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Trans-retinol Precursor and/or N-acetyl Cysteine Protects Against Monosodium Glutamte-induced Nephrotoxicity in Rats

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Abstract: The main objective of this study is to investigate the ameliorating effect of N-acetyl cysteine (NAC) and/or trans-retinol precursor (β-carotene) on monosodium glutamate (MSG)-induced oxidative damage in the kidney of rats. For this purpose, oxidative status was monitored through estimating renal contents of malondialdehyde (MDA, index of lipid peroxidation), L-ascorbic acid, reduced glutathione (GSH) and serum nitric oxide (NO) levels. Histopathological examination of renal tissue was also assessed. In MSG group, the convoluted tubules showed cloudy swelling and the interstitial tissue showed congestion & oedema in both cortex and medulla with a hyaline cast in the latter. Co-administration of NAC, β-carotene and their combination to MSG-treated rats returned the renal cortex and medulla to their normal architecture. The increase in serum levels of urea, creatinine & NO and renal MDA and the decrease in renal GSH and L-ascorbic acid induced by subcutaneous injection of MSG were reversed by treatment with NAC and/or β-carotene. Combination of these two antioxidants showed the most promising results in ameliorating some renal disorders induced by MSG. This may be helpful in reducing renal congestion and oedema induced by other nephrotoxins and other chemical drugs.

Key words: Nephrotoxicity, trans-retinol precursor, N-acetyl cysteine, monosodium glutamte, rats

INTRODUCTION

Additives have been used for many years for many purposes; to maintain product consistency such as alginate, methyl cellulose and pectin, to enhance palatability for example ascorbic acid, butylated hydroxyl anisole and sodium nitrite, to improve the nutritional value of certain food such as vitamin A & D, iron and riboflavin or to control acidity/alkalinity such as lactic acid, sodium bicarbonate and tartrates. In addition, flavor enhancement is achieved by fructose, saccharine and monosodium glutamate[1].

Monosodium glutamate (MSG) is the most commonly used flavoring agent allover the world. The major adverse reaction of MSG might be either immunological reactions such as urticaria, angioedema, cutaneous allergic reactions and asthma, or non-immunological reactions, which include variety of symptoms such as headache, myalgia, backache, neck pain, tingling, and lushing and chest heaviness. The non-immunological reactions contribute to "Chinese restaurant syndrome"[2,3].

Alterations in the levels of lipid peroxides (LP) and antioxidants such as reduced glutathione (GSH), catalase and superoxide dismutase were observed in adult mice during MSG treatment[4,5]. The production of tumor necrosis factor- α (TNF-α), an inflammatory mediator, was significantly increased by glutamate treatment[6,7].

In addition, glutamate receptors have been demonstrated outside the CNS so that glutamate has systemic effects including oedema, hydropic degeneration and necrosis of kidney as well as increased blood levels of glucose, leptin, insulin and triglyceride. It impaired glucose tolerance test and lowered glycogen content which were due to morphological and biochemical changes in the liver[8,9]. Moreover, some findings have been suggested that, there is an elevation of interstitial glutamate concentration that might alter sensory perception and pain sensitivity in human.

In recent years, antioxidants have gained a lot of importance because of their potential as prophylactic and therapeutic agents in many diseases. The discovery of the role of free radicals in cancer, diabetes, cardiovascular and autoimmune diseases has led to a medical revolution that is promising a new paradigm of healthcare.

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N-acetyl-L-cysteine (NAC) is the N-acetyl derivative of the naturally occurring amino acid L-cysteine[10]. Because of its ability to reduce disulphide bonds it is widely used to reduce viscosity and elasticity of mucus and is virtually non-toxic[11]. NAC has the potential to interact directly with oxidants[11,12]. Like many thiols, such as GSH, it is an excellent scavenger of hydroxyl radical[12]. In addition to oxidant scavenger function, there is plenty of evidence showing that NAC promotes cellular GSH production, and thus, NAC reduced or even prevented oxidant mediated damage to cell culture or animals[12,13].

A number of studies have indicated that NAC has a power to prevent cancer and other mutation- related conditions. It has an impressive array of mechanisms and protective effects towards DNA damage and carcinogenesis, which are related to its nucleophilicity, antioxidant activity, modulation of metabolism, effects on mitochondria, decrease of the biologically effective dose of carcinogens, modulation of DNA repair, inhibition of genotoxicity & cell transformation and modulation of gene expression & signal transduction pathways. Its effects are extended to regulation of cell survival and apoptosis, anti-inflammatoy activity, anti-angiogenetic activity, immunological effects, inhibition of progression to malignancy, influence on cell cycle progression, inhibition of invasion and metastasis, and protection towards adverse effects of other chemopreventive agents or chemotherapeutical agents[14].

NAC has been found to protect renal proximal tubules from in vitro simulated reperfusion injury; it seems that it protects kidney tissue against oxidative damage[15].

Carotenoids of dietary origin have recently been the subject of renewed research interest because of epidemiological evidence indicating a relationship between intake of carotenoids-rich plant substances and risk of certain substances[16].

A natural antioxidant, β-carotene is a vitamin A precursor carried in plasma and LDL[17]. Like other antioxidants, β -carotene protects the body against free radicals, quench singlet oxygen, and reduce peroxyl radicals[18]. β-carotene also induces hepatic enzymes that detoxify carcinogens[19]. 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[20].

β-carotene supplementation may also help to strengthen the immune system[21], increase lung capacity[22], reduce cholesterol levels[23], reduce the skin's risk to sun and protect against UV light-induced erythema[24]. Adequate amounts are essential for a healthy body and its benefits can increase when taken with other antioxidants like vitamins C and E. ß-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[25].

The aim of this study is to evaluate the protective effect of NAC and/or β-carotene against dramatic conditions resulting from S. c. injection of MSG. For this purpose, parameters of renal function including serum albumin, creatinine and urea were estimated. Moreover, those of oxidative stress including malondialdehyde (MDA), GSH, L-ascrbic acid and nitric oxide (NO) levels were measured in all treated groups. In addition, the biochemical analysis was supported by histopathological examination of renal tissues in both cortex and medulla.

MATERIALS AND METHODS

Chemicals: MSG was purchased from BDH laboratory (Poole, UK), β-carotene and NAC from Pharco CO. Egypt. All chemical reagents were of analytical grades purchased from Sigma Chemical Co. (St. Louis, Mo, USA).

Animals: This study was performed on eighty adult male albino rats (150-200 gm) provided from animal house of King Saud University. They were kept in individual metabolic cages and allowed free access to slandered diet and water. The rats were left for two weeks for acclimatization. All animals were fasted for 3 hours prior to drugs administration.

Experimental Design: MSG toxicity was induced by S. c. injection of 2g/kg daily for three weeks[26]. NAC and β-carotene were injected daily by the same route along with MSG by doses20 and 10 mg/kg, respectively[27,28]. Eighty rats were divided into eight groups (10 animals each) as follows: I: Normal control group (without any treatment), II: Normal group treated with β-carotene, III: Normal group treated with NAC, IV: Normal group treated with combination of β-carotene and NAC, V: Control group treated with MSG, VI: MSG group treated with β-carotene, VII: MSG group treated with NAC, VIII: MSG group treated with both β-carotene and NAC.

Blood and Kidney Separation: Twenty-four hours after the last dose injection, rats were decapitated and trunk blood samples were collected, allowed to coagulate and centrifuged. Serum was stored at -80° C for determination of urea, creatinine, NO and albumin levels. One kidney was homogenized in phosphate buffer for malondialdehyde (MDA), glutathione (GSH) and L-ascorbic acid determination. The other kidney was kept in 10% formaldehyde for histopathological evaluation of renal injury.

Biochemical Assays:

Serum Determinations:

Determination of Albumin Level: Serum albumin was estimated according to the method of Doumas *et al*. The obtained color was measured spectrophotometrically at 628 nm[29].

Determination of Creatinine Level: Serum creatinine was determined according to the kinetic method described by Moss *et al,*[30].

Determination of Urea Level: Serum urea was assayed enzymatically according to the method of Lespinas *et al.,*[31].

Determination of Nitrite Level: Nitrite, a stable end product of nitric oxide radical, is mostly used as indicator for the production of nitric oxide. Nitrite level was determined using Griess reagent according to the method of Moshage *et al.,*[32].

Renal Tissue Determinations:

Determination of L-ascorbic acid:

L-ascorbic acid was estimated according to the method adopted by Kleszczewski[33]. The developed blue color during the reaction was read at wavelength 760 nm[33].

Determination of Lipid Peroxide Levels: Lipid peroxides expressed as malondialdehyde (MDA) were estimated using thiobarbituric acid reagent as described by Ohkawa *et al.,*[34].

Determination of Reduced Glutathione (GSH) Levels: Renal content of GSH was estimated according to the method of Moron *et al.,*[35].

Histological Study: The pathological changes in kidney were observed microscopically after hematoxylin and eosin staining.

Statistical Analysis: Data were expressed as mean SD. Statistical analysis was performed using Instat-3 computer program (Graph pad software Inc, San Diego, CA, USA). Differences between groups were analyzed using one way analysis of variance (ANOVA) followed by Bonferroni multiple test. The level of significance was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

Results: Values of the analyzed parameters and the statistical differences between the groups are shown in Table (1) and Figure 1 (a, b, c and d).

Administration of MSG significantly increased the serum urea and albumin levels as compared to normal control group, while, no significant change was observed in serum creatinine level. Administration of ß-carotene, NAC and their combination significantly normalized serum urea and albumin in MSG-treated groups with the best improvement by β-carotene.

Levels of renal MDA and serum nitrite showed a significant increase in MSG-treated control group in comparison with normal control. β-carotene alone or with NAC showed the best decrease in nitrite level compared to NAC alone. While, in case of MDA level, the order of the decrease was the combination, NAC and β-carotene, respectively.

A significant decline in renal levels of GSH and L- ascorbic acid was seen in MSG treated rats in comparison with normal rats. Administration of β-carotene, NAC and their combination significantly elevated these decline levels induced by MSG treatment. β-carotene and NAC combination exhibited the most significant improvement particularly with respect to L-ascorbic acid.

The histopathological examination of the normal groups (control, β-carotene and/or N-acetyl cysteine) revealed normal structure of the renal cortex in the form of the renal glomeruli and the proximal convoluted tubules. The cell lining of the proximal convoluted tubules appear in the form of single layer of pyramidal cells on a basement membrane. In addition, normal structure of the renal medulla in the form of loop of Henle (narrow tubules), collecting tubules (lined with cubical epithelium), and capillaries (tubules containing RBCs) was observed (photo 1-4 A and B). On the other hand, rats treated with MSG showed cloudy swelling of the convoluted tubules of the renal cortex and congestion and edema of interstitial tissue. Also, tubules of renal medulla showed cloudy swelling with fat and hyaline cast as well as congestion and edema of the interstitial tissue.

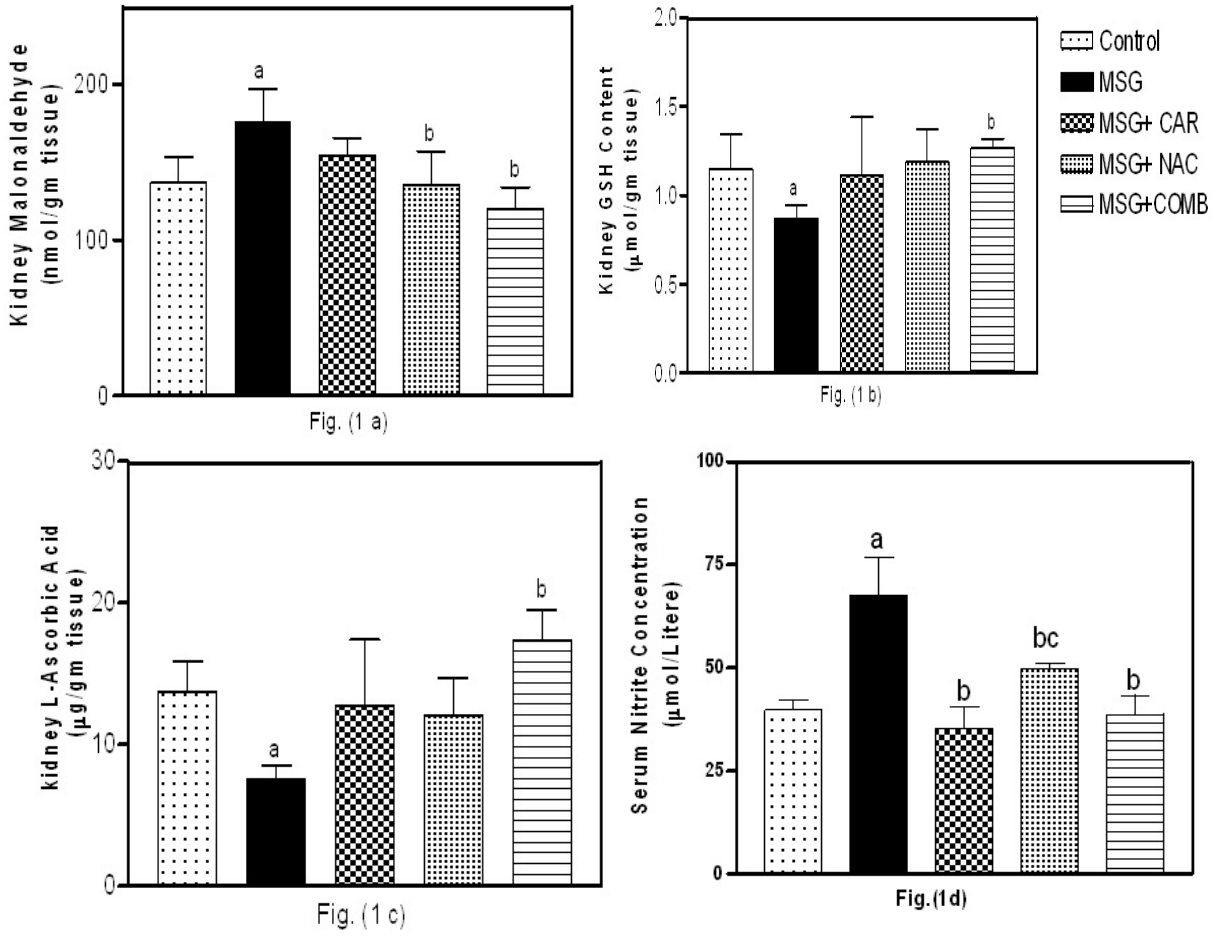
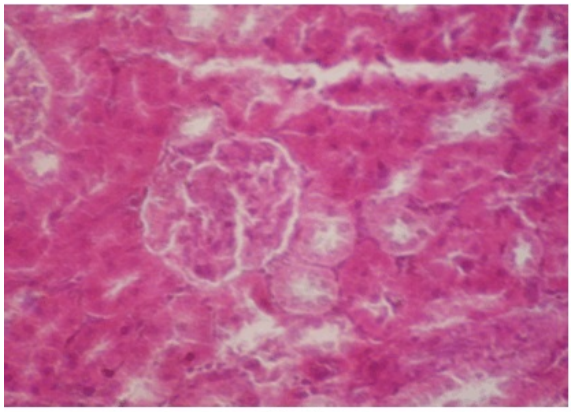


Fig. 1: Effect of β carotene, NAC and their combination on renal levels of malondialdehyde (MDA), GSH and L-ascorbic acid Fig 1 (a, b and c, respectively) as well as serum nitrite levels (Fig. 1d) in normal and MSG-treated groups.
Values are expressed as mean ± SD of ten rats in each group.
Differences between groups were analyzed using one way ANOVA followed by Bonferroni multiple comparison tests. $p \leq 0.05$ was considered significant.
a: Significance from control (G1), b: Significance from MSG control (G5), c: Significance from MSG treated with β-carotene + NAC (G8)

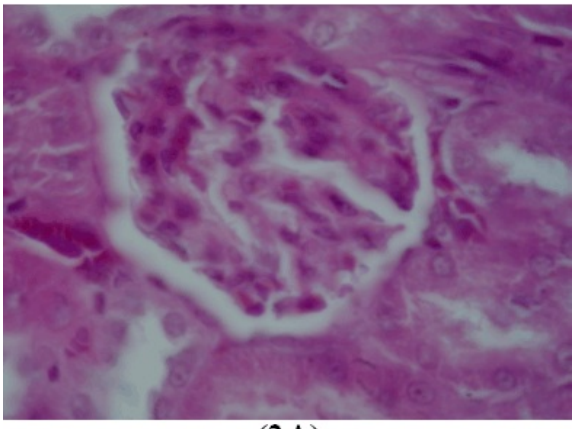
Treatment of MSG groups with β-carotene and/or N-acetyl cysteine restored the normal pathology of the kidney. Histopathological examination of these groups showed the normal structure of the renal cortex in the form of the renal glomeruli and the proximal convoluted tubules as well as the normal structure of the renal medulla in the form of loop of Henle (narrow tubules), collecting tubules (lined with cubical epithelium).

Discussion: Dietary nutritional inadequacy, deficiency and toxicity are the results of many lifestyle disorders. Thousands of agents are internationally added into food we consume. Monosodium glutamate (MSG) is considered one of the most commonly used food enhancer in many types of food. MSG treatment provokes hormonal alternations and specific intestinal changes in smooth muscle reactivity to agonists. The administration of MSG in high concentrations or for long period of time may cause tissue damage and mediate inflammation. In addition, it triggers the production of reactive oxygen species (ROS) coupled with impaired oxidant/ antioxidant balance leading to a state of oxidative stress[36]. Oxidative stress and decreased antioxidative capacity participate in the progression and complications of renal disease such as hyperlipoproteinemia or cardiovascular diseases[37].



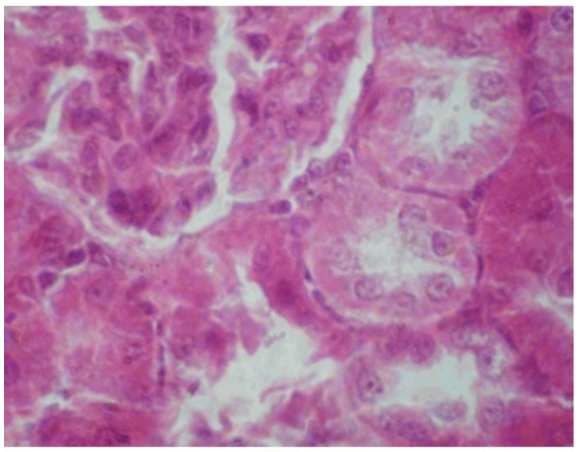
(1A)

Photo 1A: A photomicrograph of a section in kidney of a control albino rat showing the normal structure of the renal cortex in the form of the renal glomeruli and the proximal convoluted tubules. The cell lining of the proximal convoluted tubules appear in the form of single layer of pyramidal cells on a basement membrane. HX & E X 1000



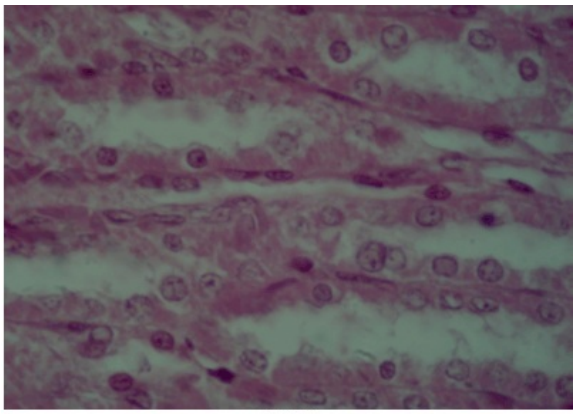
(2A)

Photo 2A: A photomicrograph of a section in kidney of albino rat administered β-carotene showing the normal structure of the renal cortex. The cell lining of the proximal convoluted tubules appear in the form of single layer of pyramidal cells on a basement membrane. The glomerulus contains glomerulus capillaries arranged in form of lobulus and surrounded with capsular space. Hx & E X 1000



(1B)

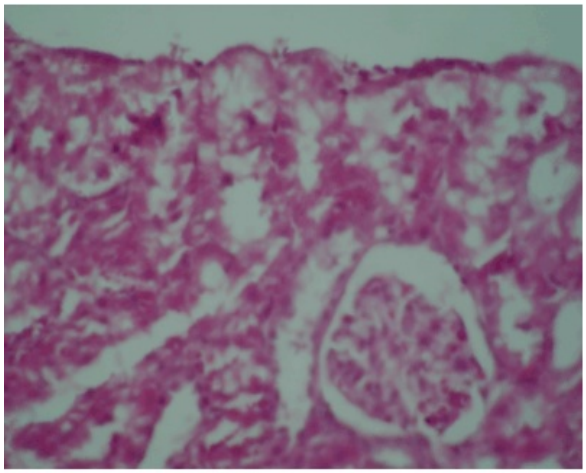
Photo 1B: Higher magnification showing the normal structure of the renal medulla in the form of loop of Henle (narrow tubules), collecting tubules (lined with cubical epithelium), and capillaries (tubules containing RBCs).



(2B)

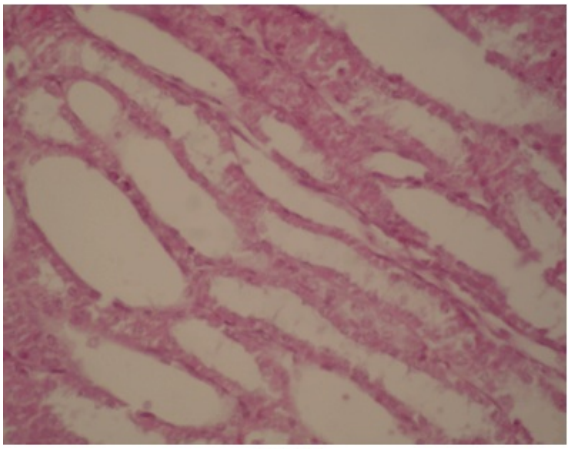
Photo 2B: A photomicrograph of a section in kidney of albino rat administered β-carotene showing the normal structure of the renal medulla in the form of loop of Henle (narrow tubules) and collecting tubules (lined with cubical epithelium). Hx & E X 1000

In the present study, administration of MSG resulted in impairment of some renal biomarkers reflected by the significant increase in urea and decrease in albumin serum levels. While, serum creatinine level was not affected. Administration of NAC and/or β-carotene along with MSG significantly decreased urea and increased albumin serum levels with the most pronounced improvement by ß-carotene administration.



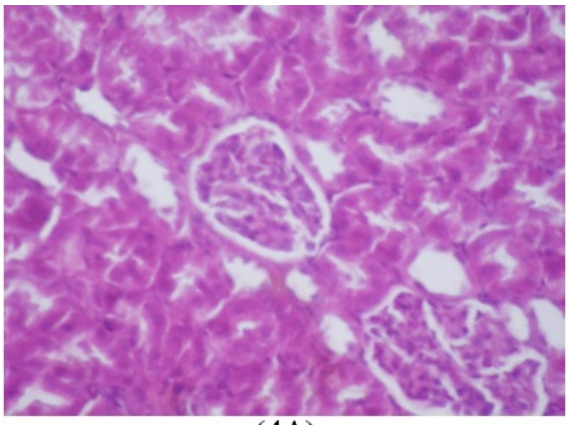
(3A)

Photo 3A: A photomicrograph of a section in kidney of albino rat administered NAC showing the normal structure of the renal cortex. The cell lining of the proximal convoluted tubules appear in the form of single layer of pyramidal cells on a basement membrane. The glomerulus contains glomerulus capillaries arranged in form of lobulus and surrounded with capsular space. Hx & E X 1000



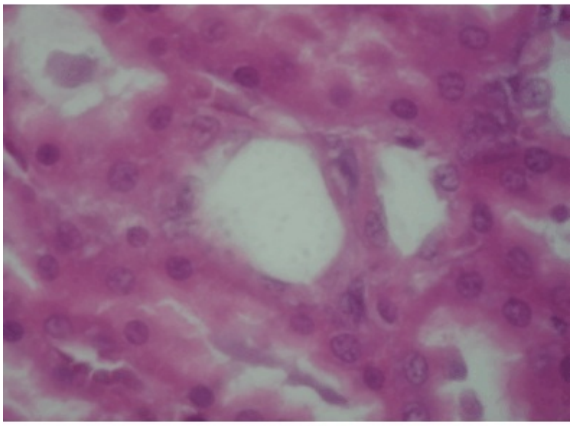
(3B)

Photo 3B: A photomicrograph of a section in kidney of albino rat administered N-acetyl cysteine showing the normal structure of the renal medulla in the form of loop of Henle (narrow tubules), collecting tubules (lined with cubical epithelium), and capillaries (tubules containing RBCs). Hx & E X 1000



(4A)

Photo 4A: A photomicrograph of a section in kidney of normal albino rat co-administered with β-carotene and N- acetyl cysteine showing the normal structure of the renal cortex in the form of the renal glomeruli and the proximal convoluted tubules. The cell lining of the proximal convoluted tubules appear in the form of single layer of pyramidal cells on a basement membrane. (Hx & E X 1000).



(4B)

Photo 4B: A photomicrograph of a section in kidney of normal albino rat co-administered with ß-carotene and N- acetyl cysteine showing the normal structure of the renal medulla in the form of loop of Henle (narrow tubules) and collecting tubules (lined with cubical epithelium). Hx & E X 1000

In the current study, histopathological examination of the kidney of rats treated with MSG showed cloudy swelling of the convoluted tubules of the renal cortex, congestion and edema of interstitial tissue.

Table 1: Serum levels of creatinine, urea and albumin in normal and different treated groups.

	Normal groups				MSG - treated groups			
	Control	β-caro	NAC	β-caro + NAC	Control	β-caro	NAC	β-caro + NAC
	G1	G2	G3	G4	G5	G6	G7	G8
Creatinine (mg/dl)	0. 46 ± 0.018	0. 45^{cd} ± 0.025	0. 42^{bd} ± 0.016	0. 34^{ab} ± 0.045	0. 49 ± 0.004	0. 39^{ab} ± 0.019	0. 42^{bd} ± 0.031	0. 37^{ab} ± 0.0008
Urea (mg/dl)	44.37 ± 2.65	45.71^{bd} ± 2.00	54.3^{b} ± 1.62	46.25^{b} ± 4.04	80.19^{a} ± 8.18	49.65^{b} ± 4.61	53.48^{b} ± 7.54	57.28^{abc} ± 3.74
Albumin (gm/dl)	5.62 ± 0.23	5.95^{b} ± 0.28	5.1^{bc} ± 0.39	5.13^{b} ± 0.59	2.8^{a} ± 0.34	5.93^{b} ± 0.23	5.97^{b} ± 0.44	6.4^{b} ± 0.58

- Values are expressed as mean ± SD of ten rats in each group.
- Differences between groups were analyzed using one way ANOVA followed by Bonferroni multiple comparison tests. $p \leq 0.05$ was considered significant.

a: Significance from control (G1)
b: Significance from MSG control (G5)
c: Significance from MSG treated with β-carotene + NAC (G8)
d: Significance from ß-carotene + NAC (G4)

Also, tubules of renal medulla showed cloudy swelling with fat and hyaline cast as well as congestion and edema of the interstitial tissue. These findings were coincided with the previous biomarker's results.

The circulating MSG was dissociated into sodium (Na^+) and L-glutamate. L-glutamate cross the mesothelial peritoneal cells and arrives at the blood stream by means of a transport system using ATP. A part of the L-glutamate in the cell conjugates, in order to be eliminated, and another part is transformed into glutamine[38]. When this occurs, the cells try to repair some of the damages by using enzymes that are present in the smooth endoplasmic reticulum but the cell is not able to completely remove the excess glutamine. Probably for this reason, the convoluted tubules showed cloudy swelling[38].

When L-glutamate arrives in high concentrations through the renal artery, the kidney tries to excrete it. The renal corpuscle receives the L-glutamate through the afferent arteriole, it is absorbed, filtrated and across the membrane damaging the cell. The convoluted proximal tubules were more susceptible to damage in comparison to the distal convoluted tubules[38].

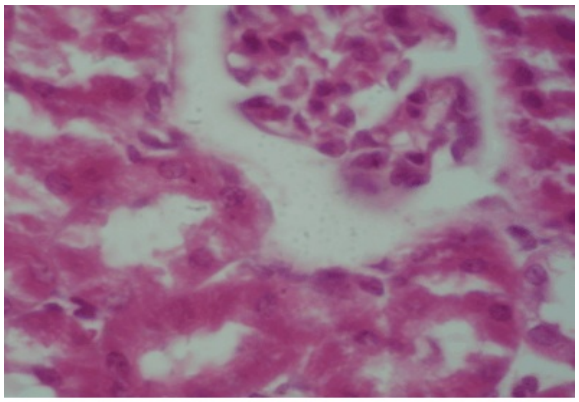
MSG rats treated with ß-carotene and/or N-acetyl cysteine showed the normal structure of the renal cortex in the form of the renal glomeruli and the proximal convoluted tubules as well as the normal structure of the renal medulla in the form of loop of Henle (narrow tubules), collecting tubules (lined with cubical epithelium).

Glutathione (GSH) has been found to act as a natural scavenger for the superoxide anion and protects protein thiol groups against oxidation, which is essential for maintaining cellular integrity. Thus, it protects cells from oxidative damage and maintain cellular integrity. GSH also has a major role in restoring other free radical scavengers and antioxidants such as vitamin E and L- ascorbic acid[39]. GSH is able to conjugate with endogenous or exogenous substances and prepares them to eventual excretion. This occurs enzymatically by series of enzymes called glutathione transferase or non- enzymatically. This detoxification of compounds by conjugation with GSH occurs mainly in kidney and liver[40]. L-ascorbic acid and GSH are antioxidants linked tightly to each other.

Normal L-ascorbate level has a therapeutic benefit due to its ability to reduce the oxidative stress by reacting with superoxide and hydroxide radicals as well as alkyl, peroxyl and alkoxyl radicals, thereby it can neutralize these radicals and stop the initiation and propagation of chain reaction[41,42].

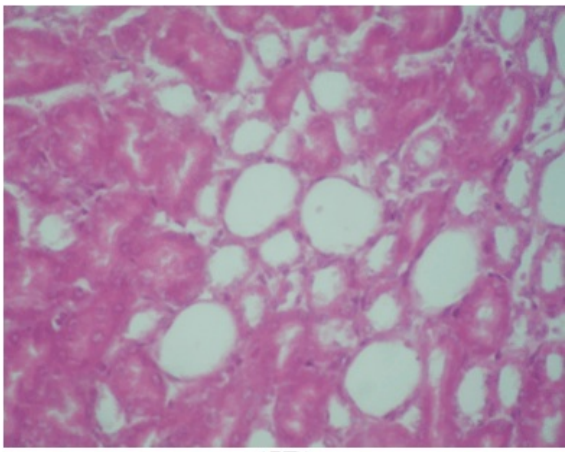
In the present work, administration of MSG led to impairment of the cellular oxidative state was indicated by a significant increase in renal MDA and a decrease in renal GSH and L-ascorbic acid levels. The decrease in both L-ascorbic and GSH presented in the current study might reflect their direct reaction with the reactive oxygen species generated by MSG.

In consistent to our results, MSG has been found to markedly increase MDA formation in rats' kidney



(5A)

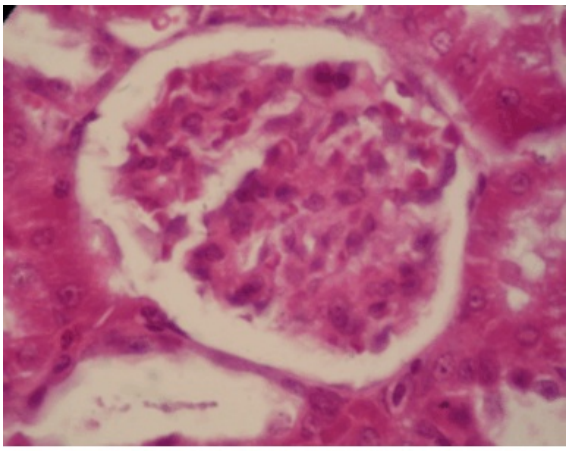
Photo 5A: A photomicrograph of a section in kidney of albino rat after treatment with MSG showing the structure of the renal cortex in the form of the renal glomeruli and the proximal convoluted tubules. The convoluted tubules show cloudy swelling. The interstitial tissue shows congestion & oedema. Hx & E X 1000



(5B)

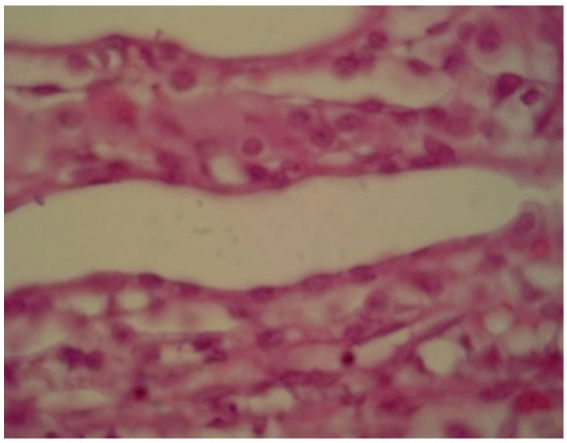
Photo 5B: A photomicrograph of a section in kidney of albino rat after treatment with MSG showing the renal medulla. The tubules show cloudy swelling. The tubules contains fat and hyaline cast. The interstitial tissue shows congestion & oedema. Hx & E X 1000

and as well exhibited a significant decrease in renal GSH level[43]. Glutamate toxicity involves an imbalance in the hemostasis of cysteine, the precursor of GSH, leading to depletion of intracellular GSH levels and



(6A)

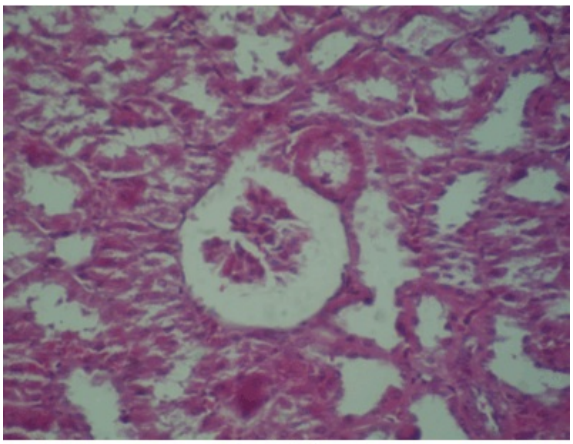
Photo 6A: A photograph of a section in kidney of albino rat treated with MSG + β-carotene showing the normal structure of the renal cortex in the form of the renal glomeruli and the proximal convoluted tubules. (Hx & E X 1000).



(6B)

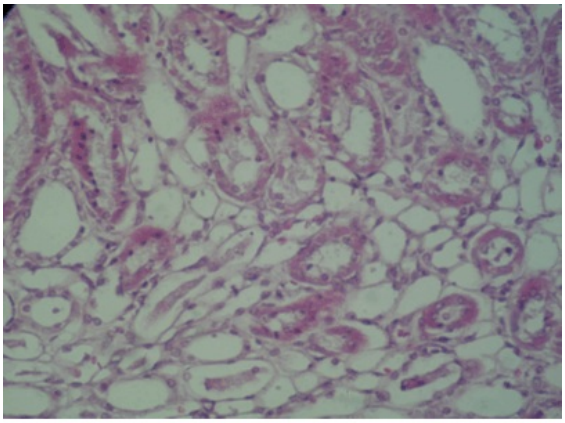
Photo 6B: A photograph of a section in kidney of albino rat treated with MSG + β-carotene showing the normal structure of the renal medulla in the form of loop of Henle (narrow tubules), collecting tubules (lined with cubical epithelium). (Hx & E X 1000)

reduced ability to protect against oxidative injury in the cell and ultimately, cell damage. Moreover, lipid peroxidation may eliminate the active sulfhydryl group of GSH and other enzymes. Thus, oxidative stress and accumulation of free radicals seems to be responsible for MSG toxicity.



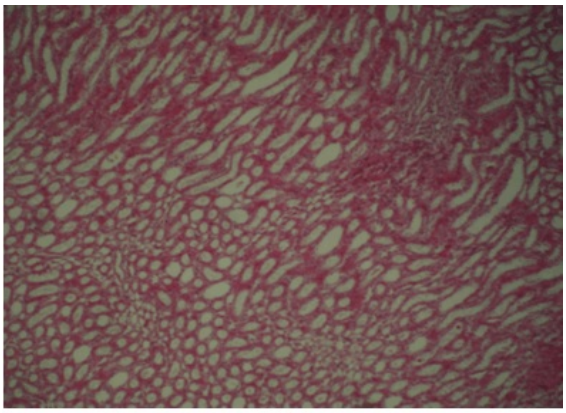
(7A)

Photo 7A: A photograph of a section in kidney of albino rat treated with MSG + NAC showing the normal structure of the renal cortex in the form of the renal glomeruli and the proximal convoluted tubules. (Hx & E X 1000).



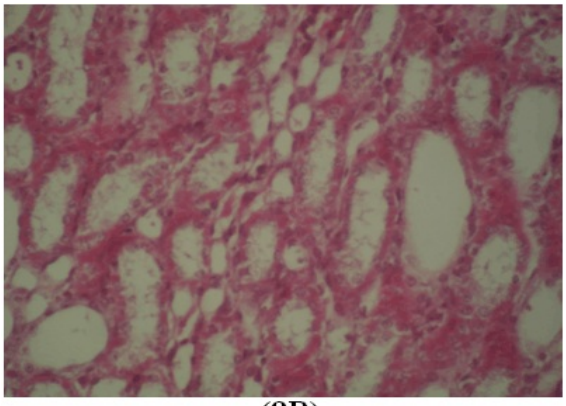
(7B)

Photo 7B: A photograph of a section in kidney of albino rat treated with MSG + NAC showing the normal structure of the renal medulla in the form of loop of Henle (narrow tubules), collecting tubules (lined with cubical epithelium). Hx & E X 1000



(8A)

Photo 8A: A photograph of a section in kidney of MSG- treated albino rat co administered with β-carotene and NAC showing the normal structure of the renal cortex in the form of the renal glomeruli and the proximal convoluted tubules. Hx & E X 1000



(8B)

Photo 8B: A photograph of a section in kidney of MSG- treated albino rat co administered with β-carotene and NAC showing the normal structure of the renal medulla in the form of loop of Henle (narrow tubules), collecting tubules (lined with cubical epithelium). Hx & E X 1000

Nitric oxide (NO) has a double- edged knife in pathopysiology, since both the abundance and paucity of NO cause diseases[44]. The gas phase of cigarette smoke contains high levels of nitric oxide that have been shown to interact with lipid membranes to induce lipid peroxidation and protein oxidation to form protein-bound carbonyl groups[45]. The direct toxicity of NO is enhanced by reacting with superoxide radical to give powerful secondary toxic oxidizing species, such as peroxynitrite ($ONOO^-$) which is capable of oxidizing cellular structure and causes lipid peroxidation, a process leading to membrane damage[46].

In the present work, MSG significantly increased serum NO levels as compared to control. Thus, both lipid peroxidation and elevated NO levels may contribute to MSG renal toxicity.

Increasing understanding the role of free radicals in disease is opening new area for the antioxidants manifest in prevention and therapy of the health care system, along with promising role as supportive remedies in many regimens of mainline. Nevertheless, epidemiological studies have been suggesting strongly that antioxidants can decrease the incidence of diseases. However, more number of animal and human studies is required to establish the efficacy and safety of antioxidants.

As a defense mechanism, the body produces a number of endogenous antioxidants capable of scavenging the harmful free radicals to maintain an optimal oxidant:antioxidant balance, thereby maintaining normal cellular function and health. However, under conditions of high oxidative stress, the ability of these antioxidants to eliminate free radicals are often exceeded and, therefore, dietary sources of antioxidants or drugs are required. The most widely used dietary antioxidants include vitamin E, vitamin C, carotenoids, flavanoids, zinc and selenium. The use of NAC as an antioxidant drug has gained popularity in recent years due to its ability to inhibit proinflammatory molecules and HIV replication. The antioxidant actions of carotenoids are based on their singlet oxygen quenching properties and their ability to trap peroxyl radicals[47].

Supplementation with β-carotene resulted in improvement of anti-oxidative status of kidneys of rats with streptozotocin-induced diabetes[48]. Also, carotenoids protect liposomes against lipid peroxidation[49] and can influence immune function through their ability to regulate membrane fluidity, and gap junctional communication[50].

Previous studies showed that, NAC corrected the reduction in glutathione concentration and results in a significant preservation of membrane fluidity and of the activities of catalase, mitochondrial superoxide dismutase and the different forms of glutathione peroxidase in biliary obstructed rats. In addition, NAC appears to support the synthesis of GSH under conditions when the demand for GSH is increased, such as during the metabolism of acetaminophen[51].

The previous findings confirmed the results of our study in which simultaneous administration of β-carotene and/or NAC to MSG-treated rats significantly reduced the increase in MDA and NO levels induced by MSG and they were effective in ameliorating its effects on GSH and L-ascorbate.

These results agreed with those obtained by Sehirli *et al.,*[15] who found that, NAC protects renal proximal tubules from in vitro simulated reperfusion injury and protects kidney tissue against oxidative damage. In addition, β-carotene accumulation in mouse tissue including renal tissue has been found to be protective against lipid peroxidation *in vivo*[52].

Conclusion: Treatment with ß-carotene and/or NAC has a synergistic effect in protecting tissue GSH and decreasing lipid peroxidation following administration of MSG in rat's kidney. It is hopeful that these antioxidants protect kidney against other chemicals or toxins.

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