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Role of Beta-Carotene on the Histological Features of Neonatal Albino Rat Cardiocytes Treated with Monosodium Glutamate.

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Abstract

The objective of this study was to analyze the cardiac effects of exposure to monosodium glutamate for a period of 7 days alone and in combination with beta-carotene supplementation. Rats were allocated into: control (I), experimental group (II) exposed to 4 mg of monosodium glutamate for 7 days and experimental group (III) exposed to monosodium glutamate and 300 mg/kg/diet beta-carotene for 7 days. After three days of randomization, cardiac structure was assessed by electron microscopy. The myocardial cells after exposure to monosodium glutamate illustrated branching cytoplasmic network of cardiac muscle with cross striations which are retracted around the nuclei. These nuclei showed dense and electron dense heterochromatin that associated with nuclear covering and dense chromatin distributed in the nucleoplasm. Delicate supportive tissue filling the intercellular spaces containing blood was also seen. Ultrastructurally, vacuolation of the mitochondria with fragmentation of its cristae with loss of its striations were observed. The myofibrils were dissociated with irregularities of its sarcomere. Empty spaces in the cytoplasm with disrupted intercalated disc were observed. In beta-carotene plus monosodium glutamate, most fibers showed normal morphological aspects. In conclusion: One week monosodium glutamate exposure induces morphological cardiac alterations and beta-carotene supplementation attenuates this ventricular remodeling process.

Key words: Beta-carotene, Monosodium Glutamate, Histology of cardiocytes, Neonatal Albino Rats

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Introduction

Monosodium glutamate (MSG), a widely used food additive, is known to induce neural damage in the immature nervous system [1]. The amino acid glutamate is abundantly present in the mammalian central nervous system (CNS) and is the principal excitatory neurotransmitter in the brain [2]. Glutamate receptors (GluRs) have been implicated in brain function and pathology. Their presence in peripheral tissues suggests a vital role in the pathophysiology of various organ systems [3]. Gill et al. [4] have hypothesized that GluRs play a role in mediating the cardiac effects of these glutamate analogues.

There are many other exogenous substances acting as glutamate analogues that possess excitatory properties and potential excitotoxic effects. Glutamate and its analogues may enter the food supply during food preparation and food processing, as contaminants and/or additives [5].

Domoic acid is one of the most potent excitatory amino acids and neurotoxins that can enter the food chain. It is produced by a phytoplankton that is ingested and accumulated in the digestive system of seashells such as mussels [6]. A variety of excitatory amino acids can also be present in foods including aspartate and monosodium glutamate [7]. The association of arrhythmias and other cardiovascular symptoms in humans intoxicated with DA and on individuals susceptible to MSG prompted us to focus our attention to the investigation of GluRs in heart [8].

The antioxidant nutrients such as carotenoids [9] are attractive agents that could protect against cardiovascular injury, assuming that they are capable of preventing oxidative damage. Therefore, several observational and prospective epidemiological studies have consistently shown an inverse relationship between dietary intakes or blood evels of beta-carotene (BC) and cardiovascular diseases [10].

Therefore, the objective of this study was to investigate the effects of monosodium glutamate injection, isolated or in association with dietary BC, on the neonatal cardiac muscle fibers in rats.

Materials and Methods

Twelve neo-natal male Sprague-Dawley rats were housed with their mothers individually with food and water available ad libitum. All animals were housed in clear plastic cages in a colony room. The neo-natal rats were divided into three groups: Group (I) (control; n = 4), control rats neither exposed to MSG nor supplemented with BC; experimental group (II) (n = 4) were pretreated by four milligrams of monosodium glutamate per gram body weight was injected subcutaneously in the neonatal rats, every day for 7 days and experimental group (III) (n = 4) fed with the standard diet, supplemented with BC (obtained from International Vitamin Corporation) received orally (300 mg/kg/diet) dissolved in saline daily and MSG injected as in the experimental group (II) simultaneously for one week. The rats were anesthetized by intraperitoneal injection of 50 mg/kg pentobarbital sodium and sacrificed. The cardiac muscles from the left ventricle were removed. Cardiac muscle tissue was perfused with normal saline solution followed by phosphate-buffered 2% glutaraldehyde and 4% paraformaldehyde.

The cardiac muscle was carefully removed and immersed in the same fixative, and 2-mm coronal slices were prepared. Each tissue sample was then post-fixed in 1% potassium-ferrocyanide reduced osmium tetroxide, dehydrated in graded acetones, and embedded in Epon 812. Semi-thin (0.5 um) sections were cut from tissue blocks and stained with 0.5% toluidine blue. The sections were stained with 0.25% lead citrate and 5% uranyl acetate in 50% methanol and were observed and photographed by an electron microscopy technician using a JEOL 100CX electron microscope [11].

Results

Group I (control)

With regard to touilidine blue stain, the rat cardiocytes showed clarified branching cytoplasmic network, cross striations, delicate supporting tissue filling the intercellular spaces that contain a network of blood capillaries and many rows of mitochondria. Ultrastructurally, the rat cadiocytes were noticed the striation pattern, typical alteration of myofilaments, normal appearance of mitochondria with well-preserved mitochondrial cristae and transversely oriented parts of intercalated disc. (Figs. 1, 2 and 3).

Experimental groups II (the rats treated with MSG alone)

With regard to touilidine blue stain, the rat cardiocytes illustrated branching cytoplasmic network of cardiac muscle with cross striations which are retracted around the nuclei with dense and electron dense heterochromatin, associated with nuclear covering and dense chromatin distributed in the nucleoplasm and delicate supportive tissue filling the intercellular spaces containing blood. Ultrastructurally, vacuolation of the mitochondria with fragmentation of its cristae myofibrils with irregularities of the sarcomere, empty spaces in the cytoplasm and disrupted intercalated disc were observed (Figs. 4, 5, 6, and 7).

Experimental group III (the rats treated with MSG and BC)

With regard to touilidine blue stain, the rat cardiocytes were noticed most of fibers had normal morphological aspects except disorganization of myofilaments with irregular-shaped nuclei are evident. Ultrastructurally, the rat cardiocytes show vacuolation of the mitochondria with fragmentation of its cristae, dissociation of myofibrils with irregularities of the sarcomere, empty spaces (Figs. 8 and 9).

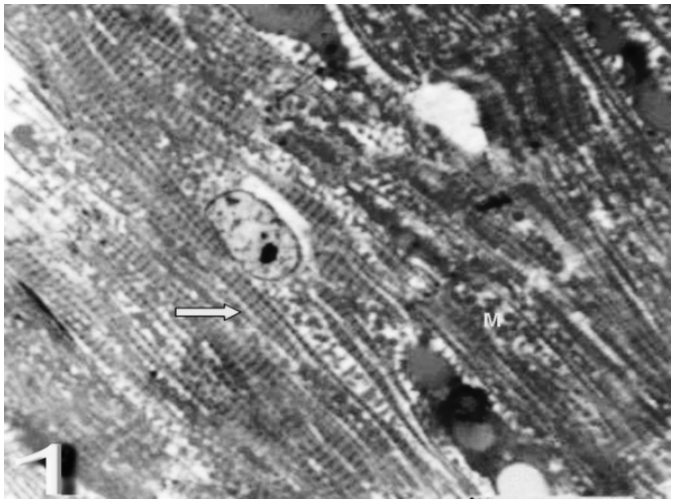

***Figure 1**: Photomicrograph of a cardiocyte from the neonatal rats of the control group. The rat cardiocytes show clarified branching cytoplasmic network (arrow), cross striations, delicate supporting tissue filling the intercellular spaces that contain a network of blood capillaries (BC) and many rows of mitochondria (M).*
Toluidine blue: Final magnifican, X 1000.

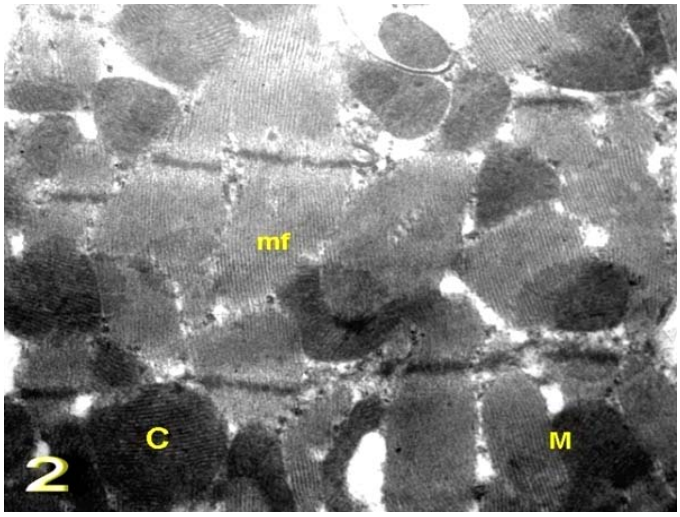

Figure 2: *Electron micrograph of a cardiocyte from the neonatal rats of the control group. The rat cardiocytes show typical alteration of myofilaments (mf) that consists of prominent A (dark) and I (light) bands that bisected by a thick dark Z band. The myofilaments are subdivided by rows of mitochondria (M) with well-preserved cristae (C). Uranyl acetate and lead citrate: Final magnifican, X 20,000.*

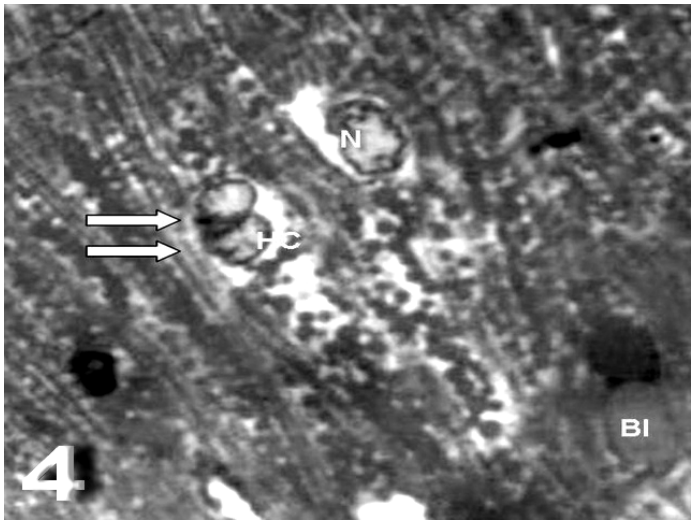

Figure 4*: Photomicrograph of a cardiocyte from the rats treated with monosodium glutamate. The rat cardiocytes illustrates an extremely thin resin-embedded section showing branching cytoplasmic network of cardiac muscle with cross striations (arrows) which are retracted around the nuclei (N) with margination of its heterochromatin (HC) with some myofibrils that appear disrupted. Also, note the delicate supportive tissue filling the intercellular spaces containing blood (Bl).Toluidine blue: Final magnifican, X 1000.*

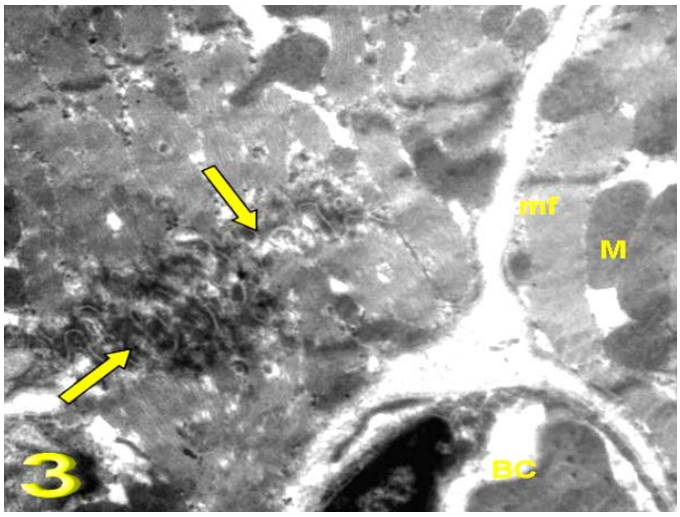

Figure 3*: Electron micrograph of a cardiocyte from the normal neonatal rats. The rat cardiocytes show transversely oriented parts of intercalated disc (arrows) and intercellular spaces that contain a network of blood capillaries (BC). Note the presence of typical alteration of myofilaments (mf) and rows of mitochondria (M). Uranyl acetate and lead citrate: Final magnifican, X 20,000.*

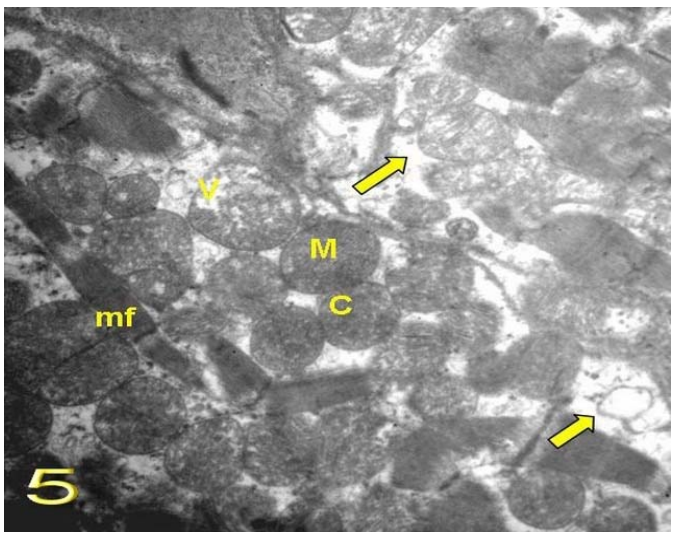

Figure. 5: *Electron micrograph of a cardiocyte from the rats treated with monosodium glutamate. The rat cardiocytes show many swollen mitochondria (M) with disorganized crests (C) and vacuoles (V) inside it. Some disrupted myofibrils (mf) appear in the left corner of the figure with its striations. Many spaces (arrows) inside the cytoplasm also appear.Uranyl acetate and lead citrate: Final magnifican, X 20,000.*

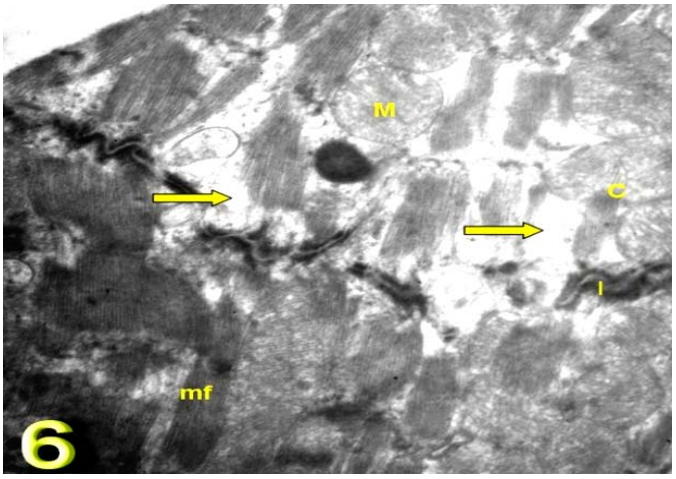

Figure 6: *Electron micrograph of a cardiocyte from the rats treated with monosodium glutamate. The rat cardiocytes show vacuolation of the mitochondria (M) with fragmentation of its cristae (C), dissociation of myofibrils (mf) with irregularities of the sarcomere, empty spaces (arrows) and disrupted intercalated disc (I). Uranyl acetate and lead citrate: Final magnifican, X 20,000.*

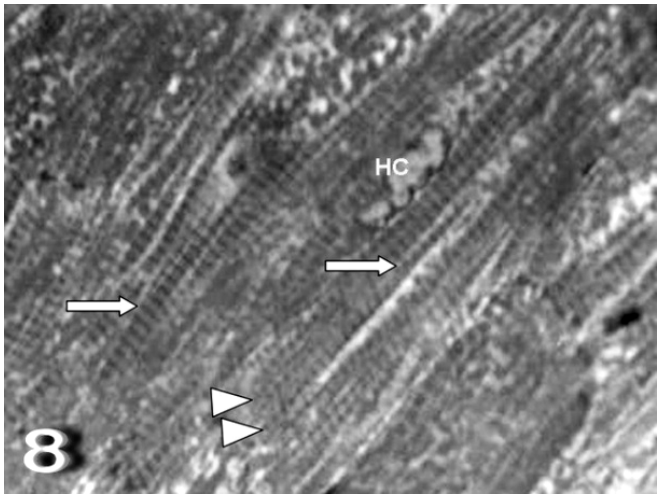

Figure 8: *Photomicrograph of a cardiocyte from the rats treated with beta-carotene and monosodium glutamate. The rat cardiocytes show most of the fibers has normal morphological aspects (arrows) except some disorganization of myofilaments (arrow heads) and margination of heterochromatin (HC) of the nucleus still present. Toluidine blue: Final magnifican, X 1000.*

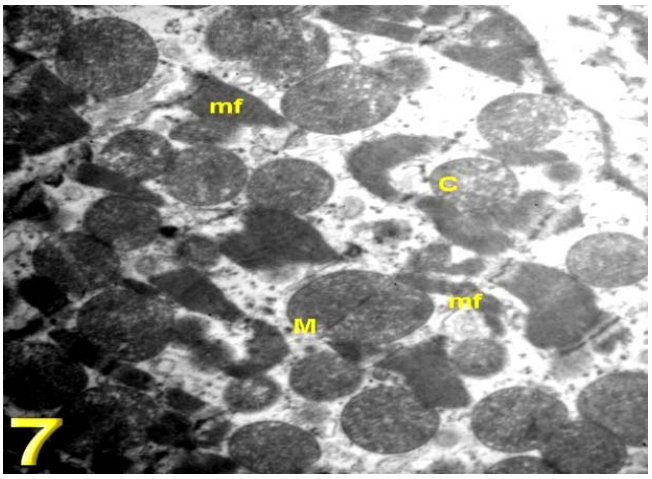

Figure 7: *Electron micrograph of a cardiocyte from the rats treated with monosodium glutamate. The rat cardiocytes show mitochondria (M) with condensed cristae (C). The myofibrils (mf) are dissociated and scattered in the cytoplasm. Uranyl acetate and lead citrate: Final magnifican, X 20,000.*

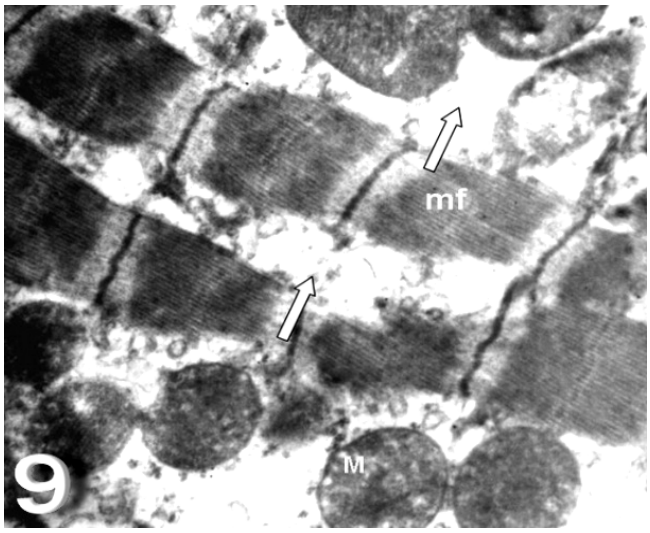

Figure 9: *Electron micrograph of a cardiocyte from the rats treated with beta-carotene and monosodium glutamate. The rat cardiocytes show swollen mitochondria (M) with some vacuoles inside one of them (V). The myofibrils (mf) appear more or less normal. Some spaces (arrows) appear in the cytoplasm. Toluidine blue: Final magnifican, X 1000.*

Discussion

An investigation was carried out on the effects of MSG, a commonly used as food additive, on the cardiac muscle of neo-natal rats [12].

In the present study, the ultra-structural results revealed that the cardiocytes submitted to MSG showed vacuolation of the mitochondria with fragmentation of its cristae with loss of its striations. The myofibrils were dissociated with irregularities of its sarcomere. Empty spaces in their

cytoplasm with disrupted intercalated disc were observed. The presence of vacuoles inside the mitochondria of group II is indicative of irreversible injury, however oncocytic or apoptotic cells were not found, although without receiving oxygen to maintain the aerobic metabolism, the structures remained in a quiescent state [13]. However, Kennedy and Rice [14] found sublethally damaged cardiocytes that had lysed contractile material; vacuolated sarcoplasm; altered mitochondria and pyknotic nuclei. Necrosis was followed by macrophage invasion and phagocytosis of necrotic debris. Repair of the lesions was by deposition of collagen and elastic fibers.

Regarding the current work, the experimental group III (the rats treated with MSG & BC), the rat cardiocytes revealed that most of the cardiac muscle fibers had normal morphological aspects except disorganization of myofilaments with irregular-shaped nuclei are evident with touilidine blue stain. Ultrastructurally, the rat cardiocytes of this group were noticed vacuolation of the mitochondria with fragmentation of its cristae, dissociation of myofibrils with irregularities of the sarcomere and empty spaces in the cytoplasm also appeared.

Antioxidants have been studied for their capacity to prevent the chronic disease. However, the use of antioxidants in cardiovascular diseases remains controversial. Discrepancy among results from their use may be related to patient characteristics or doses of antioxidants [15].
Beta-carotene and other carotenoids are mainly considered as belonging to the group of micronutrients. As they are contained in fruit and vegetables and thus part of human diet, a regular low-dose intake from natural sources is normally assured. In the last decade high-dose supplementation with synthetic carotenoids has been used successfully in the treatment of diseases believed to be associated with oxidative stress. However, in a few clinical studies harmful effects have been observed as well, e.g., a higher incidence of lung cancer after BC was given in high doses to smokers [16]. Beta-carotene has been better characterized with regard to its antioxidant capabilities; observational and prospective epidemiologic studies have shown an inverse relationship between cardiovascular disease and dietary intake of carotenoids and/or blood levels [17, 18]. Four large interventional trials were designed to test the hypothesis that BC protects against cancer and/or cardiovascular disease development in humans. In these studies, doses of BC were higher than those that could be achieved from the habitual diet, and the BC blood levels were 26 times higher than the 95th percentile level of BC in the Health and Nutrition Examination Survey of the United States [19, 20, 21, and 22]. Three out of these four intervention trials did not show any protective effect of BC against cancer or cardiovascular disease; actually, some adverse effects were found.

In fact, the risk of fatal coronary heart disease was increased in the groups that received either beta-carotene or the combination of alpha-tocopherol and beta-carotene [23] and the relative risk of death by lung cancer also increased by 17% in the beta-carotene-supplemented heavy smokers vs. placebo group [21]. Then, it can be speculated that BC is health promoting when taken at a physiologic level, but may take on circumstantially adverse properties when given at a high dose or in the presence of highly oxidative conditions [24]. Antioxidant vitamins reduce cardiac oxidative stress and cardiomyocyte apoptosis produced by exogenous norepinephrine and attenuate cardiac dysfunction in animals with pacing-induced congestive heart failure [25]. The findings observed by Qin et al. [26] suggest that NE-induced myocyte apoptosis is mediated by oxidative stress, and that antioxidant vitamins may be beneficial in heart failure in which cardiac NE release is increased.

In conclusion, our findings suggest that one-week MSG exposure induces morphological cardiac alterations and that BC supplementation attenuates this ventricular remodeling process in rats.

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