

Efficacy of Ascorbic Acid against Toxicity Initiated by Mono-Sodium Glutamate on Albino Mice

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Abstract: Mono-Sodium Glutamate (MSG) is well-known in most fast food restaurants and houses as taste enhancer. Yet it has different damaging effects that target various organs. The present examination was designed to investigate the impact of MSG (4mg/g & 8mg/g b. wt.) on the liver and bone marrow of mice and the augmenting effect of ascorbic acid (100mg/ kg b. wt.). In this study 36 swiss albino male mice (*Mus musculus*) were used. Animals were treated daily by oral gavage (p. o.) with MSG and/or ascorbic acid for 8 consecutive weeks. The results revealed that the destructive effects of MSG in treated groups compared with control group and the protected groups with ascorbic acid, which was dose dependent. Hepatotoxicity and genotoxicity were evidence for the deleterious effect of MSG. In MSG groups showed DNA fragmentation in liver tissue and induction micronucleus in bone marrow polychromatic erythrocytes (PCEs). On the other hand, mice treated with ascorbic acid, their liver and bone marrow samples showed significantly decreased deleterious effects of MSG.

Keywords: Mono-Sodium Glutamate(MSG), micronucleus(MN), polychromatic erythrocytes (PCEs), hepatotoxicity

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I. Introduction

There is no doubt that there are many food additives which are used safely without caring about their considerations of health-related dietary changes, these food additives are chemicals which may cause lots of side effects and attracting people to eating more and more amount of food. The most worldwide used is Monosodium Glutamate (MSG) which is used as taste enhancer. Monosodium Glutamate (MSG) is a sodium salt of glutamic acid, a naturally occurring non-essential amino acid, which found in all protein containing foods such as meat, poultry, seafood, vegetables and milk¹. MSG is known in Egypt as China salt and gives a special aroma to processed food in all restaurant. It gives a taste sensation called savoury², through its stimulation of the orosensory receptors and by improving the palatability of meals. MSG influences the appetite positively, and may induce weight gains³. The US Food and Drug Administration considered MSG safe when consumed at customary level⁴. Reports indicate that MSG is toxic to human and experimental animals⁵. Many studies on animals and human stated that administration of even low dose of MSG has toxic effect⁶. MSG may validate a very bad effect on immune system, reproductive organs, liver, kidney, hematological parameters, pituitary functions⁷. In addition to these symptom complexes, ingestion of MSG has been alleged to cause or exacerbate numerous conditions, including asthma, urticaria, atopic dermatitis, ventricular arrhythmia, neuropathy and abdominal discomfort⁸. Moreover, biochemical and histological alterations observed in testis of rats exposed to MSG⁹.

The micronucleus (MN) assay is now recognized as one of the most fast, vigorous, successful and not economic assays for genotoxic test. MN has become an attractive tool for genotoxicity testing because of its capacity to detect not only clastogenic and aneugenic events, but also some epigenetic effects and its simplicity of scoring, accuracy, wide applicability in different cell types and amenability to automation¹⁰. It was solely used for the assessment of chromosomal loss, breakage, and associated apoptosis and necrosis induced by different mutagens, for instance, hydrogen peroxide¹¹. The conviction that MN was delicate biomarkers of genotoxicity was affirmed by improvement in suggested protocols, scoring strategies, dose levels, selection of species, sex contrasts statistical considerations *in vivo* and cell culture examines¹². Hayashi et al., (2007)¹³ stated that, MN can be scored easily measured in a variety of systems, *in vitro* and *in vivo*. Many studies have confirmed that MSG is toxic to the liver cells through generation of excess ammonium ions and reactive oxygen species (ROS)^{3,14}. The ammonium ion overloads is known to trigger the formation of ROS which led to leakage of liver enzymes such as alanine amino transferase (ALT) and aspartate amino transferase (AST)¹⁵. This was an indication of increased oxidative stress which might have played a mediatory role in exerting the toxic effect on genetic level. Finally, this might led to DNA damage and cell death¹⁶.

Antioxidant agents like vitamins act on the reduction harmful effects of many toxic substances due to their easy, effective and safe dietary administration. Ascorbic acid being a strong antioxidant effectively inhibits the formation of lipid peroxides as it forms the first line of antioxidant defense mechanisms in human plasma¹⁷. According to the same author, both natural and synthetic ascorbic acid are chemically identical and there are no known differences in their biological activities or bio-availability. Ascorbic acid has a therapeutic advantage because of its capacity to diminish the oxidative stress by reacting with superoxide and hydroxide radicals as well as alkyl, peroxy and alkoxy radicals, thereby it can neutralize these radicals and stop the initiation and propagation of chain reaction.^{18,19}

The aim of this study is to evaluate the protective effect of oral administration of ascorbic acid against the genotoxicity and hepatotoxicity of Monosodium Glutamate at two doses.

II. Materials & Methods

Chemicals: L-Glutamic acid monosodium salt hydrate > 99% (HPLC) powder and Ascorbic acid were purchased from Sigma Aldrich Co. (U.S.A).

Animals & experimental design: The Swiss albino male mice (*Mus musculus*) were used in this study. They were supplied by Central Animal House of the National Research Center, Dokki, Giza, Egypt. The used mice aged 4 weeks and weight 20 to 30 gm. The animals received basic laboratory meal and tap water ad libitum. Room temperature and a cycle of 12h light / 12 h dark was maintained. Mice were let on acclimate for at least one week. The animals were treated daily doses by oral gavage (p. o.) with MSG and/or ascorbic acid. They were killed by cervical dislocation 8 weeks after the first injection. Animals were divided into six groups, each one consists of six animals.

Group 1 served as control group;

Group 2 treated with ascorbic acid 100 mg/kg¹⁷;

Group 3 treated with 4mg/g MSG;

Group 4 treated with 8mg/g MSG;

Group 5 treated with both small dose of MSG and ascorbic acid and

Group 6 treated with the high dose of MSG and ascorbic acid.

Sample collection and studies: At the end of experiment, mice killed and a mid-line abdomen cut was performed. The liver of every animal was immediately got out and kept at -25 degree Celsius for determination of DNA fragmentation. Bone marrow was obtained from both femora to determine the micronuclei in polychromatic erythrocytes.

DNA fragmentation assay: DNA fragmentation in liver tissues carried out according to²⁰. DNA isolation was performed using Proteinase K method²¹. The samples of DNA were displayed under 1.2% Agarose gel electrophoresis containing ethidium bromide (0.03%)

Micronucleus test: Bone marrow micronucleus test were done according to the modified method of (Schmid, 1976)²².

Statistical analysis: Statistically significant was evaluated using an ANOVA (one-way) test with the help of SigmaPlot 14.0. Ink. In cases where ANOVA showed significant differences, post hoc analysis Tukey HSD was performed. P values less than or equal to 0.05 were considered statistically significant.

III. Results

DNA fragmentation assay

The present outcomes were observed in Figure no 1 and demonstrated that lane (1) control sample showed genomic DNA band without smears or DNA fragmented bands. Also, ascorbic acid group lane (2) showed genomic DNA band without smears or DNA fragmented bands. On the other hand, MSG group (4mg/g) lane (3) showed shadowing of fragmented DNA with 4 bands of fragmentation. MSG group (8mg/g) lane (4) showed six bands of fragmented DNA. While co-administration of ascorbic acid with MSG (4mg/g & 8mg/g) lanes (5 & 6), respectively inhibit the DNA fragmentation.

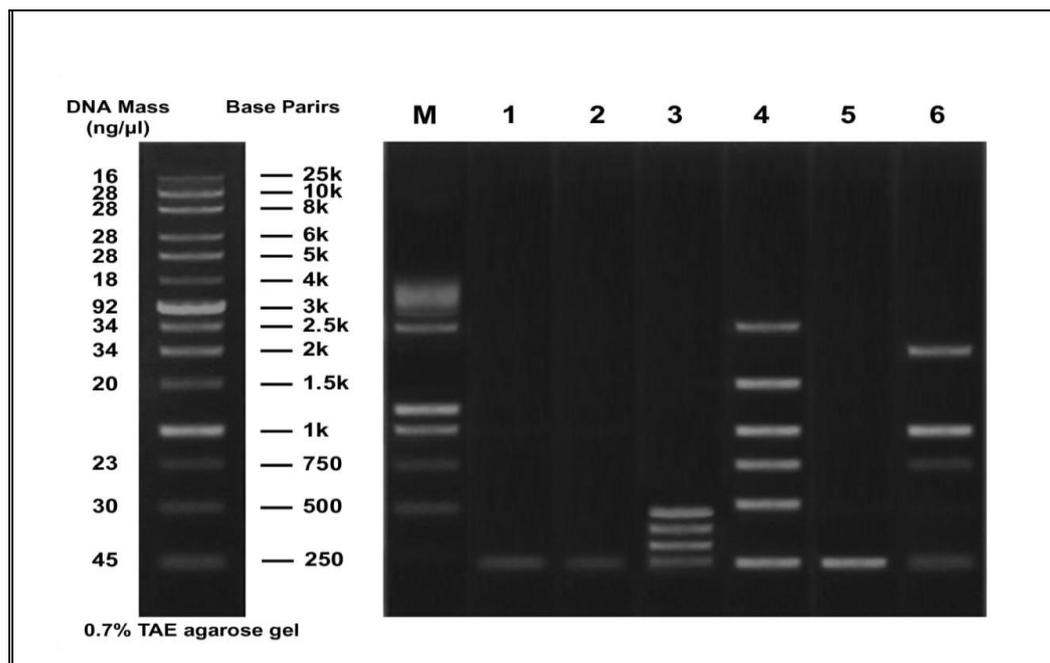


Figure no 1: Agarose gel electrophoresis demonstrating DNA fracture in liver cells of mice treated with MSG. Lane M: DNA molecular weight marker, lane 1 control samples, lane 2 ascorbic acid group (100mg/ kg), lane 3 MSG group (4mg/ g), lane 4 MSG group (8mg/ g), lanes 5 & 6 (MSG 4mg/ g + ascorbic acid & MSG 8mg/ g + ascorbic acid), respectively.

MN assay

The highest number of MNEs was noticed in both doses (4 mg/g and 8 mg/g). It was 1.13 % and 1.33 % low and high dose, respectively. The highest significant were observed in 8mg/g MSG treated group compared to control group. When ascorbic acid administered concurrent with MSG, the frequency of MNEs decreased to 0.77 % and 1.00 % in low and high treated groups, respectively. Administration of MSG produced 68 and 80 MN per 6000 PCEs, a greater than 6.18 and 7.27 fold increase over control group, Table no 1.

Table no 1: Micronuclei incidence in polychromatic erythrocytes of mice treated with MSG and/or Ascorbic acid.

Serial number	Groups	control	Ascorbic acid (100 mg/kg)	(4 mg/g) MSG	(8 mg/g) MSG	Low dose MSG + ascorbic acid	High dose MSG + ascorbic acid
1	2	2	11	14	7	11	
2	3	2	14	15	7	10	
3	1	1	10	13	6	12	
4	2	3	11	13	10	10	
5	1	3	10	13	7	8	
6	2	2	12	12	9	9	
Total		11	13 ^{n.s.}	68 ^{n.s.}	80 ^a	46 ^{n.s.}	60 ^b
Mean		1.83	2.17	11.33	13.33	7.67	9.5
S.D.		0.75	0.75	1.51	1.03	1.51	2.17
S.E.		0.31	0.31	0.62	0.42	0.62	0.89
%		0.18	0.22	1.13	1.33	0.77	1.00

a:8 mg/g MSG highly significant from 8 mg/g MSG + ascorbic acid (P < 0.001),b: 8 mgMSG + ascorbic acid highly significant from 4 mg MSG + ascorbic acid (P < 0.02); n.s., non-significant from any other group by using one way ANOVA

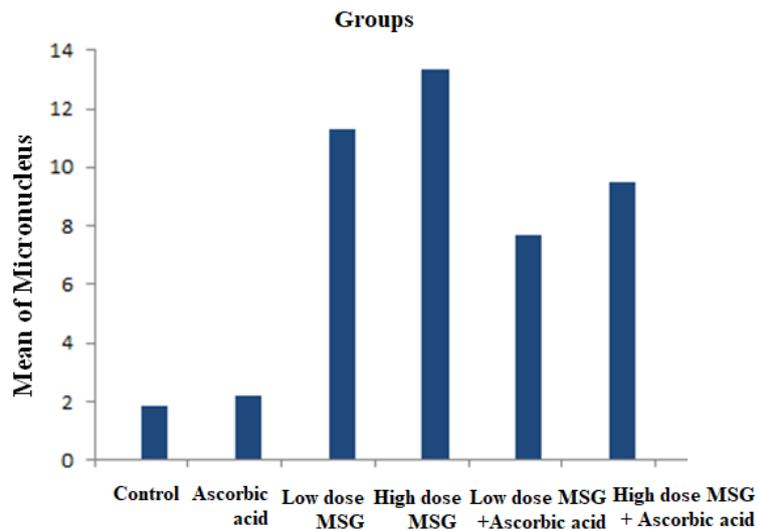
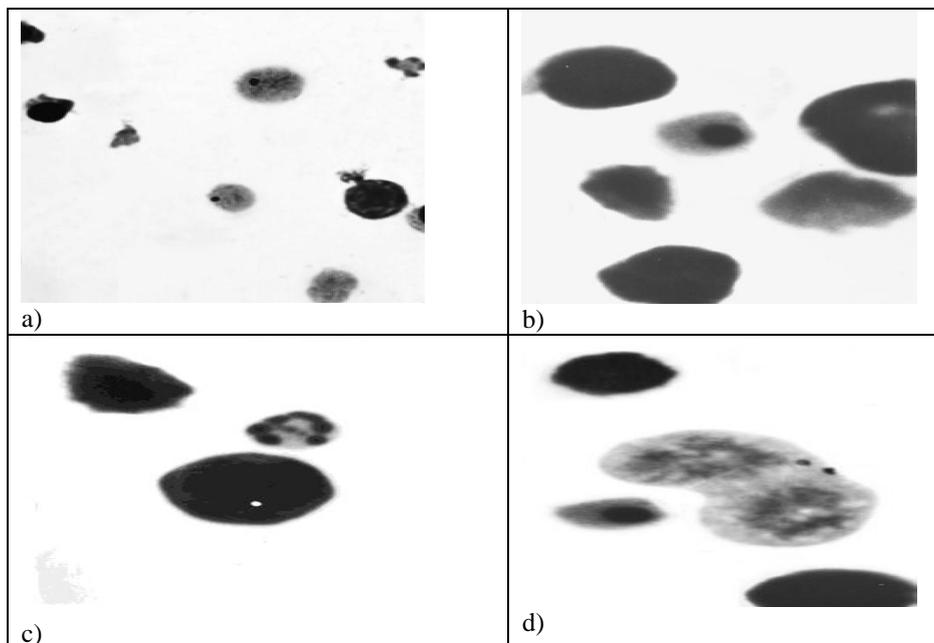


Figure no 2: Mean of micronucleated polychromatic erythrocytes induction to mice administered orally MSG and/or ascorbic acid for 8 weeks.

As regards the size of micronuclei, the majority of micronucleated cases showed up as small micronuclei, where their diameter was less than 1/5 of the diameter of erythrocyte, Figure no 3(a). A small number of micronucleated R.B.Cs were of the large size, Figure no 3(b). Moreover, R.B.Cs with several micronuclei of variable size could be spotted, Figure no 3(c). Furthermore, the chromosomal set seemed to be in aggregates around the equatorial plate while additional fragments were apart and seemed to be included in the regular set, Figure no 3(d). Occasionally, micronuclei could be spotted in normoblast. In such cases, the involved normoblast presented with several nuclei. One of them was taken as the regular nucleus, while the additional ones had a very much smaller size, Figure no 3(e). Occasionally, micronuclei could be spotted in leucocytes. Then, they occurred as rounded bodies that had no connections with the original nucleus of the involved leucocyte. However, micronucleated leucocytes were not taken into consideration or added to the micronucleated R.B.Cs., Figure no 3(f).



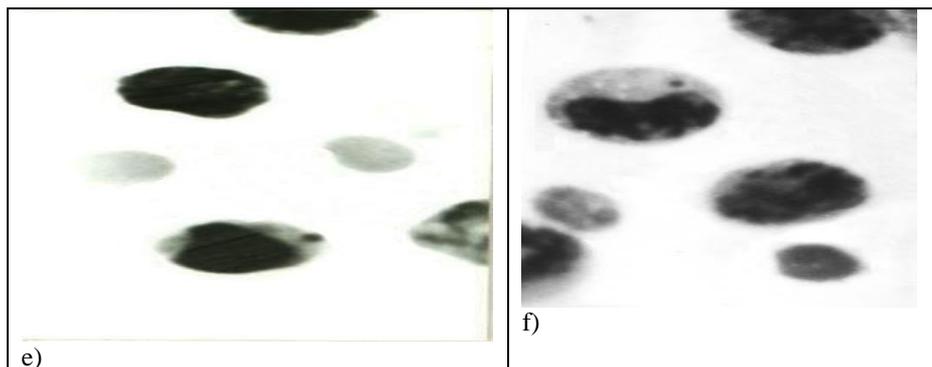


Figure no 3: Micronucleated R.B.Cs in bone marrow of mice treated with MSG and/or Ascorbic acid for 8 weeks.

- (a): Small micronucleus (b): Large micronucleus
(c): More than one micronucleus (d):The lagging chromosome
(e): Micronucleated normoblast (f): Bone micronucleated leucocytes

IV. Discussion

Many studies revealed that MSG induced Reactive Oxygen Species (ROS) with reduction of antioxidant activities²³, which in turn induce mutagenicity as noticed in micronuclei induction and DNA damage. Diniz et al. (2004)²⁴ mentioned that oxidative damage to DNA by ROS and RNS, such as hydrogen peroxide, hydroxyl radicals, singlet oxygen, and peroxyinitrite, may be important to mutagenic, carcinogenic, and aging processes. Previous studies revealed the ability of MSG to induce Reactive Oxygen Species (ROS), increase lipid peroxidation and decrease hepatic glutathione concentration and total antioxidants activities.²⁵

Nevertheless, (Bergamini et al., 2004)²⁶ explained that the oxidative stress refers to the disturbance of the redox equilibrium between the production of free radicals and the ability of cells to protect against damage caused by these species. Defense against oxidative stress could be operated by using several mechanisms. The most effective one of these is the antioxidants defense system. Though lipids are among the first cellular components which are susceptible to damage by free radicals, this in turn can spoil cellular structure and function. Pavlovic et al. (2007)²⁷ indicated that MSG (4mg/g b.wt.) enhanced apoptosis rate of thymocyte as a result of increase of oxidative stress which induce damage to DNA leading in early cell death. According to Kazmi et al. (2017)²⁸, rats treated with MSG (0.6mg/g b.wt.) for 10 consecutive days start to developed symptoms of liver damage. MSG has a clastogenic effect as reported by Dadhaniya et al.(2018)²⁹, that it decreased mitotic index at all dose treatment in a dose dependent manner. The clastogenic effects include gene mutation, chromosomal damage and induction of micronucleus (MN) in polychromatic erythrocytes (PCEs). Additionally, compound which have clastogenic action have a potential to be human carcinogens or mutagen that lead to cancer or dangerous defects³⁰.

In the present work, MSG had to initiate genotoxicity and cytotoxicity in a dose dependent manner. The outcomes showed the induction of MN in bone marrow tissue and DNA fragmentation in hepatocytes. To the author's opinion, liver is the largest gland in the body and responsible for many functions as detoxification and metabolism, so it may be affected by toxic substances and their metabolites. The obtained results are in agreement with (Shah et al., 2019)³¹ which confirmed that MSG is responsible for oxidative stress as well as genotoxicity in human neuronal cells IMR-32 cells.

No doubt that the accumulative dose of food additives led to different diseases or mutagenicity. Previous studies reported that food additives like MSG have a mutagenic effect if use in excessive doses or continuously as shown in this study. In the present result, the repeated doses (4mg/g and 8mg/g) of MSG for 8 consecutive weeks led to damage in DNA which indicates the mutagenic effect of MSG. These findings are in line with (Ataseven et al., 2016)³² who examined six concentrations (250, 500, 1000, 2000, 4000 and 8000 mg/mL) of MSG in cultured human lymphocytes. His results showed that MSG causes changes in RAPD profiles after MSG treatment includes increase or decrease from band intensity and gain or loss of bands. Also, (Kmel et al., 2019)³³ examined MSG on rats through measurement of lipid peroxidation, total antioxidant, Bcl-2, caspase-1, DNA fragmentation and comet assay. They revealed that MSG caused a significant reduction in the levels of total antioxidant activity and an increase in DNA strand breaks for the hepatic tissue cells. Husarova and Ostatnikova (2013)³⁴ stated that MSG increased the expression of several genes implicated in adipocytes differentiation and it also affected the function of liver resulting in increasing of transaminase levels and bile synthesis. Collison et al. (2012)³⁵ showed that MSG intake in extreme users led to disturbances in

metabolism with the increase in more parameters including insulin, fatty acids and triglycerides in serum. Many studies concluded that even low doses of MSG may be hepatotoxic³⁶.

According to the present results, ascorbic acid when administrated with MSG showed improvement in hepatic cells, this is in accordance with (Diab and Hamza, 2016)³⁶ who mentioned that MSG afforded hepatotoxicity, which was reduced by administration of ascorbic acid. (Wang et al., 2011)³⁷ mentioned that Vitamin C, as a water soluble antioxidant is reported to equalize ROS and minimize oxidative DNA damage and hence genetic mutations. Recent studies indicate that vitamin C is much more than just an antioxidant; it regulates the expression of some genes participating in apoptosis or DNA repair processes.^{38,39}

Moreover, (Li and Schellhorn 2007)⁴⁰ believed that ascorbic acid prevents cancer by neutralizing free radicals before they can damage DNA and initiate tumor growth and/or may act as a pro-oxidant helping body's own free radicals to destroy tumors in their early stages.

In addition, the results of the present study showed the ability of MSG to induce micronucleus in bone marrow polychromatic erythrocytes (PCEs). This result is a sign of the toxicity of MSG which agrees with (Dadhaniya et al., 2018; Renjana et al., 2013)^{29,41} who concluded that, MSG induce chromosomal aberrations and micronuclei formation in dose dependent manner. Klobucar et al. (2003)⁴² stated that, enhance in frequencies of MN is an indirect marker of structural and numerical chromosomal irregularities cause in the cells by many agents. These are in harmony with (Arslan and Parlak, 2017)⁴³ who reported that the micronucleus test is a good biomarker for determination of genetic changes in aquatic organism. Also (Fenech et al., 2011)⁴⁴ mentioned that, the micronuclei (MN) and other nuclear anomalies such as nucleoplasmic bridges (NPBs) and nuclear buds (NBUDs) are biomarkers of genotoxic events and chromosomal instability.

The study of DNA damages at the chromosome level is an essential part of genetic toxicology because chromosomal mutation is an important event in carcinogenesis⁴⁵. Furthermore he also mentioned that, the micronucleus assays have emerged as one from the preferred methods of assessing chromosomes damage because they enable both chromosome loss and chromosome breakage to be measured reliably. Induction of micronuclei in the PCEs of bone marrow cells has been considered one of the most sensitive bioassays for monitoring the mutagenic genotoxic effects of a compound⁴⁶.

The present findings showed large number of small-size MN and from the author's sight, this is a strong evidence that MSG considered as a mutagenic substance. Aneugens are agents which affect cell division and the mitotic spindle apparatus resulting in the loss or gain of whole chromosomes, in comparison to clastogens which are agents that induce breaks in chromosomes leading to sections of the chromosomes being added, deleted or rearranged, or mutagens which are agents which induce mutations.⁴⁷ Proudlock (2016)⁴⁸ mentioned that the micronucleus test can be used to detect aneugens, a class of genotoxic agents that is not consistently identified using the chromosome aberration test.

The micronuclei which arise from lagging chromosomes may be a potential to detect aneuploidy-inducing agents that are difficult to study in conventional chromosomal aberration tests (Pironon et al., 2010)⁴⁹. To the author's opinion, the present finding showed that lagging chromosomes in PCEs in animals treated with MSG is a potent sign of the toxicity of it. Pironon et al. (2010)⁴⁹ explained the mechanisms that may lead to MN from chromosome loss events, which is hypo methylation of cytosine in centromeric and peri centromeric repeat sequences such as classical satellite repeats at peri centromeric regions and higher-order repeats of satellite DNA in centromeric DNA. The previous results are in accordance with⁴⁴. Hoffelder et al. (2004)⁵⁰ showed that MN can also originate from fragmented chromosome material when nucleoplasm bridges are formed, stretched and broken during telophase.

Our findings also showed that the co-administration of ascorbic acid (100mg/kg b. wt.) and MSG attenuate the mutagenic effect of MSG on rats, which indicates the protective effect of ascorbic acid against the mutagenic effects of MSG. Moser and Bendich (1990)⁵¹ mentioned that, a dietary intake of 100 mg/day of ascorbic acid is associated with reduced incidence of mortality from heart diseases, stroke and cancer. These results are in agreeing with (Drouin et al., 2011)⁵² who stated that the toxicity of MSG can be overcome by the use of certain kinds of vitamins like vitamin C, therefore the toxic effect of MSG could possibly be counteracted by ascorbic acid. This protective effect of ascorbic acid was previously studied by (Farombi and Onyema 2007)³⁸, who demonstrated that the effects of dietary antioxidant vitamin C, vitamin E and quercetin on MSG-induced oxidative damage in the liver, kidney and brain of rats and the effect of these antioxidants on the genotoxicity of MSG (4 mg/g) in a rat bone marrow micronuclei. The previous study showed that the co-treatment of rats with vitamin C and quercetin inhibited the induction of MNPCEs by MSG but vit E failed to protect against MSG-induced genotoxicity. Pavlovic et al. (2009)⁵³ also found that, the treatment of ascorbic acid may prevent the MSG cytotoxicity in rat thymocytes by up-regulating Bcl-2 protein expression. Moreover high intracellular vitamin C was reported to prevent oxidation-induced mutations in human cells.⁵⁴

V. Conclusion

This study strongly revealed that MSG is an mutagenic agent and the possibility of ascorbic acid to attenuate its effect.

It is recommended that, our diets must contain vitamins to inhibit the mutagenic, clastogenic effects and free radicals induced by food additives, specially MSG which become more worldwide food additives.

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