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Histological and Morphometric Studies of the Effect of Monosodium Glutamate (MSG) on the Cardiac Muscle Fibres of Adult Albino Rats

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ABSTRACT

This research was to determine the histological effect of monosodium glutamate (MSG) on the cardiac muscle fibres of adult albino rats. Thirty (30) albino rats were divided into five (5) groups (A-E) of six rats each at random and administered orally with aqueous solution of MSG daily. Group A served as the control group and received normal saline. Group B served as the low dose group and received 4mg/kg body weight of MSG for 28 days. Group C served as the high dose group and received 8mg/kg body weight of MSG for 14days, while group D served as the high dose group and received 8mg/kg body weight of MSG for 28 days and group E served as the high dose group and received 8mg/kg body weight of MSG for 28 days and allowed for another 28 days post

treatment to observe for reversibility, persistence or delayed occurrence of any toxicity. The aqueous solution of MSG was at the concentration of 80g/litre daily for the periods of fourteen (14) days and twenty-eight (28) days respectively. At the end of the experimental period, the animals were anaesthetized using chloroform, sacrificed and the heart was carefully removed and weighed before fixing in 10% formalin. The heart tissue was then processed for paraffin sectioning. Administration of MSG to rats showed a significant ($p < 0.05$) increase in the thickness of myocytes, width of extracellular spaces and muscle fibers discontinuity with severity increasing with doses and time of exposure. Withdrawal of MSG for 28 days showed some degree of recovery by a reduction in thickness of myocytes and extracellular spaces as well as muscle fibers discontinuity. There was increase in size and number of fibroblasts when compared with the control. These findings suggest that the histological organization of the cardiac muscle fibers can significantly be altered with continuous and/or increased used of MSG.

1. INTRODUCTION

Glutamate is a naturally occurring amino acid that is found in nearly all foods, especially high protein foods such as dairy products, meat, fish and many vegetables. Foods often used for their flavouring properties such as mushrooms and tomatoes have high levels of naturally occurring glutamate. MSG is used as a chemical additive by food industries and is commonly marketed as a flavour enhancer (Leung and Foster, 2003). MSG is made predominantly from bacterial fermentation, in its pure form it appears as a white crystalline powder which rapidly dissociates into sodium cations and glutamate anions on contact with water (Ikeda, 1908).

MSG is one of the most extensively research substances in food supply industry. The most common source of dietary MSG is the soup, particularly maggi cube sold in the grocery stores and are used in making up the flavour and taste of soups (Eka, 1984).MSG as a food ingredient has been subjected to several health studies/researches. A report from the Federation of American Society for Experimental Biology (FASEB) compiled in 1995 on behalf of the United States Food and Drugs Administration (FAD) concluded that MSG is safe for most people when eaten at customary levels. In 1992, in a symposium held in Lagos, Nigeria organized by the Food and Drug Administration and control (FDA & C) a representative of West African Seasoning Company Ltd, makers of Ajinomoto made known that Ajinomoto which contain above 95% MSG is an ideal food enhancer that contains adequate iodized salts (Okwuraiwe, 1992).

Some abnormalities often culminate in heart failure, an extremely common result of many forms of heart diseases. In heart failure, often called congenital heart failure (CHF), the heart is unable to pump blood at the rate commensurate with the requirements of the metabolizing tissues or can do so only at an elevated filling pressure. Although usually cause by a slowly developing intrinsic deficit in myocardial contraction, a similar clinical syndrome is present in some patients with heart suddenly presented with a load that exceeds its capacity for example fluid overload, acute myocardial infarction, acute valvular dysfunction or in which ventricular filling is impaired (Cohen *et al*., 1996).

The cardiac myocytes is generally considered a terminally differentiated cell that has lost its ability to divide under normal circumstances, functionally useful augmentation of myocytes number (hyperplasia) cannot occur. Increasedmechanical load causes an increase in the content of subcellular components and a consequent increase in cell size (hypertrophy) (Pasmarthi and Field, 2002). Increase mechanical work owning to pressure or volume overload or trophic signal like hyperthyroidism through stimulation of beta-adrenergic receptors increases the rate of protein synthesis, the amount of protein in each cell, the number of sarcomere and mitochondria, the dimension and mass of myocytes and consequently the size of the heart (Pasmarthi and Field, 2002).

The pattern of hypertrophy reflects the nature of the stimulus, pressure-overloaded ventricles example in hypertension or aortic stenosis develops pressure-overload called concentric hypertrophy of the left ventricle with an increase wall thickness. In pressure overload, the predominant deposition of sarcomere is parallel to the long axis of cell; cross sectional area of myocytes is expanded but cell length is not (Robbins and Cotran, 1999). In contrast, volume overload stimulate deposition of new sarcomeres and cell width is increased, thus, volume-overload hypertrophy is characterized by dilation with increased ventricular diameter. In volume overload, muscle mass and wall thickness are increase approximately in proportion to chamber diameter (Robbins and Cotran, 1999).

The increase myocytes size that occurs in cardiac hypertrophy is usually accompanied by decreased capillary density, increased intercapillary distance, and deposition of fibrous tissue. Nevertheless, the enlarged muscle mass has both increased metabolic requirements and increased wall tension, both are major determinants of the oxygen consumption of the heart (Robbins and Cotran, 1999).

Studies have revealed some adverse/negative manifestation of MSG on different body organs and tissues. They include swollen prostate, vomiting, diarrhoea, insomnia, severe headache (Sameul, 1995). Prolonged administration of MSG caused disrupted and distorted cyto-architecture of the kidney in adult Wistar rats (Eweka, 2007). In the same study, there was varying degrees of

dilatations of the central vein of the liver with the presence of lysed red blood cells. In another study, MSG has been implicated to have had a destructive effect on the Brunner's glands of the duodenum and the small intestinal mucosa of the adult Wistar rats (Eweka and Om'Iniabohs, 2007). Injurious effect of MSG has also been linked to male infertility as it causes degeneration and alteration of sperm cell population and morphology (Oforofuo *et al.*, 1997). This negative effect of MSG on the male reproductive organ was further strengthened by a study on the testis which revealed significant oligospermia and increase in abnormal sperm morphology (Onakewhor *et al.*, 1998). In another study, oral consumption of high dose of MSG causes oxidative stress an important biomarker in the cardiac dysfunction. This was evidenced by significant increase in some biochemical markers like transaminase, creatine phosphokinase and lactate dehydrogenase activities in the serum (Paul *et al.*, 2012).

However, despite the widespread research on MSG, an extensive literature search revealed no scientific work has been reported on histological and morphometric evaluation of MSG on the cardiac muscle fibres hence the need for this study.

2. MATERIALS AND METHODS

Thirty mature male albino rats weighing 210±10g were used in this study. They were obtained from the Department of Pharmacology, University of Jos, Jos plateau state. The rats were left to acclimatize for two weeks at normal conditions with food and water freely. The MSG used was in the form of Aji-no-motto (Aji-no-motto co. Inc. Tokyo, Japan) obtained from Monday market Maiduguri, Borno state. Thirty (30) albino rats were divided into five (5) groups (A-E) of six rats each at random and administered orally with aqueous solution of MSG daily. Group A served as the control group and received normal saline. Group B served as the low dose group and received 4mg/kg body weight of MSG for 28 days, group C served as the high dose group and received 8mg/kg body weight of MSG for 14days, while group D served as the high dose group and received 8mg/kg body weight of MSG for 28 days and group E served as the high dose group and received 8mg/kg body weight of MSG for 28 days and allowed for another 28 days post treatment. The aqueous solution of MSG was at the concentration of 80g/litre daily for the periods of fourteen (14) days, and twenty-eight (28) days respectively. The rats from group B were sacrificed after fourteen (14) days of treatment while rats from groups C and D as well as those from the control group were also sacrificed after twenty-eight (28) days of MSG administration. The rats from group E were left untreated for twenty-eight (28) days of post treatment period to allow it to recover from any effect(s) of MSG before being sacrificed. The rats were anaesthetized with chloroform and the thorax opened using ventro-median incision between the jugular notch and the xyphoid process. The heart was removed and striped off of para-aotic fat and fascia before weighing using Mettler Toledo digital/sensitive weighing balance and fixed in 10% formalin. The heart tissue was then processed for paraffin sectioning. Morphometric measurements of the thickness of the cardiac muscle fibres (myocytes) and the width /distance between myocytes (extracellular spaces) using ocular micrometer were made. Measurements were taken from twenty (20) different fields in each slide, with the mean ± SEM values of 15.12 ± 0.66µm for the thickness of myocytes and 10.93± 0.59µm for the extracellular spaces of the cardiac muscle fibres. The measurements obtained were subjected to statistical analysis; the Analysis of Variance (ANOVA), resident of the InStat3 Graph Pad software for windows 2003 was used.

3. RESULTS

Width of extracellular space

There was a significant ($p<0.05$) increase in the width of extracellular spaces of the rats cardiac muscle fibres treated with 4mg/kg body weight of MSG for 28 days when compared with those in the control group. Similarly, significant ($p<0.001$) increase in the width of extracellular spaces of cardiac muscle treated with 8mg/kg body weight of MSG for 14days and 8mg/kg body weight of MSG for 28 days were observed, whereas when the recovery group was computed with the 8mg/kg body weight of MSG for 28 days group, there was no significant increase (Table 1).

Table 1 Effect of Oral Administration of MSG on the Width of Extracellular Spaces of the cardiac Muscle of Albino Rats

Groups (n=6)	Dosage (mg/kg)	Duration (Days)	Width of Extracellur spaces (µm)
A	0		10.93 ± 0.59
B	4	28	15.84 ± 1.19*
C	8	14	21.66 ± 1.10*
D	8	28	23.30 ± 1.66*
E	8	28 + 28 days PT	18.57 ± 1.37[a]

Significant relative to control: *$p<0.05$; *$p<0.001$; [a] 8mg/kg body weight of MSG for 28 days compared with 28 days post treatment; values are mean ± SEM; PT=post treatment

Thickness of myocytes

There was a significant ($p<0.05$) increase in the thickness of myocytes of rats cardiac muscle treated with 4mg/kg body weight of MSG for 28 days when compared with those in the control group. Also there was a significant ($p<0.001$) increase in the thickness of myocytes treated with 8mg/kg body weight of MSG for 14days and 8mg/kg body weight of MSG for 28 days. In comparing the 8mg/kg body weight of MSG for 28 days with the recovery group, there was no significant increase in thickness of myocytes observed (Table 2).

Table 2 Effect of Oral Administration of MSG on the Thickness of Myocytes of the Cardiac Muscle of Albino Rats

Groups (n=6)	Dosage (mg/kg)	Duration (Days)	Thickness of Myocytes (μm)
A	0		15.12 ± 0.66
B	4	28	19.42 ± 1.35*
C	8	14	25.11 ± 1.54*
D	8	28	26.22 ± 1.26**
E	8	28 + 28 days PT	21.85 ± 1.56[a]

Significant relative to control: *$p<0.05$; **$p<0.001$; [a] 8mg/kg body weight of MSG for 28 days compared with 28 days post treatment; values are mean ± SEM; PT=post treatment

Histological Analysis

Light microscopic study of the longitudinal sections obtained from the rats in the control group showed normal histological arrangement of cardiac muscle fibres, myocytes and extracellular spaces (Figure 1). Administration of 4mg/kg, 8mg/kg body weight of aqueous solution of MSG for 28 days, 8mg/kg body weight of aqueous solution of MSG for 14days and 8mg/kg body weight of aqueous solution of MSG for 28 days to the rats showed increase in thickness of myocytes, increase in width of extracellular spaces and some discontinuous muscle fibres (Figures 2-4). The section of cardiac muscle of the rats administered with 8mg/kg body weight of MSG for 28 days and allowed for another 28 days post treatment period showed some degree of recovery by reduction in the thickness of myocytes, reduction in the muscle fibres discontinuity with increasing number and size of fibroblast when compared with the sections from rats administered with 8mg/kg body weight of MSG for 28 days (Figure 5).

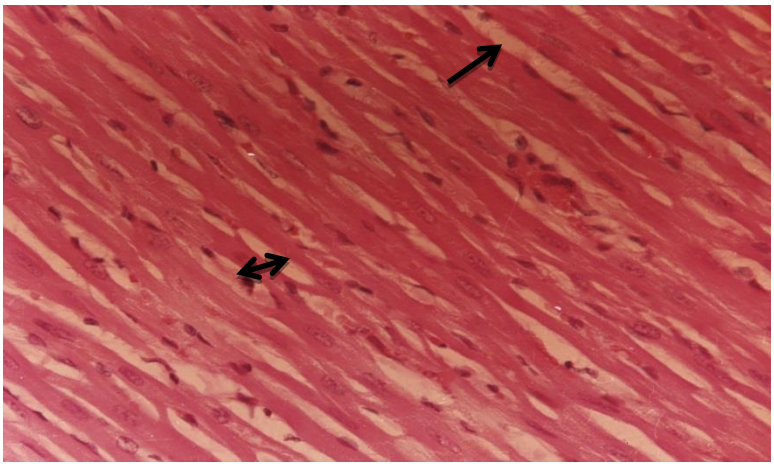

Figure 1

Photomicrograph (L/S) of cardiac muscle of control rat showing extracellular space (single-headed arrow) and thickness of myocytes (double-headed arrow) H & E x400.

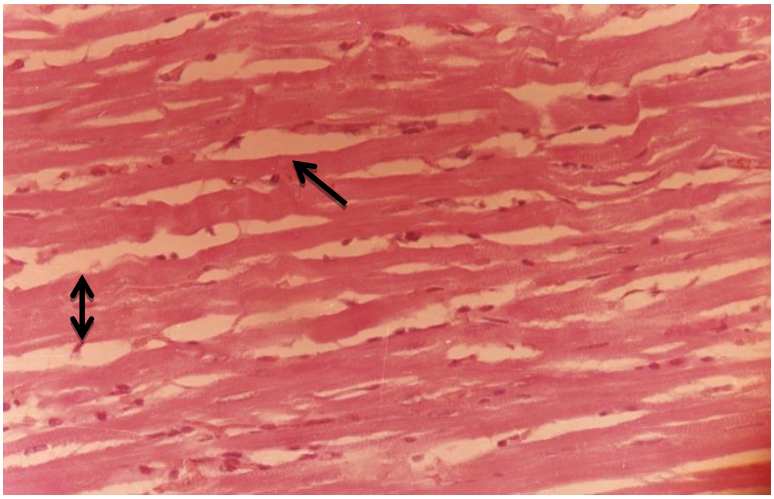

Figure 2

Photomicrograph of cardiac muscle treated with 4mgkg^{-1} body weight of MSG for 28 days showing increased extracellular spaces (single-headed arrow), and increased thickness in myocytes (double-headed arrow) H & E x400.

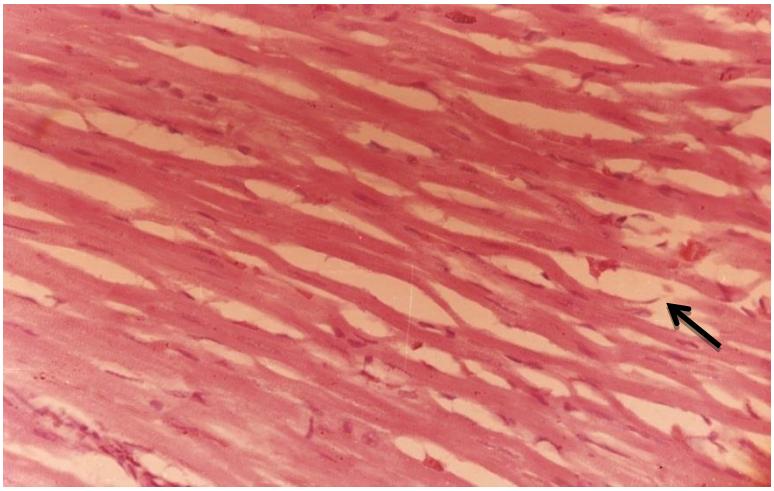

Figure 3

Photomicrograph (L/S) of rat cardiac muscle treated with 8mgkg^{-1} body weight of MSG for 14days showing discontinuous muscle fibers (arrow) H & E x400.

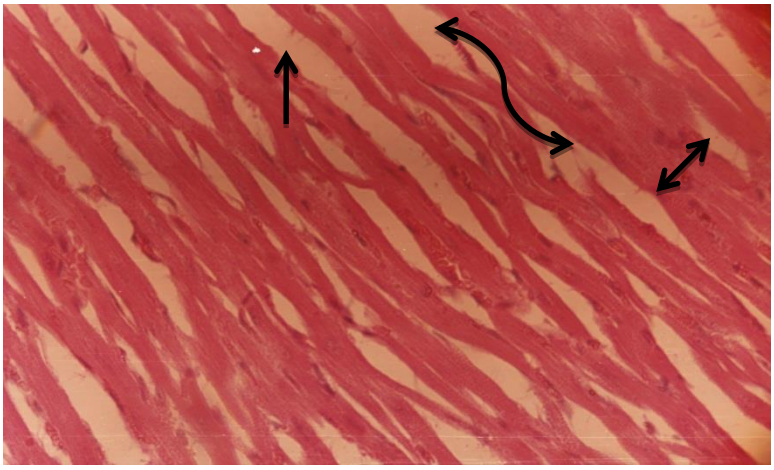

Figure 4

Photomicrograph (L/S) of cardiac muscle treated with 8mgkg^{-1} body weight of MSG for 28 days showing increased extracellular space (single-headed arrow), increased thickness in myocytes (double-headed arrow), and discontinuous muscle fiber (curved double arrow) H&E x400

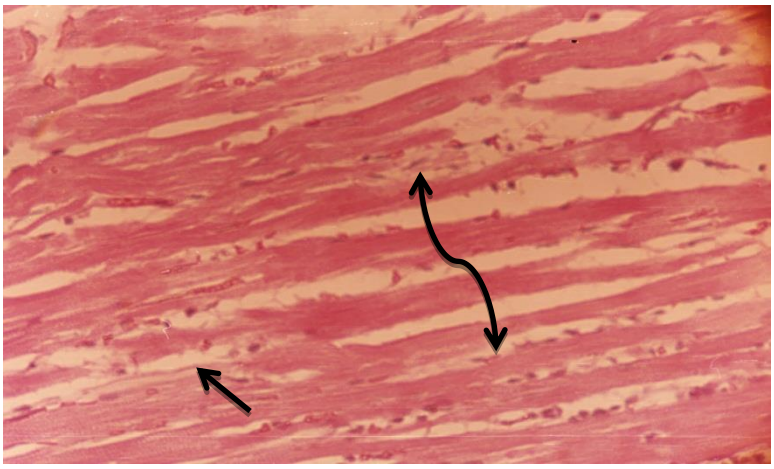

Figure 5

Photomicrograph (L/S) of the rat cardiac muscle treated with 8mgkg^{-1} body weight of MSG for 28 days, and allowed to recover for 28 days. Showing discontinuous muscle fiber (arrow) and increase in size and number of fibroblasts (curved double arrow) H & E x400.

4. DISCUSSION

Administration of 4mgkg^{-1} body weight, 8mgkg^{-1} body weight of MSG for 28 days and 8mgkg^{-1} body weight of MSG for 14days to the rats showed discontinuous muscle fibers. This shows injurious influence of MSG on the cardiac muscle as reported by Eweka (2007).

In addition to those cells of the myocardium whose primary function is contraction, there is a specialized system made up of modified muscle cells whose function is to generate the stimulus for the heart beat and to conduct the impulse to the various parts of the myocardium in such a way as to ensure the contraction of the atria and ventricle in the proper succession, so that the heart acts as effective pump. This system consists of the sinoatrial node, atrioventricular node, and atrioventricular bundle (Bundle of His). The specialized cells of the nodal tissue are distinctly smaller than ordinary cardiac muscle fibers and dense connective tissue.

The nodal fiber appears to be continuous with ordinary muscle fibers. The node is richly innervated by both the sympathetic and parasympathetic divisions of the autonomic nervous system. In cardiac muscle, which is characterised by the ability to beat rhythmically without nervous or other external stimuli, the cells with the most rapid inherent system establish the rate of beating of the rest of the myocardium. In warm-blooded animals, the fibers of the sinoatrial node have the most rapid rhythm, and this is why they are referred to as the 'pacemaker' of the heart (Sjostrand *et al,* 1958; Rhodin *et al*, 1961).

Since the nodal fibers are also embedded in the cardiac muscle fibers, discontinuous muscle fibers shown in the treated cardiac muscle with MSG may results in interrupted impulse conduction through the intercalated discs. Disease of the conduction system results in asynchrony in the beating of the ventricles or disorders in the timing of the contraction of the atria and ventricles which results in impaired efficiency of the heart (Bloom and Fawcett, 1968; Robbins and Cotran 1999).

The cardiac muscle of the rats administered with 8mgkg^{-1} body weight of MSG for 28 days and allowed to stay for another 28 days post treatment showed a degree of recovery and an increased number of fibroblasts when compared with the rats administered with 8mgkg^{-1} body weight of MSG for 28 days.

Fibroblasts are the common fixed cells of the connective tissue that elaborate the precursors of the extracellular fibrous and amorphous components. They invade an area under a condition of stimulation, as in wound healing or repairing connective tissue (Bensley, 1934). The regenerative capacity of fibroblasts and the fact that they respond so readily to injury by proliferation and fibrogenesis make them the principal agent of repair. They are involved in healing of defects, not only in connective tissue proper, but also in other tissues that have little or no regenerative capacity of their own. For example, the heart muscle that degenerates following a heart attack is replaced by a connective tissue scar. The invasion of fibroblasts in the cardiac muscle treated with MSG and allowed for recovery period of 28 days is suggestive of tissue repairs.

The significant increase observed in the extracellular spaces and myocytes are indications for cardiac muscle hypertrophy. Hypertrophy of the cardiac muscle results from mechanical overload/increased blood pressure which triggers increase in subcellular components and a consequent increase in the size of organelles of myocytes (sarcomere, mitochondria) and increased protein

synthesis which ultimately are being deposited in the extracellular spaces of the muscle (Robbins and Cotrans, 1999; Pasmarthi and Field, 2002).

The insignificant increase in the thickness of myocytes and extracellular spaces observed between the 8mgkg^{-1} body weights treated rats and the recovery animals were suggestive of decreasing thickness of myocytes and extracellular spaces following the withdrawal of the MSG.

The difference in significance between the 4mgkg^{-1} and 8mgkg^{-1} body weight treated rats (that is significant and extremely significant) respectively, which may be an indication of dose dependency as previously reported by (Palaaz *et al,* 1999).

Hypertrophy of the heart occurs in response to increased stress on the heart. The most common causes are related to increase blood pressure in the body and it's the most frequent cause of left ventricular hypertrophy (LVH) or hypertrophy cardiomyopathy (HCM). The extra work of pumping blood against the increased pressure causes the ventricle to thicken over time, the same way skeletal muscle increases in mass in response to weightlifting (Huether and McCance, 2008).

Symptoms of HCM includes dyspnea (shortness of breath) which is due to increased stiffness of the left ventricle which impairs filling of the ventricle and leads to elevated pressure in the left ventricle and atrium. Other symptoms include chest pain (angina), palpitation and fatique which are also associated with the Chinise Restaurant Syndrome as reported by Samuel (1995).

5. CONCLUSION

In conclusion, the findings revealed that the histological organisation of the cardiac muscle can be significantly altered with continuous and increased use of MSG while the morphological/structural observation shows that the cardiac muscle can significantly be thickened and extracellular spaces widened which will ultimately lead to cardiac muscle hypertrophy.

Conflicts of Interest

None

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