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Spleen Histological Changes Following Monosodium Glutamate Ingestion in Adult Male Wistar Rat

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Abstract

This study investigates the effect of ingestion of monosodium glutamate (MSG) on the spleen histology of adult male Wistar rats. The study involved 30 adult rats (140 to 180grams). They were divided into five groups of 6 rats each: A (control; placed on water and rat chow feed), while groups B to E served as the test groups and received chow feed plus solution containing 40mg/kg bwt, 80mg/kg bwt, 120mg/kg bwt and 160mg/kg bwt of MSG respectively daily for 28 days. Their weights were monitored throughout the period of the experiment and standard care for laboratory animal was practiced. At the end of the 28th day, all animals were sacrificed and the spleen harvested following standard laboratory procedures and fixed in 10% formal saline for histological processing. The results showed test groups A and B presented normal spleen histology with no alteration in architectural arrangement of cells. However, test group C and D presented mild fatty degeneration and test group E showed moderate tissue changes. The observations suggest that MSG at high dose may be toxic to the spleen and may induce spleen tissue damages in a dose dependent manner. Based on our findings, doses above 40mg/kg body weight presented adverse effect on the spleen.

Keywords

Monosodium Glutamate, Spleen, Histology

1. Introduction

Monosodium glutamate (MSG) is the sodium salt of the non-essential amino acid glutamic acid, typically marketed as a white crystalline powder and is readily soluble in water but sparingly soluble in ethanol. It is a major dietary component with the potential of intensifying the savory flavor in foods [1-3] and can produce a unique taste, known as fifth taste (umami) that improve the quality of food intake by stimulating chemosensory perception and proposed in various types of patients with cancer, radiation therapy and organ transplantation [4]. MSG is one of the world's most extensively used food additives [5] documented to has a daily consumption rate of 300–4000 mg/day; at least in developed countries [5, 6].

Although in 1959 the Food and Drug Administration categorized it to be safe, they have also commissioned a report that an unknown percentage of the population might react to it and develop complex [7]. Concerns about it toxicity and secondary physiologic effects have been widely discussed and controversial [8, 9]. While studies investigate it LD50 in rats to ranges between 15000 and 18000 mg/kg of body weight [10, 11], experimental studies have shown it prolonged ingestion to produced myriad of toxic effects, known as the Chinese restaurant syndrome [12], characterized by sweating, nausea, headache, chest tightness, and/or back of the neck burning sensation [13]. Long term

ingestion of MSG has been reported to induce hyperphagia, obesity, asthma, memory impairment, and damage to hypothalamic neurons [12, 14].

Earlier reports in neonate rats have shown the administration of the double dose (4.0 mg/kg) of MSGcompromised HPG axis functional integrity; typified by decreased weights of pituitary glands and testes [15]. Considering that alteration in the weights of visceral organs is diagnostic for the atrophicor hypertrophic dysfunctional organs [16], indicating that addition of MSG to foods can be hazardous to health.

Immuno toxicity of xenobiotic or metabolites on lymphocytes populations is represented in the spleen; the largest secondary lymphoid greatly involved in host immune response [17]. It is considered one of the organs to evaluate enhanced histopathology of the immune system [18, 19] and therefore, considered a vital organ to assess treatment-related lesions. Based on this, the main objective of this study was to investigate the histological changes induced by MSG in spleen using adult male Wistar rat as a model.

2. Materials and Method

The study was carried out in Histological Laboratory, College of Medicine, Ambrose Alli University Ekpoma, Edo state, Nigeria. Its geopolitical location is the South South part of Nigeria.

A total of thirty (30) adult Wistar rats of comparable sizes and weights ranging from 140 - 180g were gotten from the Animal Farm, College of Medicine, Ambrose Alli University Ekpoma, Nigeria. They were transferred to the Histology Laboratory where they were allowed two (2) weeks of acclimatization and were kept in wire mesh cages with tripod that separates the animal from its feces to prevent contamination. During this period of acclimatization, the rats were maintained in accordance with the standard guide for the care and use of Laboratory animals.

MSG is a salt derivation of the amino acid glutamate with daily consumption reported to vary from 3 to 5 grams. Branded sachets of commercially available MSG were bought from Ekpoma local Market in Ekpoma, Nigeria. The sachets were pulverized to fine powder using ceramic mortar and pestle and then stored in sterile bottles until used, when it for preparation for rats' administration. A large volume of monosodium glutamate stock solution was prepared by dissolving 15.2g of MSG in 380mls of distilled water.

The study is an experimental investigation using Wistar rat as a model. Wistar rat was chosen because it has been reported that the safety assessment in experimental animals of both medical and non-medical biological active chemicals have been very successful in predicting toxicity in humans [30]. In this study, a total of thirty (30) adult Wistar rats were used. They were divided into five equal groups of six rats each. Group A served as the control and was placed on water and rat chow feed only. Group B, C, D and E rats were test group receiving water and rat chow plus solution containing B=40mg/kg bwt (0.2ml per rat), C=80mg/kg bwt (0.4ml per rat), D=120mg/kg bwt (0.6ml per rat) and E=160mg/kg bwt (0.8ml).

The substance administration was given daily for 28 days (4 weeks) and the weights of both the test animals and control animals were monitored before, during and after administration of MSG. at the end of MSG administration, the rats were put under light chloroform anesthetic and sacrificed and the spleen harvested and fixed in 10% formal saline for histological processing.

The tissues were processed using automatic tissue processor according to the processing schedule used in the Irrua Specialist Teaching Hospital (ISTH), Irrua, Edo State, Nigeria. The fixed plastic cassette tissues in 10% formalin were automatically processed by passing them through different grades of alcohol as follows: 70% alcohol (2hrs), 80% alcohol (2hrs), 90% alcohol (2hrs), 90% alcohol (2hrs), 95% alcohol (2hrs), Absolute (2hrs), Xylene 1 (2hrs), Xylene II (2hrs), molten paraffin wax 1 (2hrs) and Molten paraffin Wax II (2hrs). After the last timing, the tissues were removed from their plastic cassettes and placed at the centre of the metallic tissue mould and then filled with molten paraffin wax. They were also left to solidify after which they were now placed in the refrigerator at 5oC for 15 minutes. After the blocks were cool in the refrigerator for the time stated above (15 minutes), the blocks were then removed from the metallic case using a knife and after which the paraffin wax at the side of the blocks were removed. The blocks were then trimmed and cut serially at 3nm on a rotary microtome. The sections were floated in water bath at 55^0C and picked up by the use of a clean frosted end slides. The frosted end slides were now placed on the hot plate for 40 minutes for adequate attachment of the sections on the slides after which the sections were dewaxed, hydrated, air dried and stored in a slide box ready for staining process.

Sections for general tissue structure were stained by Haematoxylin and Eosin technique. The sections were de waxed in 3 changes of xylene (5 minutes), the sections were hydrated through descending grades of alcohol (absolute, 95%, 80% and 70%), the sections were stained in Harris haematoxylin (5 minutes), the sections were rinsed in running tap-water to remove excess stain, The sections were differentiated in 1% acid alcohol (3 seconds), the sections were blued in running tap water (10 minutes), the sections were counterstained with 1% eosin (1 minute), sections were finally rinsed in water, dehydrated in ascending grades of alcohol (70%, 80, 95% and absolute), and the sections were cleared in xylene, air-dried and mounted with dibuthylphthalate propylene xylene (DPX). The slides were examined under a light microscope and photomicrographs were taken. The stained slides were read microscopically and photomicrograghs were obtained and results compared.

3. Results

Table 1 shows the mean body weight changes in each group of animals during the experiment. The all had increasing body weight that was statistically not significant from the onset of study, acclimatization and during the

experimental period to the 4th week where a significant change in body weight was observed. The average mean body weight was highest in the control (250.83±70.24grams) compared to the test groups with a statistical significant different ($p<0.05$). Comparatively, in the test groups we observed a zigzag change in mean body weight at the concentration of MSG increase with the group receiving 120mg/kg MSG presenting the highest mean body weight (235.83±45.41grams) and that receiving 80mg/kg MSG presenting the least mean body weight gain (225.83±41.77grams). However, between the test groups there was no significant different in mean body weight gain.

Table 1. *Body weight changes in rats receiving varying concentration of monosodium glutamate for 28 days.*

Weights (g)	CONTROL (Group A) (n=6)	Group B (n=6)	Group C (n=6)	Group D (n=6)	Group E (n=6)	P-value
WBA	140.00±22.36	150.00±0.00	150.00±0.00	150.75±0.00	160.00±22.36	0.32
WAA	195.00±27.38	210.00±22.36	225.00±25.00	225.5±27.38	240.00±41.83	0.17
WK 1	270.00±27.38	260.00±13.39	250.00±35.35	240.50±54.77	220.00±27.38	1.74
WK 2	290.00±22.26	255.00±27.38	250.00±35.35	240.50±54.77	220.00±27.38	0.66
WK 3	280.00±27.38	250.00±0.00	255.00±20.91	260.25±37.91	250.00±35.35	0.43
WK 4	330.00±44.72	260.00±54.77	245.00±11.18	280.00±44.72	255.00±44.72	0.03
Average BWT	250.83±70.24	230.83±47.65	225.83±41.77	235.83±45.41	232.50±47.86	

Key: n: number of sample; Values are mean ± Standard deviation; Wt= weight (grams); Significant: $p < 0.05$; Not significant: $p > 0.05$; WBA: Weight before acclimatization; WAA: Weight after acclimatization; WK 1: Weight after one week of administration; WK 2: Weight after two weeks of administration; WK 3: Weight after three weeks of administration; WK 4: Weight after four weeks of administration

Table 2 represents the observable physical and behavioural changes in the rat administered varying concentration of MSG. We observed changes in behaviour (aggressiveness) and fecal nature (consistency and diarrhea) in the test groups receiving 80mg/kg, 120mg/kg and 160mg/kg MSG. There was increased physical agility in the test group receiving 120mg/kg and 160mg/kg MSG.

Table 2. *Observable physical and behavioural changes in rats receiving varying doses of (Monosodium glutamate) for 28 days.*

Observations	Control (Group A)	Group B (40mg)	Group C (80mg)	Group D (120mg)	Group E (160mg)
Fur color	-	-	-	-	-
Behavioral changes (aggressive)	-	-	+	++	+++
Diarrhea	-	-	+	++	+
Death	-	+	+	-	+
Water rejection	-	-	-	-	-
Physical agility	Active	Active	Active	Very active	Very active
Fecal nature (consistency)	Solid	Solid	Semi solid	Semi solid	Semi solid

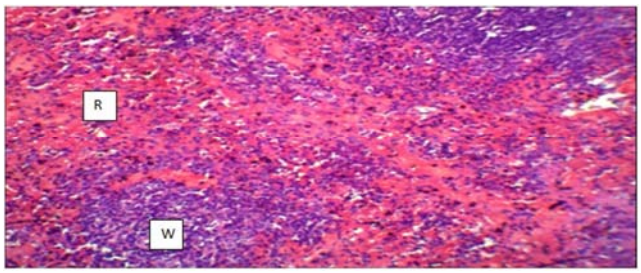
Key: + = present in trace amount; ++= present in moderate amount; +++= present in large amount; - =negative (absent).

Table 3 indicates the gross and microscopic changes in rats receiving varying concentration of MSG for 28 days. Grossly, there was no observable change in the spleen of rat receiving varying concentration of MSG for 28 days. However, microscopy shows fatty changes and loss of cell architecture in the groups receiving 80mg/kg, 120mg/kg and 160mg/kg MSG in a dose depended fashion (see table 3 and figure 1 showing tissue histology)

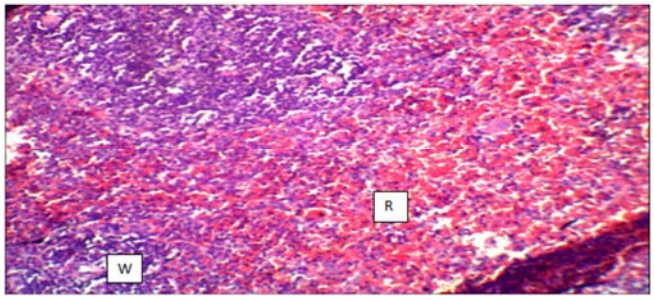
Table 3. *Gross and microscopic changes in rats receiving varying concentration of MSG for 28 days.*

Groups	Dosage	Gross findings	Microscopic Examinations		
			Vaculation	Fatty changes	Loss of Cell Architecture
A	Control (0)	Normal	-	-	-
B	40mg	Normal	-	-	-
C	80mg	Normal	-	+	+
D	120mg	Normal	-	+	++
E	160mg	Normal	-	++	++

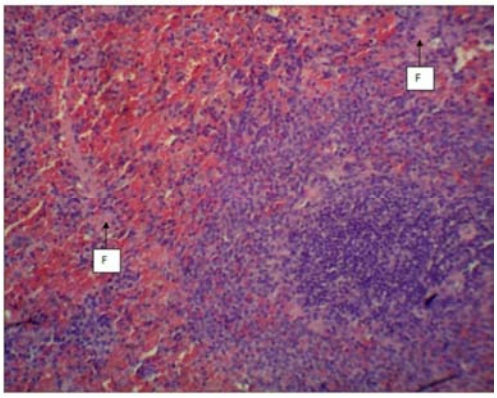
Key: Mg: Milligram; - = Absent, + = Mild; ++ = Moderate



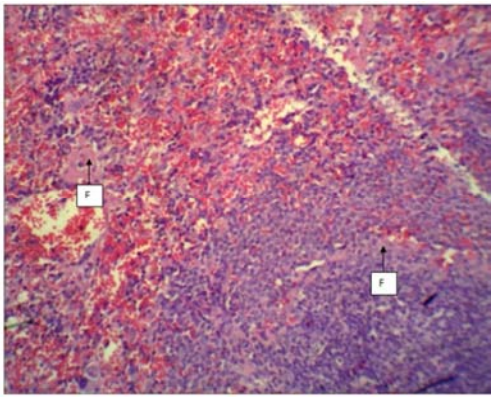
Group A (Control) Rat splenic tissue section showing normal histology composed mainly of peri-arterial lymphoid sheath with germinal centre W and the red pulp R (H&E x 100)



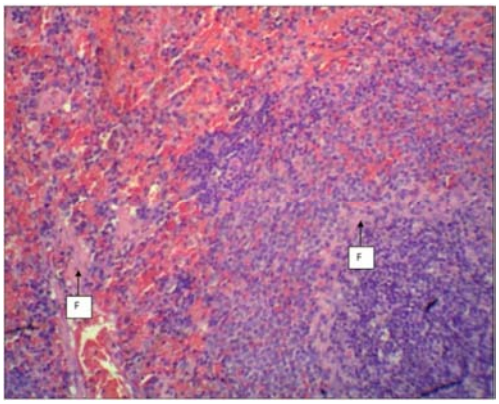
Group B; Rat splenic tissue section (Given 40mg monosodium glutamate) showing normal histology composed mainly of peri-arterial lymphoid sheath with germinal centre W and the red pulp R (H&E x 100)



Group C; Rat splenic tissue section (given 80mg monosodium glutamate) showing mild fatty changes F (H&E x 100)



Group D; Rat splenic tissue section (given 120mg monosodium glutamate) showing mild fatty changes F (H&E x 100)



Group E; Rat splenic tissue section (given 160mg monosodium glutamate) showing moderate fatty changes F (H&E x 100)

Figure 1. *Tissue microscopic changes in rats receiving varying concentrations of MSG for 28 days.*

4. Discussion

The present study showed that MSG ingestion may alters the normal body weight gain pattern considering the reduction in mean body weight gain in the test groups compared to the control (see table 1). Although there were increasing mean body weight as the ingestion of MSG progresses from the 1st week through the 4th week, it was however, lower than the control with a significant different at the 4th week. This finding disagrees with the reports by Hassan *et al.* [20] MSG intake to significant induced body weight gain and that withdrawal significantly induced body weight loss that return toward normal. Other studies have established the capability of MSG to induce adiposity and gained in body weight of experimental animals [21-24]. However, our finding correlates with the study by Kondoh and Torii [25] who reported no significant changes in weight gain in rat administered MSG.

On the physical and behavioural changes with intake of varying concentration of MSG, we observed some physical (fecal nature and consistency) and behavioural (aggressive) alterations; specifically with the intake of MSG doses ranging from 80 to 160mg/kg (see table 2). This finding indicates that MSG may alter fecal matter nature and consistency and induce diarrhea while also inducing aggressive and physical activity.

In the present study, the effect of MSG on the histology of the spleen was observed to be dose dependent with doses capable of altering spleen architecture to begin from 80mg/kg. In fact, the group receiving 40mg/kg MSG was comparable with the control and showing splenic tissue section with normal histology composed mainly of peri-arterial lymphoid sheath with germinal centre and the red pulp. However, there were mild to moderate fatty changes (steatosis or fatty degeneration or adipose degeneration) in the groups receiving 80 to 160mg/kg. This finding corroborates the study by Ciric *et al*. [26] who observed that MSG administration induced degenerative and atrophic changes in rat spleen. Our results also agreed with the studies of Onyema *et al*. [27] who indicated MSG influenced both hepatic and adipose tissue. According to the study by Hassan *et al*. (2014), there was a positive correlation among the changes in serum IL-1β levels and both thymic and splenic MDA contents after MSG treatment. By implication, the observed histological changes in 80-160mg/kg intake of MSG in the present study may be that MSG induced spleen fatty and cell changes occur due to induction of oxidation or lipid peroxidation.

Although the degree of cellular recovery after cessation of MSG was not investigated in the present study, Hassan *et al*. [20] and Ebaid and Tag[28], demonstrated that there were varying degrees of improved cellular recovery after cessation of MSG treatment. The observed steatosis in the present study is said to be a pattern of reversible cell injury resulting from hypoxia, toxic or metabolic insults, diabetes mellitus, obesity, and protein malnutrition which result in accumulation of droplets of triglyceride/ neural fat in various solid organs [29], thus our findings are in accordance.

5. Conclusion

The present study showed that MSG induced histological alterations in the spleen including fatty changes and loss of cellular architecture which we assert can disturb spleen function in immune status and response. This lead to the conclusion that high dose and prolong intake of MSG has

effect on the histology and overall immunological function of the spleen by causing degenerative fatty changes. We therefore recommend mild and discriminate intake of MSG and MSG containing foods and snacks and their used as additive.

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