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Role of Monosodium Glutamate in Antioxidant Modulations and Structural Alterations in Skeletal and Smooth Muscles of Male Wistar Rats

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ABSTRACT

MSG is a salt of sodium and glutamic acid used all over the world to enhance flavour in various food. MSG increases the appetite by stimulating the appetite centre in the brain but nowadays it has been debated for its safety and harmful effects as it affects almost every major organ in the body. The aim of this study is to assess the role of Monosodium glutamate in modulating skeletal and smooth muscle Tissue calcium levels, and histopathological changes in muscles of Male wistar Rats.30 adult 7 week old male wistar rats was used and were grouped into 6 groups which include; group A fed with regular feed + MSG Sub-cut at 6 g/kg/day, group B fed with powdered feed* + 5% MSG in feed, group C fed with Powdered feed + 2% MSG in feed, group D fed with Sub-cut at 6 mg/kg/day and groupE fed with Regular feed + water. Rats were kept for a total of 30 days and sacrificed at the end. Biochemical analysis of tissue calcium was done using commercial calcium test kits and the absorbance of the specimen and standard were measured against reagent blank using standard methods. Histopathological analysis on sections of skeletal and smooth muscle blocks of each rat were done using the standard Haematoxylin and Eosin technique and morphometric changes were assessed using light microscopy and ImageJ®. Levels of calcium in the skeletal muscles were higher in the control group showing that MSG was not associated with high levels of calcium. Levels of MSG in smooth muscles at low concentrations and dosages did not show any significant structural alterations. Catalase levels in both skeletal and smooth muscles were not affected while levels of MDA were severely affected following MSG treatment at high dosages. Concentrations of MSG used in this study were not associated with any toxic effects in rats and usage of MSG at these low concentrations through feed supplement should be encouraged.

Keyword: Monosodium glutamate, histopathological, raats, smooth muscles, antioxidant

INTRODUCTION

MSG is a salt of sodium and glutamic acid used all over the world to enhance flavor in various foods [1]. The occurrence of MSG as a food additive in various diets makes it relevant to human diet worldwide [2]. It is one of the most naturally occurring non-essential amino acids found in tomatoes, cheese and other foods [3]. MSG increases the appetite by stimulating the appetite center in the brain [4] but nowadays it has been debated for its safety and harmful effects as it affects almost every major organ in the body. The European Union classifies it as a food additive permitted in certain foods and subject to quantitative limits. International and national bodies governing food additives currently consider MSG safe for human consumption as a flavor enhance [5]. Debate over the healthiness of MSG and its associated health problems bas led to a negative public opinion of the additive. It is concluded that MSG has the potential to create several health hazards and thus advocates strict guidelines and mass awareness regarding its use [6]. Monosodium glutamate has been shown to lead to oxidative stress which is caused by the excessive production or a decreased elimination of free radicals in cells, the majority of which are oxygen radicals and other reactive oxygen species (ROS) [7]. Nutrition metabolism and several extracellular and intracellular factors such as hormones, cytokines, and detoxification processes contribute to the oxidative stress. Therefore, excessive metabolism of glutamate due to MSG intake can be a source of ROS.MSG is also known for its alteration in mitochondrial lipid peroxidation [8], and antioxidants resulting in oxidative damage in different regions of body organs as studies on experimental animals have confirmed toxic effect of MSG, mainly manifested by enhanced GPx activity, increased peroxidation, significant decline in catalase, and

reduced glutathione levels hence raising the levels of reactive oxygen species a phenomenon called oxidative stress [2]. In skeletal muscles, glutamate plays a vital role in energy metabolism by increasing the availability of Tri Carboxylic Acid Cycle (TCA) intermediates [9]. More so, nutritional supplementation of glutamate or its precursor glutamine, (ornithine) a-ketoglutarate, or the branched chain amino acids can influence muscle glutamate levels thus affecting muscle activity [10]. However, D-alanine supplementation results in improved homeostasis via release of calcium ions [11]. In the cross-bridge cycle, calcium plays a crucial role in muscle contraction [12], thus showing the possibility of glutamate altering its bio-availability in the tissue. During muscle contraction, a total of 2 ATP molecules are used and this means that mitochondria play a crucial role in this regard. Oxidative stress development is faster if mitochondrial respiratory mechanisms are altered [13], due to increased rate of glucose metabolism and accumulation of free oxygen species. Moreover, muscle glutamate and glutathione concentration are highly correlated [14], showing a positive relationship between glutamate and oxidative stress in the tissue. In skeletal muscles, ingesting MSG elevates intramuscular glutamate [15] whereas infusion of MSG elevated the influx of glutamate in the limbs [16]. In smooth muscles, evidence from human and animal studies indicate that glutamate is a major oxidative fuel for the gut and undergoes a first pass metabolism in the intestine [17]. In smooth muscle, GIT responsiveness to glutamate is associated with strong variations [18], thus showing the need to understand its role in the muscles. The role of glutamate is comparable to that of acetylcholine which both lead to increased depolarization of the smooth muscles [19]. This would be due to increased calmodulin (Cam) and myosin light chain kinase (MLCK) activity which allows for a wide variation in contractile strength and velocity [20]. Since smooth muscles use 1 ATP molecule, increased contractile strength would lead to an increased rate of mitochondrial respiration due to the activity of Na/K-ATPase enzyme [21]. This then subsequently leads to accumulation of free oxygen species, thus leading to oxidative stress in the tissues [13]. This would subsequently lead to changes in tissue architecture and function over time. This study aims to assess the role of monosodium glutamate in modulating skeletal and smooth muscle Tissue calcium levels, and histopathological changes in muscles of Male Wistar Rats.

MATERIALS AND METHODS

Study Design

This was an experimental study in which quantitative and qualitative data was collected from T2DM Wistar rats. It involved assessing the structural changes, Ca^{2+} levels as well as antioxidants in skeletal and smooth muscles.

Monosodium Glutamate (MSG)

This was procured from Ajinomoto Co. Inc, Japan through the Institute of Innovation, and Laboratory of Dr. Sakai-Ryosai (DVM, PhD). The study was an experimental study involving 30 adult 7-week-old male Wistar rats. Rats were kept for a total of 30 days and sacrificed at the end according to table 1.

Table 1: Experimental Design

Groups	Experimental groups on supplementation	Group description	No. of rats	Av. weight (g)
A	Regular feed + MSG Sub-cut at 6 g/kg/day	MSG Sub-Cut	6	120
B	Powdered feed* + 5% MSG infeed	MSG alone in feed	6	120
C	Powdered feed* + 2% MSG infeed	Low MSG in feed	6	120
D	feed*+MSG Sub-cut at 6 mg/kg/day	High MSG+feed	6	120
E	Regular feed + water	Control	6	120

Key: *Feeds will be provided ad libitum, AV. Wgt = average weight, Sub-cut = subcutaneous (route of administration).

Organ collection for Laboratory Analysis

Rats were anesthetized using chloroform and the smooth muscles of the ileum and jejenum as well as skeletal muscles of forelimb (biceps and triceps) and forelimb (quadricepsfemoris) were removed. Sections of the organs were homogenized for biochemical analysis and the other sections were used for histopathology. After homogenization, the homogenate was centrifuged at 1500 rpm for 5 minutes and the filtrate will be used for biochemical analysis.

Biochemical Analysis of Tissue Calcium

This was done using commercial calcium test kits. Vitro Scient® (from Hannover, Germany) contained three basic reagents. Calcium reagent was based on the cresolphthaleincomplexone (CPC) method of Moorehead and Briggs [22]. CPC reacted with calcium and magnesium and prevented interference from this cation. CPC was an acid-

base indicator necessitating the use of a strong buffer to stabilize the pH. Calcium reacted with cresoplhthaleincomplexone to form purple color complex in alkaline medium.
Ca^{2+} + O-Cresolphthaleincomplexone = Ca^{2+}–Cresolphthaleincomplexone complex.

Table 2: Preparation of blank and standard for calcium measurement

Cuvette	Blank	Standard	Specimen	Absorbance
R2	0.5ml	0.5ml	0.5ml	
R3	0.5ml	0.5ml	0.5ml	
Standard	--------	10µ1	--------	
Specimen	-------	--------	10µ1	

Cuvette samples were mixed and incubated for 5 minutes at room temperature. The absorbance of specimen and standard was measured against reagent blank. The color was stable for 1 minute. The intensity of color was measured photometrically between 540 and 600 nm with maximum absorbance at 580 nm being directly proportional to $[Ca^{2+}]$ in the specimen and concentrations were quantified using this formula;

$$[Ca^{2+}] = \frac{Absorbance\ of\ Specimen}{Absorbance\ of\ standard} * Standard\ value$$

Unit conversion: mg/dl*0.25 = mmoVI

Antioxidant Determination in Tissues

This was done for CAT, and lipid peroxidation was done for MDA (Malondialdehyde).

Malondialdehyde (MDA) Determination

This was done using a standard method according to Uchiyama and Mihara, (1978) [23].

Catalase Determination

This was done using Johansson & Borg, (1988) method for small animal samples [24].

Histopathological Analysis

Sections of skeletal and smooth muscle blocks of each rat were analyzed according to a systematic random embedding, random sectioning and random sampling method [25]. Morphometric and microscopic changes were assessed using a light microscopy, using an ocular integrated test system (Integrations platte, Zeiss, Oberkochen, Germany). Pathological lesions that were investigated included cellular necrosis, fibrosis and cell size. The area associated with pathological lesions were measured using Image-J® software to assess level of damage.

Data Analysis

Quantitative data was generally expressed as mean± SD. An unpaired 2-tailed Student's t-testand 2-way repeated ANOVA were performed for between-group comparisons using MS Excel 2013 Version. Tissue $[Ca^{2+}]$ was analyzed with ANOVA and t-test to determine in-group differences. Qualitative data from the histological analysis waspresented in tables and chi-square test conducted on the data set. Sections of tissue degeneration were quantified using Image-J® to determine in-group differences.

Ethical Considerations

The animals were kept under consideration of the National Institute of Health (NIH) guidelines regarding experimental animals. The rats were kept in the Animal House of the College of Veterinary Medicine Animal Resources and Biosecurity (CoVAB) after acquiring institutional ethical clearance. The number of animals were reduced to the minimal required for the study purpose and the techniques used to achieve the objectives were carefully handled. In brief; provision of sufficient space, adequate fresh air, 12-hour day light exposure, comfortable bedding materials (saw dust) provided and housed in a raised structure away from predators and secondary sources of infection. Water was provided ad libitum and the feed ration provided. Toe laboratory monitoring tool was used. After completion of the study, the carcass was properly disposed.

RESULTS

Table 3: Levels of calcium in skeletal and smooth muscles after MSG exposure.

Experimental Group	N	Skeletal muscle	Smooth muscle
		Mean ± SD calcium concentration (mMol/1)	
6 g/kg s.c	5	0.15±0.03a	0.26±0.03a
5% MSG in feed	6	0.23±0.03b	0.08±0.03b
2% MSG in feed	6	0.13±0.023	0.20±0.03c
60 mg/kg	6	0.21±0.03b	0.l 7±0.04c
Control	6	0.33±0.03c	0.18±0.04c

KEY: a and b = different superscripts indicate significant differences using Tukey's multiple comparison test.

Table 4: Antioxidant and MDA variations.

Experimental Group	N	Skeletal muscle		Smooth muscle	
		CAT (µMoI/protein)	MDA (nMol/L)	CAT (µMoI/protein)	MDA (nMol/L)
6 g/kg s.c	5	36.22±1.60[3]	801.3±37.01[3]	303.2±11.91[a]	427.4±97.92[3]
5% MSG in feed	6	35.29±4.15[a]	512.8±45.33b[b]	304.5±10.74[3]	88l.4±32.05[b]
2% MSG in feed	6	36.38±2.67[3]	790.6±33.l0[a]	309.9±7.30[3]	410.3±125.00[3]
60 mg/kg	6	36.31±0.86[3]	359.0±35.11[c]	298.4±9.02[3]	406.0±77.63[3]
Control	6	37.34±7.49[3]	224.4±37.01[d]	296.5±13.l4[8]	336.5±61.37[R]

KEY: CAT = Catalase; MDA = malondialdehyde; a and b = different superscripts indicate significant differences using Tukey's multiple comparison test

Histopathological changes

Skeletal muscle changes

The myocyte was unchanged in all experimental groups. In addition, the size and integrity of the endomysium and perimysium were shown to be consistent in all groups following MSG exposure.

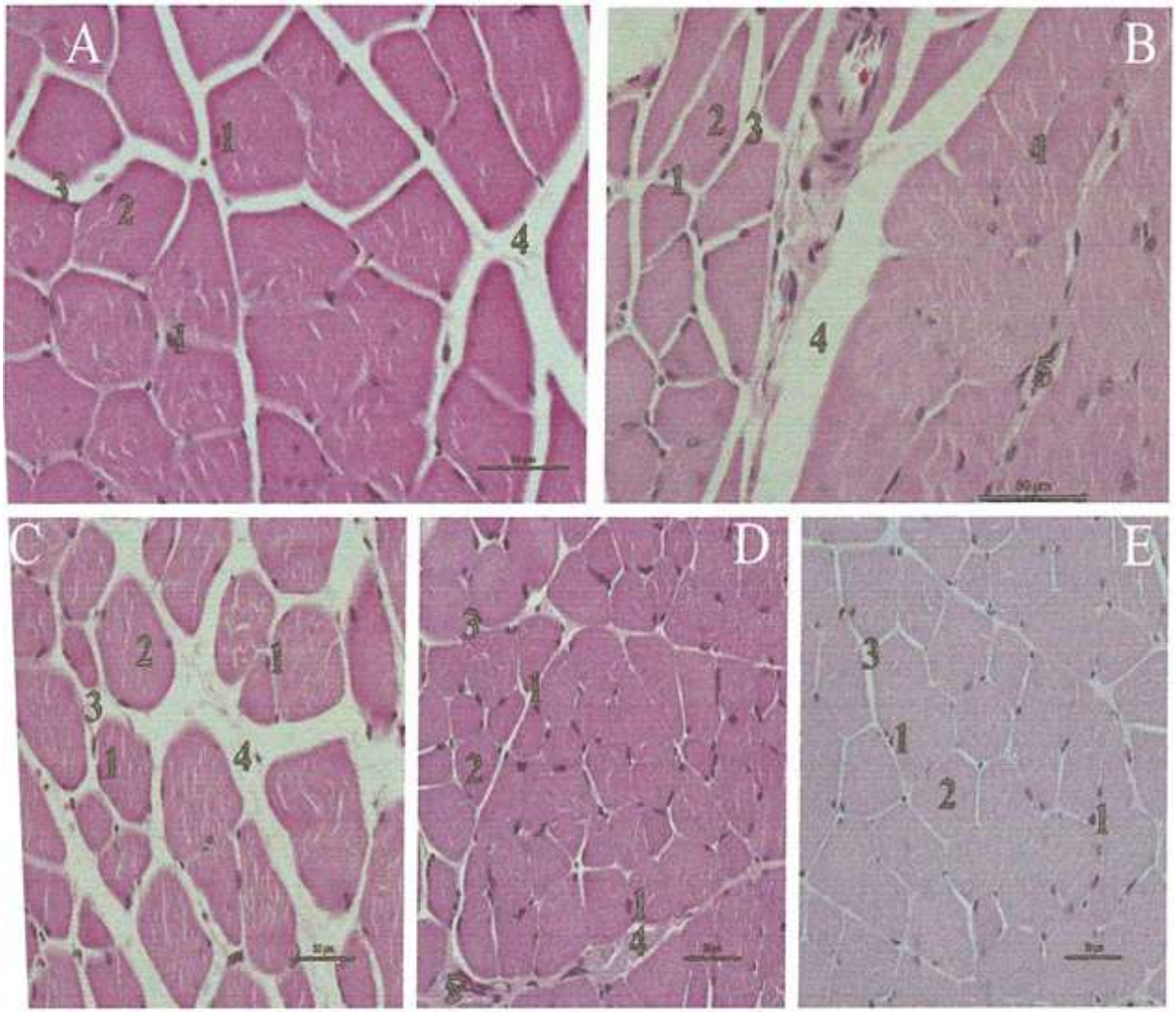

Figure 1: Structural changes in skeletal muscles following MSG treatment.

KEY: Group A= Rats treated with MSG at 6 g/kg/day s.c; B = 5% MSG in feed; C = 2% MSG feed; D = 60 mg/kg/day MSG s.c; E = No MSG. On photomicrographs: 1 = Peripheral leus; 2 = Muscle fiber (cell); 3 = Endomysium; 4 = Perimysium; 5 = Capillary.

Smooth muscle changes

No significant changes were seen in the villi, gastric pits and muscular layers of the duodenum in all groups after MSG exposure as shown in the figure below.

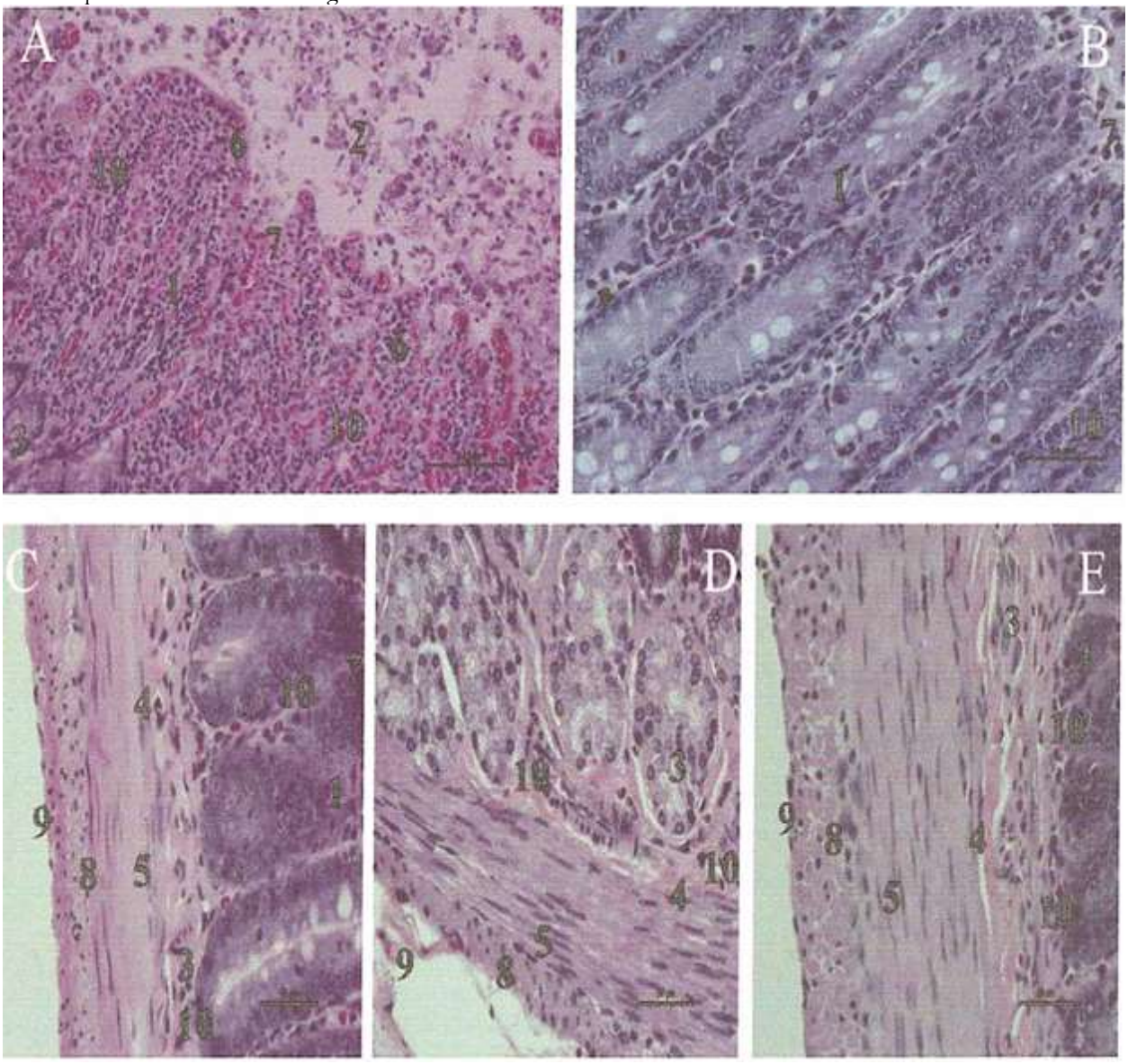

Figure 2: Structural integrity in rats after MSG exposure.

KEY: Group A = Rats treated with MSG at 6 g/kg/day s.c; B = 5% MSG in feed; C = 2% MSG in feed; D = 60 mg/kg/day MSG s.c; E = No MSG. On photomicrographs: 1 = Duodenal cypts; 2= Duodenal lumen; 3 = Brunner's glands; 4 = lamina muscularis; 5 = lamina submucosa; 6 = villi; 7 = gastric pits; 8 = tunica muscularis; 9 = serosa; 10 = lymphoid tissue.

DISCUSSION

Calcium levels following MSG supplementation were generally within normal ranges in both skeletal and smooth muscles, and this was especially amplified during low MSG treatments. In striated muscles, calcium release triggers the cross-bridge cycle which creates the energy required for skeletal muscle contraction, which shows that tissue calcium levels have to be maintained within the normal ranges for maximal activity [26].

In addition, calcium has been shown to play a pivotal role in the regeneration and development of skeletal muscles thus showing that slight deviations in calcium concentrations would affect the integrity of skeletal muscles [27].

This study has been able to provide basic information that MSG does not affect calcium levels especially when consumed at low concentrations. In humans, MSG effects on skeletal muscles have not been found [28], showing that it is safe for routine use. In addition, calcium signaling in smooth muscles involves an interplay of several transporters and the contraction is due to actions of myosin light chain kinase activation [29, 30]. In the body, contractions of the smooth muscles under the actions of the parasympathetic system leads to production of secretions and increased food metabolism and assimilation showing the need to have stable calcium levels. The concentrations used in the study were not associated with any major changes in tissue antioxidant proteins, showing a lack of sufficient evidence to imply that MSG is involved in smooth and skeletal muscle atropic changes following tissue damage which has previously been reported [31]. This implies that great differences would exist in the methodologies and what happens in man. In addition, the concentrations were not associated with any significant structural changes in both skeletal and smooth muscles. These findings are in agreement with Claire et al., (2011), who showed that 2% MSG promotes glutamate synthesis which is important for intestinal activity during food metabolism [32]. This is highly important since glutamate has been shown to promote muscle integrity [33]. This shows that the safety of MSG at these dosages has beneficial effects in animals.

CONCLUSION

Concentrations of MSG used in this study were not associated with any toxic effects in rats. Usage of MSG at these low concentrations through feed supplement appears to be safe.

Recommendations

Further studies should concentrate on determining the effect(s) on chronic use and localization of MSG in the different sections of the gastro-intestinal tract and different skeletal muscles on both acute and chronic use to provide concrete evidence on its distribution in muscle tissues.

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