ORIGINAL ARTICLE

MORPHOMETRIC ANALYSIS OF THE GASTROINTESTINAL MUCOSA IN MONOSODIUM GLUTAMATE-TREATED ALBINO RATS

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ABSTRACT

Background: The consumption of monosodium glutamate as a major constituent of food flavours or additives has been on the increase in Nigeria despite the strongly speculated side-effects.
Objectives: This study intends to determine effects of monosodium glutamate (MSG) on the morphology of stomach and small intestines of male Albino rats.
Methods: Forty-four rats weighing 40 80g were randomly divided into four groups of eleven animals each consisting of six treatment and five controls. All the treatment rats were intraperitoneally injected with 4g/kg body weight of aqueous solution of MSG daily while the controls received comparable volume of normal saline. The animals in groups I, II, III, IV were sacrificed at the end of the day one, 2nd week, 4th week and 6th week of experiment respectively. Samples obtained from the fundic and pyloric parts of the stomach, distal parts of duodenum, jejunum and ileum were carefully dissected and quickly fixed in Bouin's fluid for morphometric studies after H&E stain.
Results: In comparing the values from sections of MSG treated rats with those of controls a significant ($P<0.05$) reduction in length and circumference of the small intestines were obtained. The thickness of the gastric mucosa and mucosa of the duodenum and ileum were significantly ($P<0.05$) increased except for the thickness of jejunal mucosa which was significantly ($P<0.05$) reduced later during the course of the experiment.
Conclusion: These findings indicate that MSG induces trophic changes **that are characterized by d**ecrease in the length and circumference of the small intestine and increase in the duodenal and ileal villi length with concomitant increase in the thickness of the gastric mucosa

Key words: - Monosodium glutamate, Morphometric study, Stomach, Small Intestines, Albino rats.

INTRODUCTION

Monosodium glutamate (MSG) a sodium salt of glutamic acid, is particularly effective in flavouring food [1] as such it is manufactured on large scale all over the world and marketed under various names such as Vedan, A-one and Ajinomoto. MSG is an inexpensive and popular condiment in Nigeria[2] that is found abundant in yeast and food ingredients with most of them not appearing on the label[3] and could thus be inadvertently abused.

The safety of MSG usage has generated much controversy locally and globally.[4, 5] Being an amino acid, it has been classified as Generally Recognized As Safe "GRAS" [6, 7, 8, 9] by reputable nutritionists and international organizations, such as the Directorate of Regulatory Affairs of Food, Drug, Administration and Control (FDA&C) in the U.S.A. and National Agency for Food and Drug Administration and Control *(NAFDAC)* in Nigeria however, evidence suggests that MSG is toxic to humans and animals.[5, 10, 11, 12]

Literatures regarding toxic effects of Monosodium glutamate have shown that it can cause selective destruction of large parts of the neurons in neonatal rats and other brain damaging potentials. [12 13, 14, 15, 16.] It has also been reported that MSG has the potential to cause significant oligozoospermia, increase in abnormal sperm morphology in rats.[17] It has also been reported that MSG has hepatotoxic and destructive effects on the small intestinal mucosa.[16, 18] A case of Chinese restaurant syndrome characterized by migraine, diarrhea, weakness, vomiting, stomach ache and tightness of the chest has also been linked to MSG usage. [10,19,20]

These reported adverse effects coupled with the

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potential side-effects of extended exposure to MSG in our diet and the quantity of its consumption, which is currently on the increase in Nigerian homes, necessitated the present study to be undertaken on the primary target system in the body. Thus, the present study was designed to determine the effects of MSG on the morphometry of the stomach and small intestines in the Albino rats.

MATERIALS AND METHODS

Test Article: The Monosodium glutamate was obtained commercially from the local market in Maiduguri, Nigeria and assayed to be greater than 99.9% purity by amino acid analysis with no other amino acids found (NAFDAC).

Preparation of MSG solution: A total of eighty grams of MSG was dissolved in one liter of normal saline according to the methods of Dyer *et al.*,[21] The solution was then stored in a clean screw-corked bottle and refrigerated until used.

Animals and Husbandry: This study was carried out in the Department of Human Anatomy, University of Maiduguri, Nigeria. Forty-four adult male Albino rats weighing 40-80g were used for the study. The rats were obtained from the Department of Pharmacology of the University of Jos, Nigeria and were maintained in the Animal holdings of the Department of Human Anatomy, University of Maiduguri, Nigeria. After an acclimatization period of two weeks, the rats were individually indentified by color tattoo and weighed. The rats were then kept in plastic cages at room temperature and provided with rat pellets (Sanders Seepc, Nig. Ltd) and water *ad libitum*. The rats were cared for according to the *Guiding Principles for the Care and Use of Animals* based on the Helsinki Declaration as amended by World Medical Assembly, Venice, Italy.[22]

Experimental Protocol: The forty-four adult male albino rats were divided using simple random sampling into four groups (I, II, III and IV) of eleven rats each. The grouping was based on experimental periods of 24 hours, two, four, six weeks respectively with each group consisting of six treated, and five control rats.

The twenty- four rats in the treatment groups received intraperitoneal injection of the aqueous solution of MSG at a dose of 4g/kg body weight daily [21,23] at a concentration of 80g/liter[21] while the twenty control rats were injected with comparable volume of normal saline daily. The animals were observed for behavioral changes such as food intake, bowel habit, respiration, physical appearance of their hair coat, signs of abdominal cramps and mortality. At the end of the experimental periods of one day, 2,4 and 6 weeks respectively the animals in groups I, II, III and IV were sacrificed by cervical dislocation under ether anaesthesia respectively.

The abdominal cavity was opened using ventro-medial incisions and the stomach was removed and dissected according to the method described by Eric and Susumu.[24] The intestines were dissected according to the method described by Makanya *et al*.[25] The junction between the foregut (small intestines) and hindgut was identified and severed. After washing, the unstretched length of the intestines from the gastroduodenal junction to the ileocaecal junction was measured by suspending it vertically along a measuring rule.[24] The gut circumference was determined from the average width of the transverse sections of the different parts of the small intestines.[25, 26] Specimens were collected from the fundic and pyloric parts of the stomach, distal parts of duodenum, jejunum and ileum for histological processing.

Tissue Processing: The specimens were fixed in Bouin's fluid (containing 1% acetic acid) for 8hrs, dehydrated and embedded in paraffin wax with the mucosal surface perpendicular to the cutting surface. From each specimen, 5µm serial sections were cut, with two sections separated by 20µm on each glass slide. All sections were stained with Haematoxylin and Eosin.

Morphometric Analysis: The thickness of the gastric mucosa was taken as the distance from the mucosal surface to the bottom of the gastric glands[27]. In the sections of the small intestines, the height of villi and the depth of crypts of Lieberkhun were measured. Several measurements were carried out on the sections from each rat organ using an eyepiece micrometer in a light microscope and at low magnification (x40). All measurements from each tissue section were obtained from a maximum of two coded sections, each being separated from the previous one by 20µm of the tissue.

Statistical Analysis: The data obtained were recorded and analyzed for Duncan multiple comparison test using computer based statistical package (GraphPad Instat version 4.0) .The results were expressed as mean ± standard error of mean (SEM). The significance of the results was tested using Student's *t*-test and ' p' value of less than 0.05 was considered as significant.

TABLE 1: Morphologic Data in MSG-treated and Control Rats

Variables	Group I (24hrs)		Group II (2 weeks)		Group III (4 weeks)		Group IV (6 weeks)	
	Control	MSG	Control	MSG	Control	MSG	Control	MSG
Intestinal length (cm)	92.10 ± 0.90	79.05 ± 1.93*	93.26 ± 0.63	89.47 ± 1.11*	96.00 ± 0.91	86.25 ± 1.07*	91.50 ± 1.23	90.30 ± 1.37
Intest. circumference(cm)	7.7 ± 0.4	5.8 ± 0.2*	6.1 ± 0.4	6.3 ± 1.4	7.0 ± 1.1	4.5 ± 0.4*	6.5 ± 1.0	6.0 ± 1.1
Thickness of fundic mucosa (mm)	0.17 ± 0.01	0.12 ± 0.01*	0.17 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.19 ± 0.01*	0.15 ± 0.01	0.18 ± 0.01
Thickness of pyloric mucosa (mm)	0.61 ± 0.03	1.14 ± 0.05*	0.54 ± 0.01	0.70 ± 0.05*	0.56 ± 0.01	0.63 ± 0.02*	0.61 ± 0.02	0.60 ± 0.01
Duodenal villus height (mm)	0.18 ± 0.01	0.30 ± 0.01*	0.24 ± 0.004	0.29 ± 0.01*	0.33 ± 0.01	0.35 ± 0.01	0.33 ± 0.01	0.38 ± 0.01*
Duodenal crypt depth (mm)	0.10 ± 0.004	0.10 ± 0.001	0.09 ± 0.004	0.09 ± 0.004	0.11 ± 0.004	0.11 ± 0.004	0.10 ± 0.004	0.11 ± 0.004*
Jejunal villus height (mm)	0.45 ± 0.01	0.49 ± 0.01*	0.40 ± 0.01	0.29 ± 0.02*	0.41 ± 0.01	0.31 ± 0.01*	0.46 ± 0.01	0.36 ± 0.01*
Jejunal crypt depth (mm)	0.12 ± 0.004	0.12 ± 0.004	0.11 ± 0.004	0.11 ± 0.004	0.12 ± 0.004	0.11 ± 0.004*	0.12 ± 0.004	0.11 ± 0.004*
Ileal villus height (mm)	0.27 ± 0.004	0.33 ± 0.01*	0.36 ± 0.01	0.41 ± 0.01*	0.37 ± 0.01	0.40 ± 0.01*	0.29 ± 0.01	0.32 ± 0.01*
Ileal crypt depth (mm)	0.11 ± 0.004	0.11 ± 0.004	0.11 ± 0.004	0.11 ± 0.004	0.11 ± 0.004	0.12 ± 0.004*	0.11 ± 0.004	0.12 ± 0.004*

All values are mean ± SEM; *significance relative to control and at $P<0.05$.

RESULTS

Effects of MSG administration on behaviour

The MSG-treated rats developed diarrhea after two days of treatment, which became less pronounced during the course of the experiment. The treated animals were weak and showed signs of abdominal cramps. However, there was no mortality.

Effects of MSG administration on Morphometric parameters

Gastric Mucosa: The MSG- treated rats had a significantly thinner (0.12 ± 0.01mm, p=0.01) fundic mucosa at the end of day one but became thicker (0.19 ± 0.01mm, p=0.04) at the end of the 4th week of the experiment when compared to their controls (0.17 ± 0.01mm and 0.16 ± 0.01mm, respectively). The rats administered MSG also had significantly thicker pyloric mucosa at the end of day one (1.14 ± 0.05mm, p=0.00), 2nd week (0.70 ± 0.05mm, p=0.02),and at the end of 4th week (0.63 ± 0.02mm, p=0.02) of the experiment compared to their controls with mean thickness of 0.61 ± 0.03mm; 0.54 ± 0.05mm and 0.56 ± 0.01mm, respectively (Table 1).

Small Intestines: Both the mean length and mean circumference of the small intestines became significantly reduced on the day one (p<0.001) and the 4th week (p=0.02) in the MSG- treated rats when compared to their controls (Table 1).

The mean height of the intestinal villi of the duodenum in the MSG-treated animals showed increment at 0.30 ± 0.01mm (p<0.001) on the day one; 0.29 ± 0.01mm (p=0.01) on the 2nd week, and 0.38 ± 0.01mm (p=0.01) on the 6th week of the experiment. These results were significantly higher than their controls at the respective periods. The mean villus height in the jejunum of MSG-treated animals only increased initially on the day one, that is, from 0.45 ± 0.01mm in the control to 0.49 ± 0.01mm (p=0.02) in the treated rats. However, at the end of the 2nd week, 4th week and the 6th week there were significant reductions (P<0.001) in the mean villus height in the jejunum of MSG-treated animals. In the ileum the mean villus height of the MSG-treated rats significantly increased at the end of all the periods, on the day one (0.33 ± 0.01mm, p<0.001); the 2nd week (0.41 ± 0.01mm, p=0.01), the 3th week (0.40^{-+}0.01mm, p=0.02) and on the 6th week (0.32 ± 0.01mm, p=0.02) of the experiment when compared to the animals that were given normal saline (Table 1).

In the duodenum the mean depth of the crypts remained almost the same between the controls and the MSG-treated animals except at the end of the 6th week where the mean crypt depth increased from 0.10 ± 0.004mm in the controls to 0.11 ± 0.004mm (p=0.04) in the treated group. The mean crypt depth in the jejunum of the treated groups (Groups I & II, p=0.09) either remained the same with the controls or reduced significantly (Groups III, & IV, p=0.04). However, in the ileum the crypt depth increased significantly (P=0.04) in the Groups III & IV treated animals. In all cases, the crypt to villus ratio was not affected.

DISCUSSION

Physical signs of adverse reactions observed in all the animals administered monosodium glutamate (MSG) were general weakness, abdominal cramps and diarrhea. These signs were consistent with some of the reported adverse reactions to MSG and considered as signs of the Chinese restaurant syndrome.[10, 19, 28, 29]

Because of MSG administration, both intestinal length and circumference were reduced thus shortening and occluding the lumen. The monosodium glutamate might have in part caused a stimulatory contractile effect on the longitudinal and circular muscles of the small intestines. This in turn caused cramping pains that elicited intense enterointestinal reflexes resulting in inhibition of gastro-intestinal motility leading to irritable bowel, nausea and vomiting.[30] These adverse reactions to MSG were also reported.[20, 29, 31, 32, 33]

In general, the thickness of the gastric mucosa and the mucosa of the duodenum and ileum were significantly increased, except for the jejunal mucosa, which was significantly reduced during the course of the experiment. Thus, monosodium glutamate might have acted in part as a β-agonist that caused stimulation of cAMP and consequently led to a relaxant effect on the gastric mucosa, duodenal and ileal mucosae. The relaxant effect on the duodenal mucosa contributed to a compensatory contractile effect on the jejunal mucosa during the course of the study.

The present study indicated that MSG induced trophic changes in the gastro-intestinal mucosa. This result however, is contrary to the findings of Eweka and Om'Iniaboh[18] in which the histopathological study of the small intestines of rats treated with 6g of MSG revealed degenerative and atrophic changes. MSG could also be an allergen that could cause adverse reactions. It is known that minute amounts of allergens could trigger adverse reactions including anaphylaxis in people who are acutely sensitive to these allergens.[34, 35, 36]

CONCLUSION

The findings of this study suggest that consumption of monosodium glutamate can cause trophic changes

that are characterized by decrease in the length and circumference of the small intestine and increase in the duodenal and ileal villi length with concomitant increase in the thickness of the gastric mucosa. Further studies with humans are recommended to support these findings.

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