</td>
<td>1143.62±65.15</td>
<td>0.000*</td>
</tr>
<tr>
<td>Peripheral</td>
<td>Control</td>
<td>3148.35±250.43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MSG treated</td>
<td>4278.80±419.40</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

*p-value=Significant

**Table 2: Comparison of mean area (µ²) of the follicular cells in the central and peripheral regions of the thyroid gland in control and MSG treated rats**

<table>
<thead>
<tr>
<th>Region</th>
<th>Group</th>
<th>Mean± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central</td>
<td>Control</td>
<td>300.86±27.90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MSG treated</td>
<td>690.66±50.46</td>
<td>0.000*</td>
</tr>
<tr>
<td>Peripheral</td>
<td>Control</td>
<td>765.96±94.56</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MSG treated</td>
<td>1955.48±221.73</td>
<td>0.000*</td>
</tr>
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</table>

*p-value=Significant
The average area of follicular cells in central and peripheral region was 300.86±27.90 micron² and 765.96±94.56 micron² in control and 690.66±50.66 micron² and 1955.48±221.73 micron² in experimental group and both showed a significant increase (p<0.000) in the MSG treated animals (Table 2). A significant increase (p<0.000) in the average height of the follicular cells was also noted in both the central and peripheral regions of the thyroid gland. 4.32±0.39 microns and 4.83±0.59 microns and in the experimental group was 9.75±1.21 microns and 10.09±0.91 microns in the central and peripheral regions respectively (Fig 5).

**Fig 5**: Comparison of mean height (µ) of follicular cells in the central and peripheral regions of the thyroid gland in control and MSG treated rats

**Discussion**

In the present study, the short term effects of monosodium glutamate evaluated after a period of one month in adult rats showed a statistically significant increase (p< 0.001) in the mean body weight of rat. Obesity without hyperphagia and stunted growth after MSG treatment was a common outcome of numerous studies. Obesity induced after administration of MSG in newborn mice had caused lesions in hypothalamic arcuate nucleus and impaired leptin and insulin signaling in this region resulting in hyperleptinemia and hyperinsulinemia. Leptin, an appetite-suppressing hormone, regulates energy, controls appetite and body weight. Intra-hypothalamic arcuate-paraventricular nuclear axis acts as the principal factor of food-intake regulation by neuropeptide-Y (NPY). MSG-induced ARCN(expansion?) damage might have resulted in loss of NPY secreting neurons which explains the decrease in food intake or hypophagia.

Striking changes were observed in the histomorphometry of the thyroid gland following administration of MSG in rats. The shape of the thyroid follicles showed alterations varying from triangular to rectangular to semilunar in MSG treated rats as compared to control rats which were round to oval. The larger follicles were usually found in the periphery and small to medium sized follicles were found centrally in both control and MSG treated groups which is in accordance with the observations made by Menna and Mohamed.

The short-term effects (one month) of MSG administration in adult rats have not been reported earlier. Most of the studies conducted in the past, have reported long term effects of neonatally administered MSG in rats and mice. No significant changes were reported in thyroid morphology, number of follicles, in the thickness of follicular epithelium planimetric fractions occupied by epithelium, colloid and stroma after a period of 6 to 18 months in rats treated with MSG neonatally. However, Dhindsa et al. observed large follicles distended with colloid accumulation and lined by squamous cells in young female mice treated with MSG with different doses (2, 4 and 6 mg/g) for seven days and the effects seen after 13 and 52 weeks. These changes indicative of a typical hypothyroid condition were consistent whether observations were made after 13 or 52 weeks.

Cekićl et al. observed thyreocytic atrophy with colloid hypersecretion, while in some of the follicles hyposecretion of colloid was seen in rats treated neonatally with monosodium glutamate and sacrificed after 6 months. Some of the thyroid portions expressed nodular hyperplasia in the form of microadenoma. They also demonstrated degenerative changes in the nerve cell perikaryons and the surrounding neuropil in the hypothalamic region. Severe edema was observed in the neuropil, as well as the reduced number of dendritic extensions. They suggested that the hypothalamic-hypophyseal-thyroid axis was very important for the thyroid function, since the damage of hypothalamic structures is reflected in thyroid hypofunction.

Contrary to the previous observations, distinct hypertrophy and hyperplasia of the follicular cells accompanied by diminished colloid in some of the follicles and increased stromal vascularity was seen in the present study, after a period of one month following MSG treatment which resembled more or less the histological changes produced by the goitrogens. According to Thomas et al., the growth response of the thyroid to goitrogens is triphasic. There is an initial sharp rise in the mitotic activity in the epithelial and stromal cells of the thyroid gland. This increased proliferation peaks at about 2 weeks and returns to pretreatment levels within 3 months. Hyperplasia and hypertrophy of...
the follicular cells observed after one month of the last dose of MSG is in accordance with their statement. The duration of one month may not be adequate for the thyroid gland to return to its pre-treatment levels. Miskowiak and Partyka did not observe any significant change in the structure of the thyroid gland after a period of 6-18 months. It is possible that this long period was sufficient for the histological changes of the thyroid gland to revert back leading to fall in the height of the epithelium, accumulation of colloid and the follicular cells to resume their normal size and architecture.

Research over the past few decades has undeniably demonstrated that glutamic acid in excess can act as a neurotoxin, causing neuronal degeneration and causes several neuroendocrinological disorders. In the present study, MSG has induced some adverse effects on the thyroid gland of rat even after a short period of time, as amply demonstrated by the histopathological changes. MSG, a flavor enhancer is being widely used in countless processed and branded foods. With change in the lifestyle nowadays, we are depending more and more on the processed foods which contains chemicals which increase the shelf life, kill bacteria and improves taste. Keeping in mind the potential toxic effects of MSG, it should be consumed with caution.

Acknowledgments: None

Conflict of interest: None

References