Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 4 (5): 178-193, 2009
ISSN 1816-496X

Protective Effect of N-acetyl Cysteine and/or Pro Vitamin A against Monosodium Glutamate-Induced Cardiopathy in Rats

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Abstract: In the present study the prophylactic effects of the antioxidants, β-carotene and/or N-acetyl cysteine (NAC) in ameliorating the metabolic abnormalities and oxidative damage induced cardiopathy under the effect of the flavor enhancers, monosodium glutamate (MSG) toxicity were studied. Animals were divided into 5 groups; G1: normal control, G2: MSG-treated group, Gs 3,4 and 5: animals pretreated with either NAC or β-carotene or their combination prior MSG administration, respectively. The present results revealed that, chronic administration of MSG caused metabolic dysfunction characterized by significant increases in the levels of serum glucose, total lipids, triglycerides (TG), total cholesterol (TCh) and Low Density Lipoprotein (LDL) and a decrease in the high density lipoprotein (HDL), parameters have important role in MSG induced cardiovascular disorders. The adverse effects of MSG may be related to an imbalance between the oxidant and antioxidant systems. This was indicated by marked increased levels of serum nitric oxide (NO) accompanied by pronounced increased level of thiobarbituric acid reactive substances (TBARS, marker of lipid peroxidation) and decreased levels of the antioxidants, L-ascorbic acid, glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) in cardiac tissue versus normal animals. Significant inhibition in cardiac Na^+/K^+ ATPase with increase in serum activities of creatine phophokinase (CPK) and aspartate aminotransferase (AST) were also observed in MSG treated animals as biomarker enzymes of cardiac tissue damage. This result was supported by myocardial infarction (necrotic lesion) observed by histopathological examination. Administration of either β-carotene or NAC prior MSG injection significantly modulated the alteration in most of the previously mentioned parameters to near their normal levels. Administration of synergistic combination of the these antioxidants showed the most significant effect as it has the ability to restore all of the studied parameters to their normal levels. The biochemical results were supported by the improvement in histological architecture of heart tissue, implicating that these antioxidants either alone or their combination may protect heart from the harmful effects of cardio-toxic agents.

Key words: β-carotene, N-acetyl cysteine, monosodium glutamate, heart, lipids

INTRODUCTION

Monosodium glutamate (MSG) [CsHaNO4NaH = O], the sodium salt of glutamate, is one of the widely-used food addictives in our daily diet (Walker and Lupien, 2000). Its consumption has increased throughout the world as flavoring agent in cooking (Chaudari and Roper, 1998) to increase palatability and food selection in a meal (Bellisle *et al*., 1996). However, consumption of this food addictives has been shown to cause metabolic disorders including, hyperlipidemia, hyperglycemia and

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hence oxidative stress (Diniz *et al*., 2005; Nagata *et al*., 2006) which may responsible for the pathophysiology of many diseases like cancer, diabetes, endothelial dysfunction (Lall *et al*., 1999; Naderali *et al*., 2004; Diniz *et al*., 2005), brain lesions (Mallick, 2007) and coronary heart disease (CHD) (Diniz *et al*., 2005; Nagata *et al*., 2006; Singh and Pushpa, 2005). Oxidative cardiac damage and alterations in the levels of thiobarbituric acid reactive substances (TBARS) and antioxidants like reduced glutathione, catalase and superoxide dismutase were reported in adult mice during MSG treatment (Ahluwalia *et al*., 1996; Choudhary *et al*., 1996; Singh and Pushpa, 2005; Farombi and Onyema, 2006). Oxidative stress, owing to increased production of free radicals and decreased levels of antioxidants in the myocardium plays a major role in cardiovascular diseases such as ischemic heart disease, atherosclerosis, congestive heart failure, cardiomyopathy and arrhythmias (Diniz *et al*., 2002; Faine *et al*., 2002).

In recent years, antioxidants have gained a lot of importance because of their potential as prophylactic and therapeutic agents in many diseases. It was reported that the prevention of cardiovascular diseases has been associated with the ingestion of natural antioxidants (Argolo *et al*., 2004).

Carotenoids are a widespread group of naturally occurring fat-soluble pigments. They are especially abundant in yellow-orange fruits and vegetables and in dark green, leafy vegetables (Mangels *et al*., 1993). In human beings, carotenoids can serve several important biological activities (Maiani *et al*., 2008). The most widely studied and well understood nutritional role for carotenoids is their provitamin A activity. Vitamin A, which has many vital systemic functions in humans, can be produced within the body from certain carotenoids, notably β-carotene (Britton, 1995). Deficiency of vitamin A is a major cause of premature death in developing nations, particularly among children. In addition, it was reported that carotenoids potentially play an important role in human health by acting as biological antioxidants, protecting cells and tissues from the damaging effects of free radicals which might be useful in preventing lipid oxidation (Beutner *et al*., 2001; Yeh *et al*., 2009). β-carotene and other carotenoids have been thought to have anti-cancer activity, either because of their antioxidant activity or because of their ability to be converted to vitamin A (Russell, 2004). Also, it was reported that β-carotene supplementation may help to strengthen the immune system (Chew and Park, 2004), increase lung capacity (Schünemann *et al*., 2002), reduce serum lipid profiles, attenuate atherogenesis (Hu *et al*., 2008) and improve the glucose tolerance ability of streptozotocin-induced diabetic rats (Furusho *et al*., 2002). In both observational and case control studies, the intake of carotenoid-rich fruits and vegetables has been found to be inversely correlated with risk for cardiovascular disease (Tavani and La Vechia, 1999).

N -acetyl cysteine (NAC) is precursor of glutathione which plays an essential role in preventing the oxidative damage (Prakash and Kumar, 2009). In particular, NAC is known to increase the intracellular stores of glutathione thereby enhancing the endogenous antioxidant level (Yalcin *et al*., 2008). Its antioxidant properties have recently been reviewed (Harvey *et al*., 2008). It is well-reported that the thiol group in N AC interacts directly with reactive oxygen species leading to cellular protection against oxidative damage (Zafarullah *et al*., 2003). As an antioxidant, NAC has been found to have the beneficial effects of reducing cardiovascular events in haemodialysis patients (Tepel *et al*., 2003). In addition, a number of studies have indicated that NAC has hypolipidemic, hypoglycemic, antifibrogenic and antiatherogenic activities (Murata *et al*., 2003; Lin *et al*., 2004; Kopp *et al*., 2006; Adachi *et al*., 2007; Voghel *et al*., 2008).

In the present study, our interest has been focused on the possibility of the protective influence of either β-carotene, NAC or their combination against metabolic disorders and oxidative stress induced cardiac damage in rats due to chronic administration of toxic MSG. This can be achieved through measuring serum glucose and lipid profiles as well as some oxidative stress markers (NO in serum and lipid peroxidation in cardiac tissue), in addition to some antioxidant markers including, L-ascorbic acid (vitamin C), GSH, SOD and CAT in cardiac tissue. cardiac Na+/K+ATPase, serum CPK and AST as well as histopathological study were also conducted as indices of cardiac tissue injury.

MATERIALS AND METHODS

Chemicals and Drugs

All chemical reagents were of analytical grades purchased from Sigma Chemical Co. (St. Louis, Mo, USA), Merk (Germany) and BDH (England). MSG was purchased from BDH laboratory (Poole, UK) and dissolved in bidistilled water before administration. β-carotene and NAC were purchased from Pharco CO., Egypt. β-carotene was suspended in gum acacia, while NAC was dissolved in bidistilled water before administration.

Animals

Fifty adult male albino rats (150-200 g) were obtained from animal house of National Research Centre, Dokki, Giza, Egypt. The animals were housed in cages under standard hygienic condition and were fed with rat chow and water *ad libitum*. In order to optimize drug absorption, all animals were fasted for 1 h prior to drug administration.

Experimental Design

Rats were divided into five groups, each of ten rats as follow:

- **Group 1:** Normal control rats (not received any medication)
- **Group 2:** MSG-treated animals
- **Group 3:** MSG animals treated with β-carotene
- **Group 4:** MSG animals treated with NAC
- **Group 5:** MSG animals treated with synergistic combination of β-carotene and NAC

MSG cardio-toxicity was induced by intraperitoneal injection of 4 mg g^{-1} b.wt. (Singh and Pushpa, 2005; Farombi and Onyema, 2006) three times a week for three weeks to the rats of different experimental groups except normal control group. β-carotene and NAC (10 and 20 mg kg^{-1}, respectively) (Ritter *et al*., 2004; Dene *et al*., 2005) were administered intraperitoneally three times a week for three weeks one hour before MSG injection. At the end of the experiment, animals of different experimental groups were fasted overnight (12-14 h), the blood samples were collected from each animal into sterilized tubes for serum separation. Serum was separated by centrifugation at 3000 x g for 10 min and used for biochemical serum analysis. After blood collection, rats of each group were sacrificed under ether anesthesia and the hearts from different animal groups were immediately removed, weighed and homogenized in ice cold bidistilled water to yield 10% homogenates using a glass homogenizer. The homogenates were centrifuged for 15 min at 10000 g at 4°C and the supernatants were used for different biochemical tissue analysis.

Biochemical Analysis

Serum Analysis

Fasting blood glucose was measured according to method adopted previously by Miwa *et al*. (1972) using a glucose kit (enzymatic method) (Wako). Total lipids were measured according to the method described by Frings and Dunn (1970), TG was determined using enzymatic colorimetric kits (Wahelfed, 1974). Both TCh and HDL-C were estimated in serum according to the method described by Stein (1986). From the results, LDL cholesterol, TCh/HDL and LDL/HDL ratios were calculated. According to Friedewal *et al*. (1972), LDL can be calculated as follows:

$$\text{LDL} = \text{Total cholesterol-HDL-TG/5}$$

Atherogenic index was calculated from serum HDL and cholesterol levels using the equation previously reported by Gillies *et al*. (1986).

$$\text{Atherogenic index} = \frac{\text{Serum cholesterol level-Serum HDL level}}{\text{Serum HDL level}}$$

Nitrite concentration (an indirect measurement of NO synthesis) was assayed using Griess reagent (sulfanilamide and N-1-naphthylethylenediamine dihydrochloride) in acidic medium (Moshage *et al*., 1995). CPK was assayed using the method described by Rosalki (1967). The AST activity was determined according to the method described by Bergmeyer *et al*. (1986).

Heart Tissue Analysis

Lipid peroxidation was determined by measuring the formed malondialdehyde (MDA an end product of fatty acid peroxidation) by using thiobarbituric acid reactive substances (TBARS) method (Buege and Aust,1978). This assay is based on the formation of red adduct in acidic medium between thiobarbituric acid and MDA, the product of lipid peroxidation was measured at 532 nm. The MDA concentration was calculated using extinction coefficient value (ε) of MDA-thiobarbituric acid complex (1.56×10^5/M/cm). L- ascorbic acid (vitamin C) was estimated by the method of Jagota and Dani (1982) using Folin-Ciocalteu reagent. The colour developed was read at 760 nm. The vitamin C content is expressed as μg g^{-1} tissue. Glutathione was determined using the method of Bentler *et al*. (1963) based on its reaction with 5, 5'-dithiobis (2-nitrobenzoic acid) to yield the yellow chromophore, 5-thio-2-nitrobenzoic acid at 412 nm. SOD activity was determined by monitoring the decrease in absorbance at 340 nm using the method of Paoletti *et al*. (1986). The activity was expressed in terms of percentage inhibition of NADH. CAT was determined by monitoring the decomposition of hydrogen peroxide as described by Aebi (1984). Na+,K+-ATPase activity was assayed using the method of Tsakiris and Dliconstantinos (1984) through measuring released inorganic phosphate (Fiske and Subbarow, 1925).

Histological Evaluation

Representative slices from heart tissue were taken from the eviscerated animals of different experimental groups and fixed in 10% formalin. For light microscopy examination, the tissues were embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin (H and E).

Statistical Analysis

Data were analyzed by comparing values for different treatment groups with the values for individual controls. Results are expressed as Mean±SD. The significant differences among values were analyzed using analysis of variance (one-way ANOVA) followed by Bonferoni as a post ANOVA test (Zar, 1999).

RESULTS

From the Table 1, it can be observed that injection of MSG to rats induces anomalous changes of serum glucose and lipid profile indicated by marked increase in the levels of glucose, total lipids, TG, TCh and LDL accompanied by decreased level of HDL versus normal animals. The abnormalities in these lipid profiles were associated with increase in TCh/HDL and LDL/HDL ratios as well as in atherogenic index (Table 2) in MSG-treated rats in relation to control ones. Administration of either β-carotene, NAC or their synergistic combination prior MSG injection effectively down regulated the abnormal increase in the above serum parameters and the related alteration in the ratios of atherosclerosis indices.

The levels of oxidative stress and antioxidant markers in control and different MSG-treated rat groups are depicted in Fig. 1a-e. In rats injected with MSG there was a significant increase in serum NO accompanied with elevated thiobarbituric acid reactive substances level and marked decrease

Table 1: Levels of serum glucose and serum lipids and lipoproteins in normal and different experimental treated groups

Groups	Glucose (mg dL^{-1})	Total lipids (g dL^{-1})	TG (mg dL^{-1})	TCh (mg dL^{-1})	LDL (mg dL^{-1})	HDL (mg dL^{-1})
Control	83.96±5.35	0.423±0.055	78.88±9.9	168.06±5.4	100.50±4. 9	51.95±2.6
MSG	232.02±7.9[a]	2.160±0.23[a]	257.83±8.3[a]	261.65±9.6[a]	183.98±5.2[a]	22.59±3.5[a]
MSG+β carotein	91.29±4.14	0.480±0.09	84.97±4.9	161.45±9.8	105.02±2.2	45.30±3.7[c]
MSG+N-acetylcysteine	89.63±5.5	0.370±0.07	83.04±6.3	167.54±6.5	100.75±1.35	50.23±3.4
MSG+Snergesitic	82.85±6.6	0.440±0.022	86.22±6.9	163.45±3.9	108.15±2.8[c]	42.83±2.6[b]

Data are Mean±SD of 5 independent experiments, [a]p<0.0001, [b]p<0.01, [c]p<0.05 compared to control

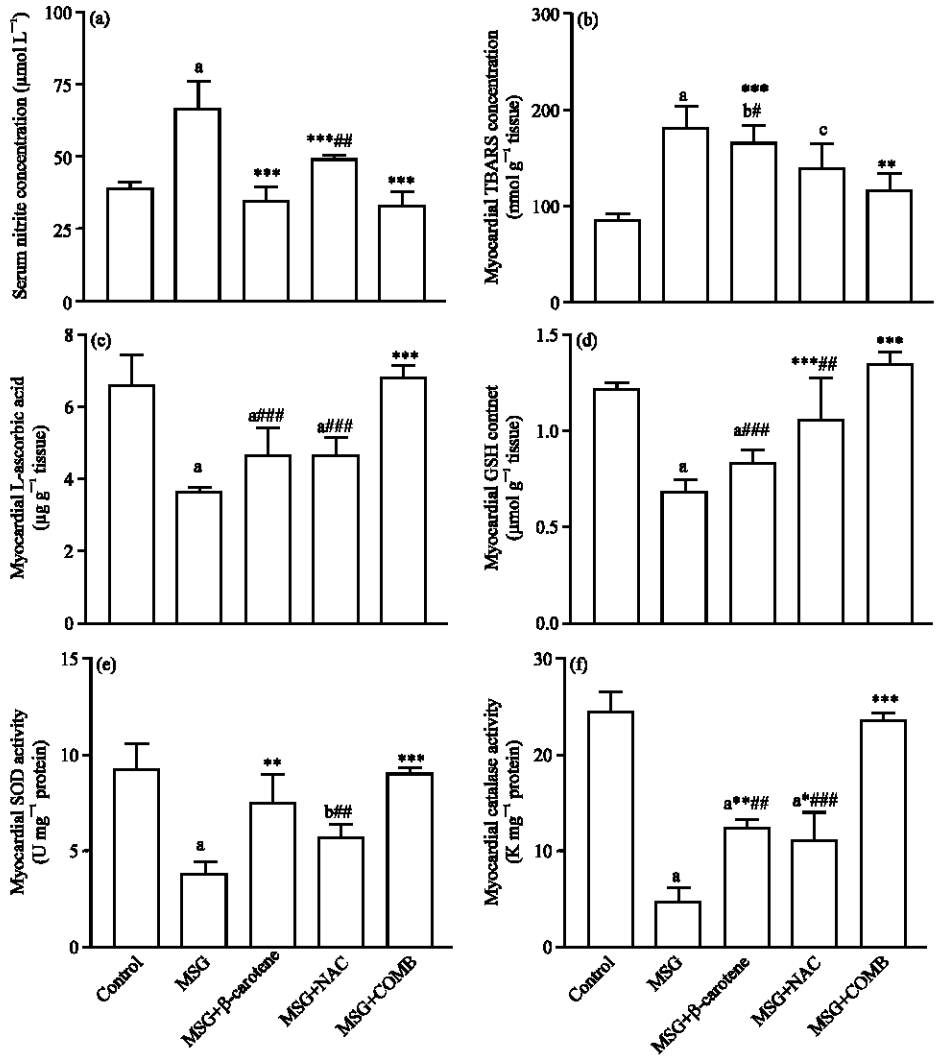

Fig. 1: Effect of β-carotein, N-acetyl cystiene and their combination on the (a) serum nitrite level as well as levels of (b) thiobarbituric acid reactive substances, (c) L-ascorbic acid, (d) GSH, (e) SOD activity and (f) CAT activity in cardiac tissues of normal and different MSG-treated rat groups. Data are presented as mean±SD of 5 independent experiments. [a]p<0.001, [b]p<0.01, [c]p<0.05 compared to control, ***p<0.0001, **p<0.01, *p<0.05 compared to MSG treated group, and [###]p<0.001, [##]p<0.01, [#]p<0.05 compared to MSG+COMB treated group respectively, using ANOVA followed by Bonferoni as a post ANOVA test

Table 2: Ratios of atherosclerosis indices in normal and different experimental treated groups

Groups	TCh/HDL ratio	LDL/HDL ratio	Atherogenic index
Control	3.19±0.95[a]	1.93±0.02[a]	2.16±0.03[a]
MSG	11.70±1.40	8.20±1.08	10.66±1.40
MSG+β carotein	3.56±0.10[a]	2.20±0.15[a]	2.50±0.10[a]
MSG+ N-acetylcysteine	3.33±0.08[a]	2.00±0.10[a]	2.30±0.10[a]
MSG+Snergesitic	3.70±0.15[a]	2.30±0.15[a]	2.45±0.11[a]

Data are Mean±SD of 5 independent experiments, [a]p<0.0001 compared to MSG treated group

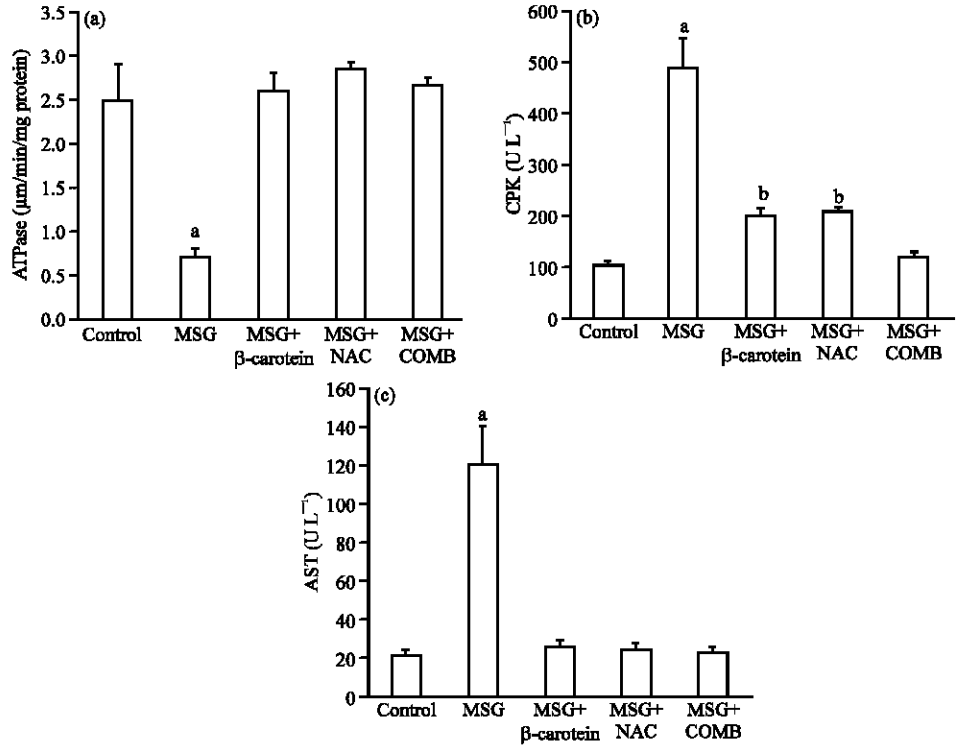

Fig. 2: Levels of cardiac Na/K ATPase (a) and serum marker enzymes, CPK (b) and AST (c) in normal and in MSG different treated rats. Data are mean±SD of 5 independent experiments, [a]p<0.0001, [b]p<0.05 compared to control

in L-ascorbic acid, GSH, SOD and CAT in cardiac tissue compared with control animals. Administration of the current used antioxidants, each alone or in combination successively ameliorated the alteration in most of the above studied markers. The best result was obtained with the synergistic combination of the two antioxidants as it modulated most of these parameters to near their normal levels.

Figure 2a-c show the activities of cardiac Na/K ATPase and serum enzymes, CPK and AST, in different experimental groups as biomarkers of cardiac tissue damage. From the figure, it can be noticed that injection of MSG developed significant cardiac damage as observed from decreased Na/K ATPase activity and elevated levels of the above mentioned marker enzymes in serum. Administration of the two candidate antioxidants each alone or in combination successively modulated the disorder in these biomarkers enzymes within their normal activities.

Figure 3a-e show the histology of heart in normal and MSG- treated animals of different groups. Normal untreated rats showed normal cardiac fibres (Fig. 3a). Figure 3b shows the histopathological findings of MSG induced myocardial infarction observed as areas of necrotic lesion.

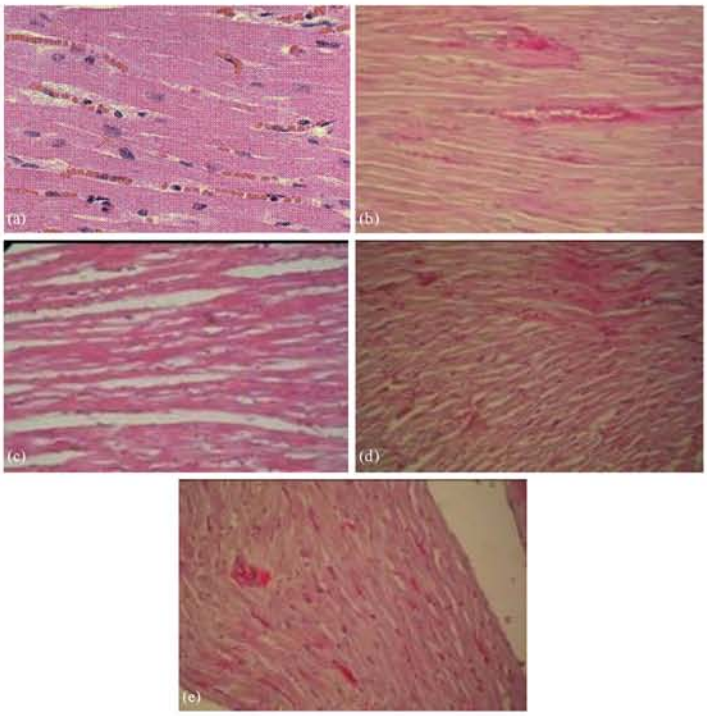

Fig. 3: (a) Group 1: Light microscopic picture of normal control heart showing normal cardiac muscle fibres, central nuclei, intercalated discs cross some of the fibers and red blood cells are seen in single file in capillaries between the fibers. (b) Group 2: Heart of MSG- treated animals showing areas of necrotic focal lesion. (c) Group 3: Heart of rats received β-carotene prior to MSG injection showing more or less normal muscle fibres. (d) Group 4: Heart of rats received NAC prior to MSG injection showing normal muscle fibres. (e) Group 5: Heart of rats received the combination of the two antioxidants showing normal muscle fibres (H and E x400)

Administration of β-carotene prior to MSG injection showing more or less normal muscle fibres (Fig. 3c), however administration of NAC or the combination of these antioxidant drugs prior to MSG injection showing normal muscle fibres (Fig. 3d and 3e, respectively).

DISCUSSION

β-carotene and NAC have been known to have antioxidative and antiatherogenic properties (Beck *et al*., 2008; Voghel *et al*., 2008), however their possible protective role against metabolic disorders and oxidative damage induced cardiopathy under the effect of MSG toxicity has not been successfully studied until now.

The results of this study showed that, administration of MSG to rats induced hyperglycemia indicated by elevated level of fasting serum glucose versus normal control animals. Our result is coped with some authors who attributed the increase in serum glucose in response to MSG toxicity to hypertrophy of pancreatic islets which associated hyperinsulinemia, an early marker of insulin resistance, together with impaired glucose uptake by tissues due to the decrease in the number of glucose transporter-4 (GLUT 4) (Seraphim *et al*., 2001; Diniz *et al*., 2005; Nagata *et al*., 2006). Insulin resistance with hyperinsulinemia is often associated with clustering of coronary risk factors, which leads to an increased risk of cardiovascular disease, presumably due to promotion of atherosclerosis

(Zavaroni *et al*., 1989; Roberts and Sindhu, 2009). Administration of either β-carotene or NAC or their synergistic combination to MSG treated rats downregulated the blood glucose level. The use of synergistic combination was found more effective in lowering the blood glucose level, indicating their beneficial hypoglycemic effect. This result is consisted with previous investigation revealed that administration of β-carotene suppresses the elevation of blood glucose level and reduces the symptoms of Diabetes Mellitus (DM) in the STZ-induced diabetic rats (Furusho *et al*., 2002). Also, NAC was reported to exhibit a reduction of the blood glucose level in type 2 diabetic C57BL/KsJ-db/db mice (Kaneto *et al*., 1999) and suppress the spontaneous development of diabetes in young NOD mice (Murata *et al*., 2003). The possible mechanisms which may explain the hypoglycemic action of these compounds including enhanced insulin sensitivity, increased glucose uptake by modulating the alterations in the GLUT and accelerated glucose utilization by peripheral tissues.

In line with previous study, the current study also demonstrated that injection of MSG to rats caused metabolic abnormalities in lipid metabolism which represented by marked increase in total serum lipids, TG, TCh and LDL (hyperlipidemia) with concomitant decrease in HDL level in relation to control ones. Alteration in these lipid profiles was reflected by increased TCh/HDL and LDL/HDL ratios as well as atherogenic index (Nagata *et al*., 2006). Hypertriglyceridemia induced in MSG-treated rats may be a response to the increased blood glucose level. It was found positive correlations between hypertriglyceridemia and hyperglycemia induced by MSG. MSG induces oxidative stress and Reactive Oxygen Species (ROS) production which reported to have specific roles in the modulation of cellular events (Berner and Stern, 2004; Diniz *et al*., 2005). The ROS react with protein thiol moieties to produce a variety of sulfur oxidations, thus diminishing the insulin receptor signal and inhibiting cellular uptake of triacylglycerol from the blood (Chen *et al*., 2003). Hyperlipidemia which represented by hypertriglyceridemia identified a highrisk dysmetabolic situation (Ginsberg, 2002; Blackburn *et al*., 2003). Some studies have linked hypertriglyceridemia to higher serum small dense LDL particles, atherothrombosis and impaired endothelial function, the hallmarks of several prevalent cardiovascular diseases as well as their complications (Lupattelli *et al*., 2000; Lundman *et al*., 2001; Ginsberg, 2002).

The enhanced serum concentration of TCh noted in the rats treated with MSG implied that this food addictive causes hypercholesterolemia, whereas the increase in the levels of LDL with a simultaneous decreased concentration of HDL reflected that MSG-induced abnormalities in lipoprotein metabolism. The changes in the cholesterol profile and other lipid compounds noted in the present study in the MSG-treated rats may be explained by the ability of MSG toxicity to inhibit the activity of hydroxy 3-methylglutaryl-coenzyme. A reductase (HMG-CoA) which plays an important regulatory role in cholesterol biosynthesis, inhibition of its activity is known to alter the metabolism of all lipids, including cholesterol (Ness and Chambers, 2000). Hypercholesterolemia and abnormalities in lipoprotein metabolism are considered other serious risk factors and important early events in the pathogenesis of atherosclerosis in both peripheral and coronary circulation (Maxfield and Tabas, 2005; Mallika *et al*., 2007; Paez and Omez, 2009). Lipid compounds and products of their oxidation especially LDL accumulate during formation of atherosclerotic lesions (Mallika *et al*., 2007). The LDL functions in the atheroma formation whereas the HDL plays an important role in inhibiting the formation of atheroma (Maxfield and Tabas, 2005; Mallika *et al*., 2007). The antiatherosclerotic action of HDL consists in its ability to remove cholesterol from vascular wall, stimulate prostacyclin formation and inhibit the synthesis of adhesive molecules (Pal, 2009). So, lowering the plasma lipid levels through dietary or drug therapy may be beneficial in decreasing the risk of vascular disease.

Administration of either β-carotene or NAC or their synergistic combination presented in the current work, effectively abrogated the alteration in the lipid profile in MSG- treated rats, implying their hypolipidemic potential action. Similar result was obtained with administration of the carotenoid, lycopene to rabbits fed a high-fat diet (Hu *et al*., 2008). Also, treatment of rats fed a high-fat diet with NAC can improve the activity of lipoprotein lipase and reduce blood lipid profiles (Yang *et al*., 2006).

In addition, it was reported that cysteine-containing agents effectively reduced the activity of lipogenic enzymes and suppressed TG and cholesterol biosynthesis in high saturated fat-consuming mice. These agents also improved hyperlipidemia-related hyperglycemia and oxidation stress (Lin *et al*., 2004). The ability of these compounds to prevent the MSG-induced changes in the serum concentrations of the estimated lipids and especially the accumulation of TCh with a simultaneous increase in the LDL and a decrease in the HDL concentration allows the conclusion that these drugs may protect against the development of atherosclerosis due to metabolic disorders induced by MSG toxicity. Our finding is supported by previous studies on β-carotene and NAC have been suggested that each one has important role in preventing the development of inflammation-associated diseases, such as atherosclerosis (Vivekananthan *et al*., 2003; Beck *et al*., 2008; Voghel *et al*., 2008), which may be related to their hypolipidemic beneficial effect and antioxidative properties (Wang and Ng, 1999; Vivekananthan *et al*., 2003; Bai *et al*., 2005; Hu *et al*., 2008; Voghel *et al*., 2008). Also some authors found that carotenoids supplementation protect LDL against oxidation (McEligot *et al*., 2005) which has the fundamental role in the development of severe atherosclerotic lesions related to cardiovascular disease (Yamashita *et al*., 2007).

It is generally accepted that overproduction of the inflammatory mediator, NO, is associated with oxidative stress, which is involved in the pathogenesis of many diseases including cardiovascular diseases (Moncada *et al*., 1991; Das, 2000; Sharma *et al*., 2007). The current study showed that administration of MSG to rats led to significant increase in serum NO level accompanied by marked increase in cardiac MDA (a marker of lipid peroxidation and oxidative tissue damage) compared with normal animals. Increased serum NO may be related to activation/induction of nitric oxide synthase (NOS) isoenzymes. NO gives an anti-inflammatory effect and involves in the regulation of blood pressure under normal physiological conditions., however its overproduction is considered as a pro-inflammatory mediator that induces inflammation leads to the alteration of vascular homeostasis (Lounsbury *et al*., 2000; Stauss *et al*., 2000) and plays an important role in the pathogenesis of cardiovascular diseases (Das, 2000). The direct toxicity of NO is enhanced by reacting with superoxide radical to give powerful secondary toxic oxidizing species, such as peroxynitrite ($ONO\bar{O}$) which is capable of oxidizing cellular structure and causes lipid peroxidation (Sayed-Ahmed *et al*., 2001). In this context, transformation of NO by superoxide radical to peroxynitrite diminshes the capacity of endothelial cells to generate bioactive useful NO, which is important in maintenance of normal blood pressure, thereby decreases in NO bioavailability, causing endothelial sclerosis and subsequently hypertension (Stokes *et al*., 2002). Therefore, NO inhibitors represent important therapeutic advance in the management of inflammatory diseases (Sharma *et al*., 2007).

Lipid peroxidation is coupled with many deleterious effects on the cell membrane which may include an increase in osmotic fragility (Helzer *et al*., 1980), an increase in permeability, inactivation of membrane-bound enzymes and cross-linking of membrane constituents (Vladimirov *et al*., 1980). On the other hand, products of MSG-induced lipid peroxidation in cardiac tissue presented in the current investigation have been suggested to be involved in the pathogenesis of atherosclerosis (Singh *et al*., 2003). This may be true because in the pathology of atherosclerosis there is a dependence on free radicals (Takano *et al*., 2003; Mallika *et al*., 2007). The statistically significant correlations observed in the current study between the main indices of atherosclerosis risk (TCh, HDL, LDL, the ratios of TCh/HDL,LDL/HDL and atherogenic index) and the indices of lipid peroxidation (TBARS) confirm an association between the peroxidative MSG action and the development of atherosclerosis. Based on the available literature data (Singh *et al*., 2003; Farombi and Onyema, 2006) and our own findings, MSG -induced lipid peroxidation may be explained by the damaged cardiac non-enzymatic and -enzymatic antioxidative defense barrier which indicated by marked decrease in the content of cardiac L-ascorbic acid and GSH as well as in the activities of antiperoxidative enzymes, SOD and CAT in MSG- treated rats and in this way it can induce the prooxidative state (Choudhary *et al*., 1996). Similar result was obtained with previous study revealed that MSG at dose level of 4 mg g^{-1}

body weight induced oxidative stress in the cardiac tissue by changing the activity of free radical initiating enzyme such as xanthine oxidase and scavenging enzymes like SOD and CAT (Singh and Pushpa, 2005).

Injection of the tested compounds either each alone or in combination prior MSG administration, markedly modulated the imbalance between the pro-oxidative inflammatory markers, NO and MDA and the antioxidant indices, L-ascorbic acid, $GSH_{,}$, SOD and CAT implying their anti-inflammatory and antioxidative capablities (Kheir-Eldin *et al*., 2001). Similar results were obtained from previous studies reported that carotenoids are capable of scavenging free radicals, inhibiting lipid peroxidation (Wang and Russell, 1999; Stahl, 2000; Furusho *et al*., 2002) and proinflammatory mediators and inducing detoxifying enzymes (Edes *et al*., 1989; Yeh *et al*., 2009). NAC was also reported for its anti-inflammatory and antioxidant potential actions (Zafarullah *et al*., 2003; Kopp *et al*., 2006; Yang *et al*., 2006) and has the ability to inhibit NO production in several type of cells and attenuate oxidative tissue damage (Pahan *et al*., 1998; Prakash and Kumar, 2009). The beneficial effect of NAC administration on oxidative damage is related to its activity as a direct and potent, free radical scavenger. First, N-acetyl cysteine enhances the levels of endogenous glutathione by increasing intracellular cysteine and subsequently potentiates the natural anti-oxidative cellular defense mechanism (Pocernich and La Fontaine, 2000; Zafarullah *et al*., 2003; Yalcin *et al*., 2008).

It has been reported that normal physiological level of cholesterol plays a regulatory role in myocardial ion transport (Yeagle, 1989), however hypercholesterolemia was found alters the physical properties of the membrane (Kutryk and Pierce, 1988), which are considered to be related to altered function of certain membrane- bound enzymes. Na^+/K^+ ATPase is an integral membrane protein, which is responsible for the active transport of sodium and potassium across the cell membrane and is considered the primary driving force for other membrane-associated transport systems or channels such as Na^+/Ca^{+2} and Na^+/H^+ antiporters (Kinne-Saffran *et al*., 1993). hypercholesterolemia has been shown to suppress this enzymatic activity in various tissues including heart (Yeagle 1989; Gokkusu and Oz, 1990; Chen *et al*., 1997). This finding is coincided with the results of the present study revealed that MSG induced hypercholesterolemia, significantly inhibited Na^+/K^+ ATPase in MSG-treated rat hearts in relation to normal control animals. Chen *et al*. (1997) demonstrated that hypercholesterolemia induces a high cholesterol content in the myocardial sarcolemma which is accompanied by a decrease in Na^+/K^+ ATPase activity. Since, Na+-K ATPase is essential for the regulation of ionic content and membrane excitability of myocardial cells, impaired of its function would lead to the accumulation of intracellular Na +, thus favoring an increase in levels of intracellular toxic Ca^{+2} through Na^+/Ca^{2+} exchange mechanism, possibly resulting in intracellular Ca^{+2} overload which is considered the major cause of ischemic damage and cardiac failure (Choi, 1994; Chakraborti *et al*., 2002). This contention is in concert with the some findings showed that certain cardiovascular diseases, such as cardiomyopathy and hypertension, are associated with decreased Na+-K+-ATPase activity (Ortega and Mas-Oliva, 1984; Norgaard *et al*., 1987; Chen and Lin-Shiau, 1988). Another possible explanation which may demonstrate the present decrease in the activity of such enzyme is the increase of lipid peroxidation induced in rat hearts in response to MSG toxicity, which is coupled with inactivation of membrane-bound enzymes (Vladimirov *et al*., 1980). Administeration of the tested two antioxidants, either each alone or in combination, effectively ameliorated the decrease in cardiac Na+-K+-ATPase activity which may attributed to their hypolipidemic and antioxidant beneficial actions against the metabolic lipid disorder and lipid peroxidation induced membrane damage in cardiac of MSG- treated rats.

In this study, the oxidative cardiac tissue damage induced by toxic effect of MSG in rats was ensured by pronounced increased in the activities of diagnostic serum marker enzymes, CPK and AST compared to normal rats and confirmed by the hitopathological picture which demonstrated focal myo-necrosis. These findings confirm the onset of myocardial lesion and leaking out of the marker enzymes from heart to blood (Suchalata and Shyamala, 2004; Ganesan *et al*., 2009). Pretreatment of

the used compounds each alone to MSG- treated rats, restored the activity of serum AST to its normal level and significantly improved the activity of CPK in relation to MSG- treated rats. However, injection the synergistic combination of the two compounds successively ameliorated these diagnostic serum enzymes to their normal levels. Histopathological findings of the used antioxidants (either each alone or in combination) pretreated myocardial infracted heart shows a near normal morphology of cardiac muscle with the absence of necrosis compared to MSG-induced heart. The biochemical and histological results presented in the current investigation suggest the ability of the used drugs to protect and stabilize cellular membranes against MSG cardio-toxicity.

In conclusion, the results of the present investigation indicate that β-carotene and NAC have important role in preventing the development of cardiopathy induced by MSG toxicity. The protective effect of these antioxidants is probably related to their hypoglycemic and, hypolipidemic beneficial actions as well as their abilities to strengthen the myocardial membrane by their membrane stabilizing action and to counteract free radicals by their antioxidant property and may candidate as protective drugs against cardio-toxic agents.

ACKNOWLEDGMENT

The authors are thankful to Sabic for its support.

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