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Does Monosodium Glutamate Induce Genotoxic Stress Through Altering Gadd45b Gene Expression?

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ABSTRACT

Monosodium glutamate (MSG) is commonly used as a flavor enhancer in nutritional industries. But unfortunately, it leads to various harmful conditions that affect multiple organs. This study was designed to investigate the impact of different doses of MSG on the DNA damage and gadd45b gene expression in liver of adult male Spargue dawley rats. Fourty rats, 6-8 weeks old, weighing 160-180 g were divided equally into four groups. Group I: animals served as normal control, Group II, III and IV: animals received different doses of MSG (8, 600, 1600 mg/kg b.wt./day, respectively; by gavage) for 14 days. The results of the present study revealed a gradual significant increase, in a dose-dependent manner for AST, ALT and ALP while a significant decrease in both albumin and total proteins. An exponential increase was observed among all the studied groups in both IL-8, and Bax accompanied with decrease in Bcl-2 compared to control group. The present findings revealed a significant concentration-dependent increase in the tail length, tail intensity, as well as, tail moments in the liver of rats ingested with different MSG doses. Moreover, the results showed that the expression of the DNA damage marker gadd45b was increased by 1.1, 1.3 and 2.5-fold in the livers of rats ingested with 8, 600 and 1600 mg/kg of MSG, respectively. In conclusion, the results of the present study revealed the potent genotoxic stress of MSG. Due to its harmful health implications, it was recommended to minimize the use of MSG even with its lowest dose, especially in patients with liver disorders.

Keywords: MSG, Gadd45b gene, Genotoxic stress, Apoptosis.

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INTRODUCTION

Monosodium Glutamate (MSG) is one of the most widely used food additives worldwide [1]. As a flavor enhancer, MSG increases the sapidity of food, by producing a flavor that cannot be replaceable by others. Therefore, Japanese described the unique taste elicited by MSG as “umami” translated later to “savory” [2]. The increasing MSG consumption globally in the last few years reflects its wide prevalence from Japan and china to many parts of the world like Middle East and western countries [3]. On the other hand, MSG has been used for the removal of stains from cloths and other textile materials in many countries. The excellent bleaching property of MSG attracts the attention towards its potential harmful effect on many tissues and organs of the body when ingested as a flavor enhancer in food [4]. Hence, studies on animals orally ingested MSG in doses similar to the average human intake revealed multiple complications targeting many organs including; neurotoxicity, nephrotoxicity, hepatic steatosis, inflammation, and dysplasia in addition to various disturbances and alterations in metabolism [5].

The present study focused on the harmful impacts of MSG consumption on the liver as the target organ. MSG affected the liver function resulting in elevation of transaminases` levels and bile synthesis [6]. It has been found that the inducing hepatotoxicity effect of MSG in liver was accompanied with elevation in reactive oxygen species (ROS) formation [7]. Oxidative stress induced by MSG has been well studied and previously proven [8,9]. However, the toxic effect of MSG at the genetic level needs to be elucidated. As part of the liver adaptations to different kinds of stress, it stimulates the expression of individual protein involved in various responses to different stresses including; injury, inflammation as well as DNA-damage [10]. DNA damage is crucial initial step in the carcinogenesis process and is tightly associated with the programmed apoptotic pathway [11]. Hence, quantitative assessment of DNA damage together with the expression of one of the stress response genes are essential for determining the cellular response routes and reflecting the strength of the genotoxic stress induced by MSG [12].

Stress response is an essential physiological mechanism for cells in response to any stress-inducing damage. The stress response family of genes “Growth arrest and DNA damage inducible 45” (gadd45) is comprised of three members: gadd45a, gadd45b and gadd45g. Each gene encodes for small (18 kDa) and highly acidic proteins. They are highly homologous to each other [13]. They are involved in gene expression regulation, cell cycle arrest, DNA repair and apoptosis [14]. Gadd45 proteins are able to interact with the proteins involved in both DNA excision repair and double-stranded DNA breaks repair [15]. Their expression is induced as a response to different types of stress, including oxidative and genotoxic stresses [16]. Gadd45 family proteins are universally distributed in mammalian tissues. However, each member of gadd45 genes has a distinctive expression pattern in different mouse tissues. Gadd45a is expressed in skeletal muscle, kidney, spleen, heart, lung, brain, and liver, but with low levels in testis, while gadd45b is mostly detected in liver, skeletal muscle, and lungs, but lowly expressed in kidney, spleen, brain, heart and testis, meanwhile, gadd45g is expressed in kidney, liver, heart, brain, spleen, lung and testis [13].

Gadd45b gene, also named MyD118, is rapidly induced by genotoxic stress [17]. This increased expression affects stopping cell cycle survival, DNA repair and apoptosis [18]. Its expression pattern change reveals the rate of cell damage at the gene level [19]. The primary response to DNA damage is the stimulation of DNA repair and the activation of cell cycle checkpoint to protect the damaged cell. Meanwhile, apoptosis is the alternate pathway achieved by the multicellular organisms against the damaged cell as the secondary response to DNA damage stress [20].

Based on the important role of the gadd45b gene expression as genotoxic stress response, in addition to, the involvement of DNA damage in carcinogenesis and apoptosis; the present study was designed to investigate the impact of the low, moderate and high doses of MSG on the DNA damage and the gadd45b gene expression. This aims to determine the toxic effect of MSG at the genetic level and therefore; clarify whether gadd45b gene expression can be considered as an appropriate biomarker following the MSG-induced hepatotoxicity.

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MATERIALS AND METHODS

Chemicals:

Ajinomoto brand of MSG was purchased from a regular foodstuff market online and dissolved in saline just before use (freshly prepared).

Animals:

Fourty adult male Sprague dawley rats, 6-8 weeks old, weighing 160-180 g obtained from the breeding unit of Misr University Science and Technology (Research unit of pharmacology and chemistry, Cairo, Egypt) were certified and used throughout this study. The rats were housed maximum five in polyurethane cages with wire mesh floors on wood-chip bedding in the breeding unit of the Medical Research Center (Faculty of Medicine, Ain Shams University, Cairo, Egypt). The room was well ventilated (> 10 air changes per hour) with 100% fresh air (no air circulation). A 12-hour light/12- hour dark photoperiod was maintained. Room temperature and relative humidity were set to be maintained between 20 ± 2°C and 30–70 %, respectively. The animals had free access to sterile pelleted feed of standard composition containing all macro and micro nutrients; water was passed through activated charcoal filter. Any behavioral abnormalities of the studied animals were examined at regular intervals. All animal experiments were performed according to the protocols approved by the local institutional animal ethics committee of Ain Shams University.

Experimental design:

After one week of acclimatization period, the rats were randomly divided into 4 groups of 10 rats each. Group I: animals served as normal control, Group II, III and IV: animals received different doses of MSG (8, 600, 1600 mg/kg b.wt./day, respectively; by gavage) for 14 days.

Blood and tissue collection:

At the end of the experimental period, animals were fasted overnight (water allowed) and euthanized by sodium thiopental (30 mg/kg in saline) [21], and subjected to gross necropsy by cervical decapitation. Blood was allowed to clot, centrifuged and the obtained serum was stored at -20°C until use. At necropsy, livers were collected immediately, rinsed from blood thoroughly in ice-cold isotonic saline solution and divided into two parts. The first part of liver (~100 mg) was immediately cut on ice, and then preserved at -20°C for the molecular studies. The second part of liver (~100 mg) was immediately stored in ice-cold sterile saline at -20°C for comet assay.

Determination of liver function tests in serum:

Serum alanine aminotransferase (ALT; EC 2.6.1.2), aspartate aminotransferase (AST; EC 2.6.1.1) [22] and alkaline phosphatase (ALP; EC 3.1.3.1) [23] activities were assayed by the colorimetric methods. Serum total proteins [24], albumin [25] concentrations were measured quantitatively. The kits were purchased from Diamond diagnostics kits (Hannover, Germany) and Gamma trade (San Antonio, Texas, USA).

Determination of serum proinflammatory interleukin-8:

Level of interleukin-8 (IL-8) was determined by using the corresponding commercially available enzyme-linked immunosorbent assay (ELISA) kit following protocols provided by the manufacturers (Cat. No. KT-60204; Kamiya Biomedical Company, Seattle, USA).

Genotoxicity Study:

Detection of DNA damage in the liver tissue by Comet assay:

The comet assay, also called single cell gel electrophoresis (SCGE), is a rapid, visual and sensitive technique for analyzing and quantifying DNA damage, even low levels in individual cells. The assay was carried out under alkaline conditions [26]. Liver samples (100 mg) from all groups were minced in chilled

homogenizing buffer (0.075 M NaCl and 0.024 M Na_2EDTA), and homogenized gently using homogenizer (Ikemoto Scientific Technology Company Ltd., Japan) in ice. Cells suspension was centrifuged at 4°C, 700X g for 10 min. Cells were re-suspended in the cold buffer. Slides were viewed by epifluorescence microscopy. In control and MSG-ingested rats, digital images were scanned into an image analyzer (Comet V image analyzer software) to determine the length of DNA migration (comet tail length) in response to genotoxicity. Tail length (TL; the distance from the comet head to the last visible signal in the tail), DNA tail intensity (TI; it is calculated from the fraction of DNA in the tail divided by the amount of DNA in the nucleus multiplied by 100) and DNA tail moment (TM; the product of the amount of DNA in the tail and the tail length) were obtained by observing at least 50 randomly selected cells per sample and all assays were performed in triplicate and compared with control.

Quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR):

To confirm the MSG-inducing DNA damage results obtained from the comet assay, and to detect the early changes in gadd45b gene expression, quantitative real-time reverse transcriptase PCR (qRT-PCR) was accomplished on independently derived mRNAs from the livers of the rats ingested with different MSG doses.

Total RNA extraction and cDNA synthesis:

Total RNA was isolated from the liver of rats ingested with the low, moderate and high doses of MSG as well as from the control group, using the BIOLINE TRIsure™ kit (Cat.no. BIO-38032), according to the manufacturer's instructions. For cDNA synthesis, one microgram of the total RNA was used according to the manufacturer's instructions of the BIOLINE SensiFast™ cDNA synthesis kit (Cat. no. BIO-65053).

Measurement of gadd45b mRNA expression levels:

The relative expression levels of mRNA encoding gadd45b or β-actin were measured using the SensiFAST™ SYBR® No-ROX kit (2X) (Cat. no. BIO-98005), according to manufacturer's protocol. The results were computerized using Stratagene (Mx 3000PTM) machine. The expression level of the target gadd45b gene was normalized to β-actin and presented as fold change relative to the control group (group I).

Assessment of serum Bcl-2 and Bax:

Levels of rats Bcl-2 and Bax were determined by the enzyme-linked immunosorbent assay (ELISA) kit following the protocols provided by the manufacturers (Cat. No. CSB-E08854r and CSB-EL002573RA, respectively, Cusabio Kit, USA).

Statistical analyses:

The software program, Statistical Package for Social Science (SPSS), version 23.0 for Windows (SPSS® Chicago, USA), analyzed all data. For quantitative descriptive parametric data, data were expressed as mean ± standard deviation (SD) of replicate determinations. Statistical analysis for differences in means of variables between groups was performed using Student t-test; a probability of $P<0.05$ was considered significant, $P<0.001$ was considered highly significant, while $P>0.05$ insignificant.

RESULTS

Morphological examinations:

During this study, no mortality was observed in animals. The different MSG doses did not initiate any behavioral abnormalities or side effects for all animals. Whereas, small cysts were noticed during necropsy in 50% of rats in both group III and IV ingested with MSG doses of 600 and 1600 mg/Kg, respectively, compared to the control group as shown in figure 1.

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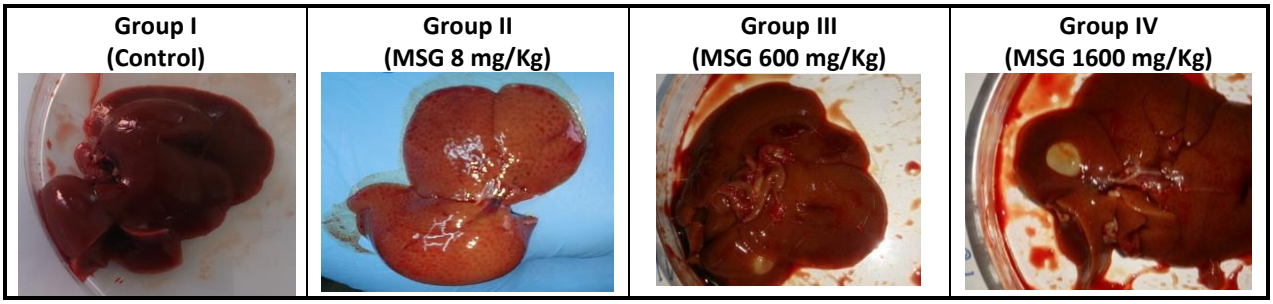

Figure 1: Morphological changes in liver of the studied groups.

Liver function tests:

The liver enzymes activities (AST, ALT and ALP) represented in table 2 showed elevated levels of AST and ALT in rats received the highest dose of MSG "Group IV" (P<0.05 and <0.001, respectively). Non-significant changes in the activity of ALT and AST were observed in both groups II and III when compared to the control group. ALP showed significant increase in group III (P<0.05) with a dramatic increase in group IV (P<0.001), although no changes was observed in group II when compared to control group.

Meanwhile, total proteins content and albumin concentration revealed significant decreases among the studied groups III and IV (P<0.05) with no alteration in the rats received the least dose of MSG "Group II, 8 mg/Kg" as compared to control group.

Serum IL-8 as inflammatory marker:

The level of IL-8 was slightly increased in group II (P<0.05, percent change:24%) and markedly increased in group III and IV (P<0.001, percent change: 53 and 60%, respectively) when compared to control group. The results were represented in figure 2.

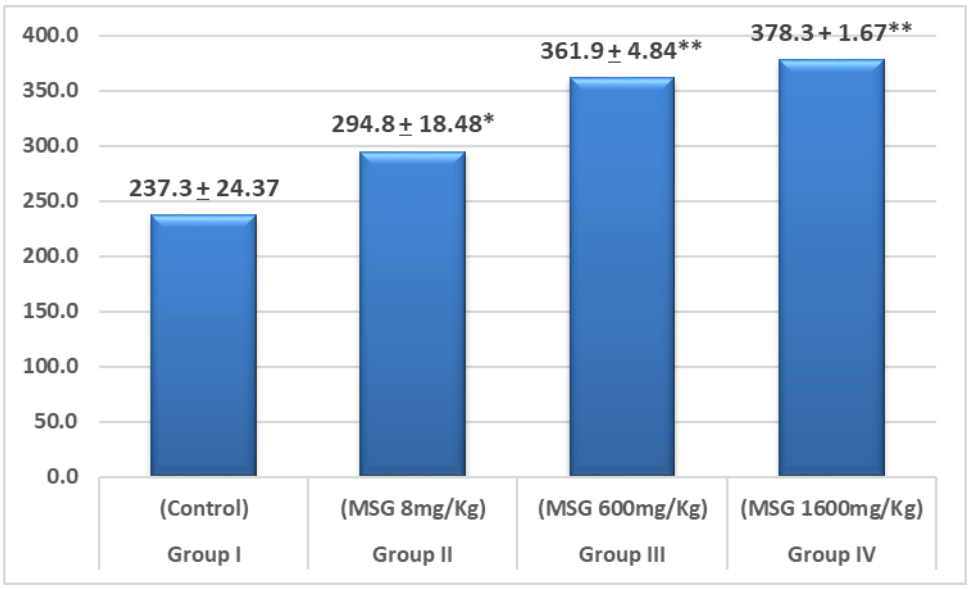

Figure 2: Levels of IL-8 (pg/ml) in the studied groups.

Data are expressed as mean ± SD. *: P value < 0.05, **: P value < 0.001 when compared to control.

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Genotoxicity Study:

DNA damage in liver:

MSG induced DNA damage in the liver of rats. As shown in figure 3, the tail length (TL) observed in group II, III and IV, indicating that chemical damage had occurred in response of the different doses of MSG. On comparing the rats administered with various MSG doses (8, 600 and 1600 mg/Kg) with control group, statistically significant differences were observed in terms of DNA tail length (TL), DNA tail intensity (TI), and DNA tail moment (TM) in the liver samples. All DNA damage parameters in the MSG groups were significantly higher than control group (P<0.001), with increasing damage upon increasing the MSG dose (46%, 100%, and 169% for TL; 36, 93, and 157 for TI; 95, 268 and 558 for TM in the liver, respectively).

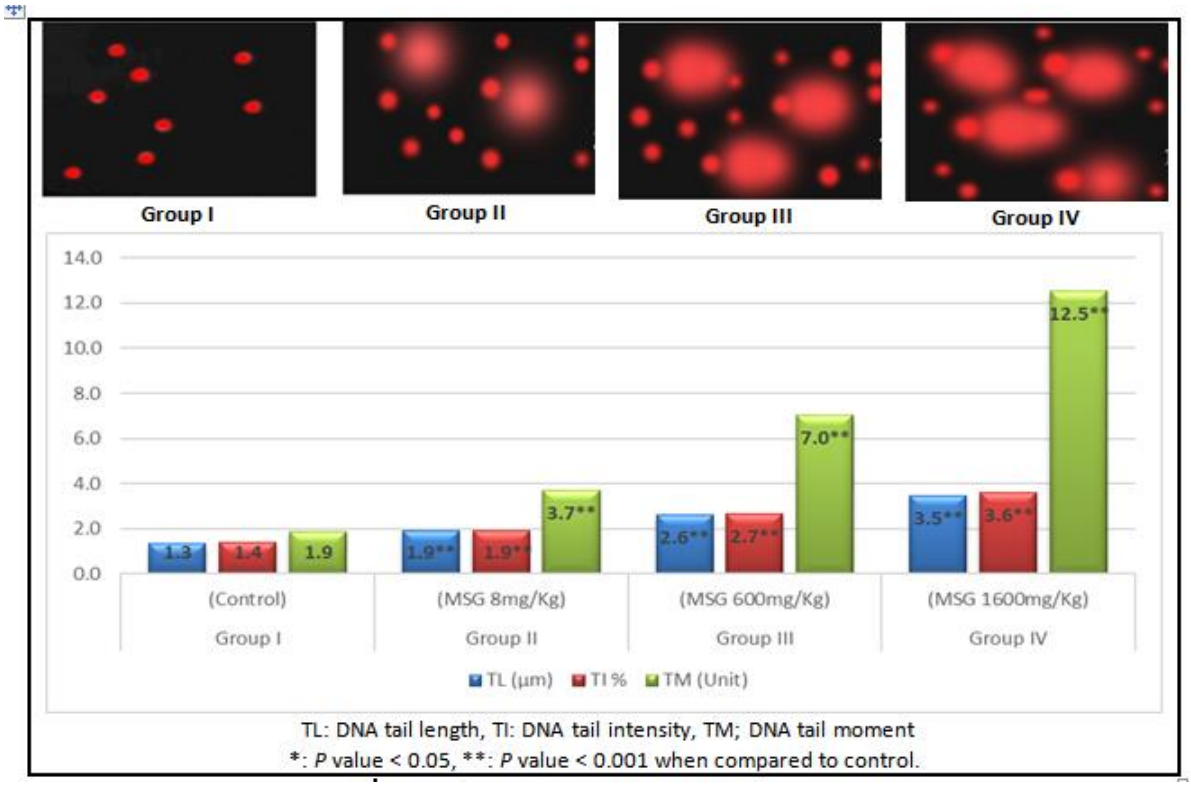

Figure 3: Comet assay in all the studied groups.

Data are expressed as mean ± SD. *: P value < 0.05, **: P value < 0.001 when compared to control.

Assessment of gadd45b mRNA expression:

The expression level of gadd45b mRNA was investigated by qRT-PCR in groups of animals ingested with low, moderate and high concentrations of MSG. The β-actin gene was used as an internal standard for normalization of target gene expression levels. As shown in figure 4, gadd45b mRNA expression was significantly upregulated 1.3 and 2.5 (P<0.001) in rats ingested with MSG doses of 600 and 1600 mg/kg b.wt. (group III and IV), respectively, as compared to the normal control group. At the same time, a marginal increase in the gadd45b mRNA expression levels was observed in rats received the lowest MSG doses (8 mg/kg b.wt.; group II) compared to the control group. These results confirm the potent genotoxic influence of MSG, especially in the two highest concentrations used via upregulating the gadd45b gene expression at the mRNA level.

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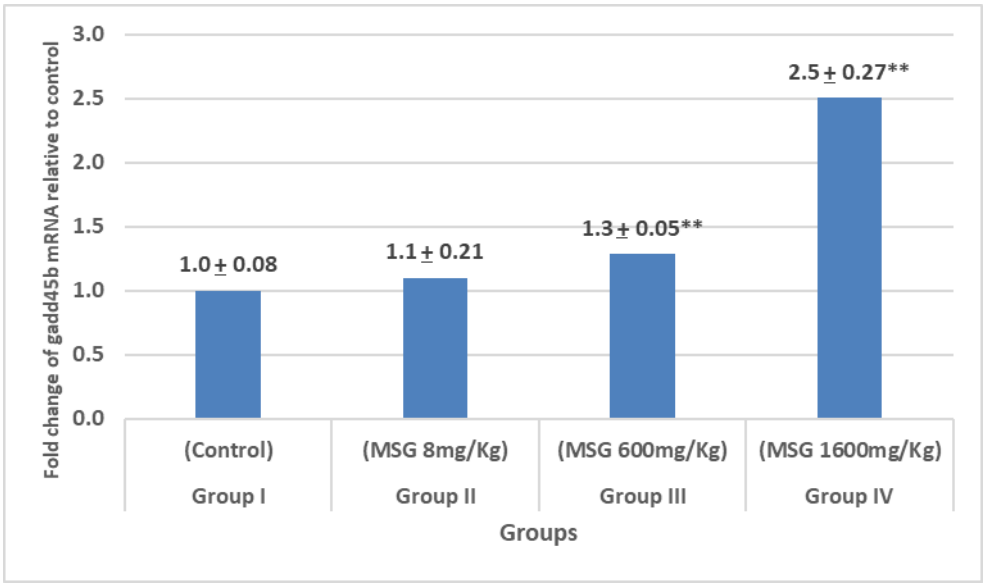

Figure 4: Effect of different concentrations of MSG on liver gadd45b mRNA expression in rats.

Data are expressed as mean ± SD. *: P value < 0.05, **: P value < 0.001 when compared to control.

Apoptotic markers:

The results shown in figure 5 indicated a gradual decrease (P<0.001) in the apoptotic marker Bcl-2 (-11, -25 and -46%) in group II, III and IV respectively. While an increase in the pro-apoptotic marker Bax was observed (P<0.001) (24, 164 and 271%) accompanied the increased doses administered of MSG among group II, III and IV, as compared to the control group.

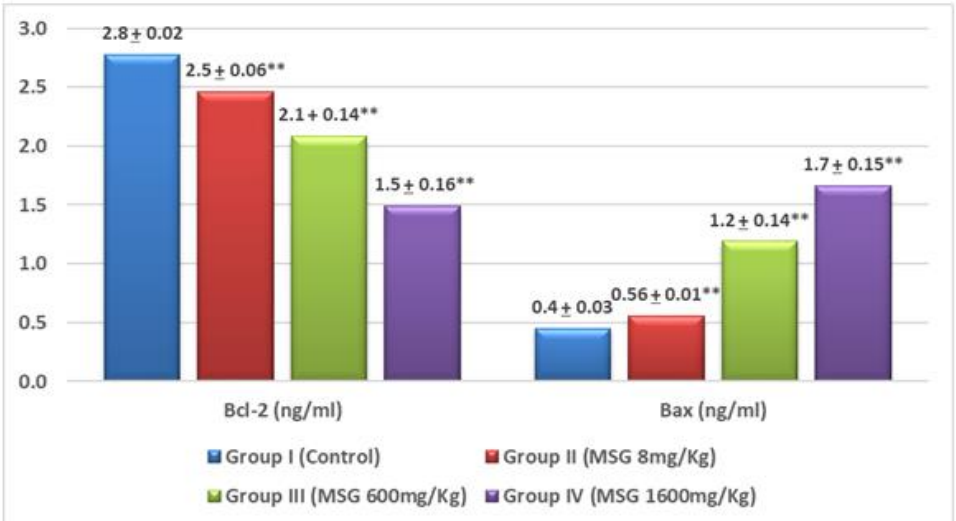

Figure 5: Levels of Bcl-2 and Bax in the studied groups.

Data are expressed as mean ± SD. *: P value < 0.05, **: P value < 0.001 when compared to control.

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Table 1: Primer sequences of target and housekeeping genes.

Gene	Primer sequence	Accession numbers
Gadd45b	Forward: 5′-CTCCTGGTCACGAACTGTCA-3′ Reverse: 5′-GGGTAGGGTAGCCTTTGAGG-3′	NM_008655
β-actin	Forward: 5′-AGCCATGTACGTAGCCATCC-3′ Reverse: 5′-CTCTCAGCTGTGGTGGTGAA-3′	NM_031144

Table 2: Some selected liver parameters with different doses of MSG.

Groups / Mean ± SD	Group I (Control)	Group II (MSG 8mg/Kg)	Group III (MSG 600mg/Kg)	Group IV (MSG 1600mg/Kg)
ALT (Units/ml)	21.33 ± 6.35	25.00 ± 2.00	36.33 ± 9.81	100.33 ± 15.01**
AST (Units/ml)	20.00 ± 8.66	25.67 ± 7.50	36.00 ± 6.55	50.00 ± 12.12*
ALP (IU/L)	85.30 ± 18.51	116.33 ± 22.33	179.00 ± 16.64*	265.67 ± 22.12**
Total Proteins (g/dL)	8.63 ± 0.20	8.13 ± 0.25	7.83 ± 0.30*	6.93 ± 0.45*
Albumin (g/dL)	5.13 ± 0.06	5.00 ± 0.10	4.87 ± 0.06*	4.63 ± 0.29*

ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase.
Data are expressed as mean ± SD. *: P value < 0.05, ** P value < 0.001 when compared to control.

DISCUSSION

MSG, so called the silent killer, is commonly used as a flavor enhancer in modern nutrition. It is easily soluble in water and doesn't catabolize like other amino acids [27]. MSG is a modified form of glutamic acid. L-Glutamic acid; the non-essential amino acid found in proteins, is referred to as "bound" or protein bound glutamic acid [28]. Meanwhile, D-Glutamic acid; known as "free glutamic acid" or "outside of protein", is artificially and chemically produced through a fermentation process outside the body. Besides, other contaminants like polyglutamic acid, mono and dichloropropanols, heterocyclic amines and peptides would also be encompassed in this process [29]. This chemically modified MSG can easily evade from the glutamate dehydrogenase enzyme. So, it leads to various harmful conditions affecting multiple organs, including; the liver, kidney, immune system, central nervous system and reproductive organs. MSG as well can trigger cognitive functions, exhibiting both cytotoxic and genotoxic effects. The stress-inducing MSG produces many alterations and disturbances in vital biochemical parameters, leading to chronic inflammation and ending with DNA damage and cell death [30].

Liver is the main organ responsible for the detoxification of foreign compounds in the body. Hence, this study focused on the toxic effect exerting by the orally ingested MSG on the selected hepatocellular functions. Morphologically, the liver of rats ingested with the two highest doses of MSG showed many cysts formation. These findings may be due to many causes, including; injury, chronic inflammatory conditions, as well as genetic stress. Moreover, MSG was shown to induce the hepatotoxicity in a dose-dependent manner. ALT and AST are sensitive markers of liver damage [31]. Consequently, the observed increase in their activities in the serum of rats ingested with MSG at the tested doses may be an indication of liver damage [32]. Following MSG intake, free glutamate dissociates easily, deaminated and producing the toxic ammonium ion (NH_4^+). This possible overload ion, unless detoxified in the liver via urea cycle, could damage the liver, and thus releasing the high ALT and AST enzymes detected in serum [33]. In addition, the detected increase in the AST activity in the serum of MSG-ingested rats confirms the liver damage along. Previous study reported that the increased activity of serum ALT and AST in male rats ingested with MSG is probably due to the MSG-induced oxidative stress in the liver [7]. The present study revealed the hepatotoxic inducing effect of MSG and hence its use should be avoided during the treatment of any liver disorders. Furthermore, oral MSG intake showed increase

in the serum ALP enzyme activity in a dose-dependent manner, which reflect its dramatic effect on the bone as previously proven that the MSG treatment induce bone loss in mice [34]. Moreover, the synthetic function of liver was altered following the MSG intake, as been revealed in the present study by the decreased levels of total proteins and albumin. This observed reduction of total proteins and albumin concentrations, which may consequently lead to enhanced retention of fluid in the tissues spaces, implied and confirmed the liver damage [35].

In addition to hepatotoxicity, the results of this study revealed that the MSG-inducing effect on the IL-8 secretion indicating that MSG can trigger chronic inflammation. IL-8 is a member of a chemoattractant chemokines family, recognized to cause the directed migration of neutrophils [36]. Previously, it has been shown that neutrophil migration can contribute to the release of ROS, which in turn is associated with many tissues damage. However, the involvement of IL-8 in hepatotoxicity can be clarified by identifying genes interacting with IL-8 signaling in the damage and repair processes. The nuclear transcription factor-kappa B (NF-kB), which is crucial to the inflammatory response [37], can transcriptionally regulate both IL-8 and TNF-α. Early study has been illustrated the significant activation of NF-kB in MSG-induced obese mice [38]. So, by referring to the previous studies showing the role of MSG in NF-kB activation and based on the present results showing the increase in the IL-8 level with increasing MSG concentration; it could be concluded that MSG-induced hepatotoxicity may increase the IL-8 expression through the activation of the NF-kB transcription factor and hence increase its level in serum. Also, several studies demonstrated that other hepatotoxicity-inducing agents, like CCl_4 can activate the NF-kB possibly via TNF-α activation providing further evidence for NF-kB–induced IL-8 expression [39]. Moreover, NF-kB showed its ability to bind to the regulatory elements in the promoter region of IL-8 following H_2O_2 exposure in lung epithelial cells [40]. So, it could be assumed that, IL-8 may contribute to the MSG-mediated damage and therefore be associated with the initiation of hepatotoxicity induction. These results make the IL-8 a potential marker for hepatotoxicity. However, this signaling mechanism needs further investigation.

Concerning the genotoxicity, quantitative assessment of DNA damage is a crucial step in evaluating the strength of the genotoxic stress. Comet assay; the short-term genotoxicity test, was selected in the present study under alkaline conditions; pH>13 [26] due to the advantages provided by this test over the other strand breaks assays. Comet assay has the ability to detect single DNA strand breakage as well as other lesions such as alkali-labile sites, DNA cross-links and incomplete excision repair events [41]. Moreover, the comet assay measurements are made on individual cells, providing an independent measure of the toxicity of a test compound. Furthermore, this test may be very useful in determining the apoptotic fragmentation cells [42], reflecting the presence of apoptotic cells. In this assay, the cell has the appearance of a comet, with a head (the nuclear region) and a tail containing DNA fragments or strands migrating towards the anode following alkaline electrophoresis, and the amount of DNA breakage in a cell was assessed from the migrated extent (tail length, TL) of the DNA [43]. Moreover, the tail intensity (TI) or the percentage of DNA in the tail has been shown to be proportional to the frequency of DNA strand breaks over a wide range of levels of damage [12]. Tail moment (TM), the derived descriptor parameter intended to combine information from both tail length and tail intensity [44, 45], is designed to take into account differences in DNA migration that may be due to the nature and extent of DNA relaxation [46]. The present findings revealed a significant concentration-dependent increase in the TL, TI as well as TM in the liver of rats ingested with low, moderate and high MSG doses, indicating significant DNA damage in all MSG-ingested groups in a concentration-dependent manner. These findings indicate the powerful genotoxic effect of MSG by increasing the DNA breaking number, making the supercoiled loops of DNA to relax, and hence more free ends can migrate, and finally a larger fraction of the DNA moves away from the comet head [47]. This genotoxicity-induced DNA damage was probably due to the direct contact of MSG with nuclear DNA [48]. In other words, MSG consumption leads to increase of free radicals in liver. The combination of such free radicals with some ions is resulting in DNA damage [49]. On the other hand, MSG genotoxicity was previously proven in neurons [50] and in rats' gingival mucosa [27]. MSG ingestion was shown to activate the glutamate receptors which are abundant in neurons and gut. Hence, this glutamate receptors activation is resulting in Ca^{+2} influx which, in turn, induces mitochondrial superoxide production and opening in the mitochondrial membranes. This nuclear DNA damage has been suggested to be a fundamental event in cell death [51,52].

The MSG-inducing genotoxicity was well evaluated and assessed quantitatively throughout this study via its DNA damaging potent effect. Consequently, the evaluation of the stress response gadd45b gene expression was essential to determine the strength of the MSG-inducing genotoxicity and therefore the response

triggered by the cells. Gadd45b, a member of the gadd45 family, is defined as a pro-apoptotic and growth-arrest protein and considered as a sensor mediating rapid responses to a variety of stimulus in mammals [53]. The response may integrate various pathways and trigger several cellular processes, including cell arrest, apoptosis, as well as metabolic changes [54], depending on the strength and nature of the stress. In other words, mammalian cells developed an intricate defense mechanism to maintain their genomic integrity by preventing the fixation of permanent damage from genotoxic stress. This involves activation of cell cycle arrest checkpoints at the G1/S and G2/M transitions, and activation of a cell death program [55]. Whether a cell undergoes either cell cycle arrest, to allow damaged DNA to be repaired, or rapid apoptosis depends on the extent of genotoxic damage and the cell type [56]. The present results showed that the expression of the DNA damage marker gadd45b was increased by 1.1, 1.3 and 2.5-fold in the livers of rats ingested with 8 mg/kg, 600 mg/kg and 1600 mg/kg of MSG, respectively. The expression of the gadd45b gene has shown to be MSG concentration-dependent. Besides, the apoptotic-inducing effect of MSG detected in the present study indicated that the cells triggered the apoptotic pathway rather than the DNA repair as a cellular response. These findings denote the severe DNA damage caused by the ingestion of different MSG concentrations and hence reflect the strength of its genotoxicity especially with the two highest used doses. The upregulatory effect of MSG on the gadd45b gene expression may be achieved via TNFα-NF-kB activation pathway [57]. MSG induces the NF-kB activation where the activated NF-kB can specifically bind to the upstream sites near the gadd45b promoter and in turn, strongly activates its transcription [58].

Apoptosis is one of the several integrated responses to DNA damage and represents the second cellular response triggered by the genotoxic-induced cells to prevent the propagation of the damaged DNA [59]. Alternatively, DNA repair is essentially needed as the primary cellular response to the common or simple DNA damage occurring to maintain the genome integrity [60]. However, the intranuclear mechanisms that signal apoptosis after DNA damage overlap with those that initiate cell cycle arrest and DNA repair, and the early events in these pathways are highly conserved. Apoptosis in response to genotoxic agents is governed and regulated by the proteins of the Bcl-2 family. The Bcl-2 family proteins, also called the DNA damage-induced apoptosis, mediate and regulate the intrinsic apoptotic pathway involving mitochondrial contribution through the interaction of their inhibitors and inducers partner [61]. Therefore, the present study concerned in the assessment of the protein level changes of the most two important apoptosis-related genes; the anti-apoptotic Bcl-2 protein and its pro-apoptotic partner, Bax, in the liver of rats orally ingested with different MSG doses to evaluate the strength of the MSG genotoxic effect via DNA damage-inducing impact. The results recorded a significantly downregulation and upregulation of the Bcl-2 and Bax proteins level, respectively in a MSG dose-dependent manner. These results reflect the severity of the DNA damage-inducing effect of MSG and explained the inability of the cells to rescue from the glutamate toxicity, especially with the two highest doses, and therefore triggering the apoptotic pathway to protect itself from the damaged DNA. Glutamate induces the Ca^{+2} influx can cause disruption of the mitochondrial inner transmembrane potential, and subsequently lead to the opening of the mitochondria permeability transition pore [62,63]. When permeability transition pore is out of control, several essential players of apoptosis, including procaspases, cytochrome c, apoptosis-inducing factor and apoptosis protease-activating factor 1 (APAF-1) are released into the cytosol. They have the ability to activate caspases, resulting in apoptosis [64]. Bcl-2 is localized on the cytoplasmic face of the mitochondrial outer membrane, endoplasmic reticulum and nuclear envelope, maintains the mitochondrial membrane integrity by preventing the release of cytochrome c which, together with APAF-1, facilitates the activation of caspase-9 [65]. On the other hand, the pro-apoptotic Bax, identified as an inhibitory binding partner of Bcl-2 [66], is activated in response to genotoxic stress, causing conformational changes, membrane-insertion, and oligomerization to form a channel in the mitochondrial outer membrane, through which cytochrome c exits to the cytosol to trigger caspase-9, initiating the caspase cascade activation and hence cell death [67]. Additionally, the homodimer formation ability of Bax has been proposed as the dominant regulator of the cell death signal [68]. Bax/Bax homodimer can antagonize the anti-apoptotic function of the Bcl-2 protein, leading to cytochrome c liberation and apoptosis initiation [59].

CONCLUSION

In conclusion, the results of the present study reveal the potent genotoxic stress of MSG. This induces liver damage and trigger chronic inflammation as detected by the DNA damage and was confirmed by the altered gadd45b gene expression in a dose-dependent manner. Hence apoptosis was triggered by the cells in response to the severe DNA damage induced by the MSG. In addition, the present results indicate that the gadd45b gene expression may be a promising marker for MSG genotoxicity. Finally, a limited use of MSG was

recommended even at its lowest dose, particularly in patients with liver disorders due to its harmful health implications.

conflicts of interest: The authors report no conflicts of interest in this work.

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