# The protective effect of *Nigella sativa* seeds against monosodium glutamate-induced hepatic dysfunction in rats

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## ARTICLE INFO

## ABSTRACT

Monosodium glutamate (MSG) is one of the most commonly used feed additives which poses a threat to public health. *Nigella sativa* is a promising natural approach in this issue due to its antioxidant, hypolipidemic, and cytoprotective characters. Here, we investigated the potential protective effect of *Nigella sativa* seed (NSS) against MSG-induced hepatotoxicity in rats. To accomplish this objective, fifteen adult Wistar albino rats were randomly and equally divided into three groups for 21 days: the control group received no treatment, MSG group supplemented with MSG at a dose of 30 g/kg feed, and MSG + NSS group supplemented with MSG at the same previous dose together with NSS at a dose of 30 g/kg feed. NSS succeeded in boosting serum alkaline phosphatase activity and total cholesterol, triglycerides, and glucose levels. It reduced lipid peroxides in the serum and down-regulated glutathione reductase and superoxide dismutase 2 immuno-expression in the hepatic cells. NSS intervention provided cytoprotection by improving the histo-architecture of the liver and reducing the number of apoptotic cells. NSS was effective in protecting against the hepatotoxicity of MSG through its antioxidant and anti-apoptotic effects. These findings are of utmost significance in directing the attention towards the incorporation of NSS in our food industry as well as a health remedy in traditional medicine to fight MSG-related hepatic abnormalities.

## 1. Introduction

Monosodium glutamate (MSG) is a naturally occurring sodium salt of glutamic acid that is widely used as a flavor enhancer in many processed foods, giving them an umami taste and improving their palatability (Foran et al., 2017; Zanfirescu et al., 2019). However, its safety as a feed additive remains highly debated. Although food safety regulatory organizations considered MSG consumption is not related to health hazard problems, several studies have confirmed its deleterious effects in relation to differences in dosage, route of administration, and duration of exposure (Chakraborty, 2019; Smriga, 2016). Nevertheless, MSG-induced hepatic impairment is evident by the detrimental changes in tissue damage biomarkers, carbohydrate and lipid metabolic pathways, antioxidant profile, and histopathological features (Eid et al., 2019; Elbassuoni et al., 2018; Quines et al., 2019).

In the light of the extensive utilization of MSG in the modern feed industry, one of the research directions is to eliminate or reduce its side effects to an acceptable level. The use of natural agents became highly accepted as a realistic option to combat food-borne toxicants and gained popularity as a promising strategy instead of traditional therapeutic drugs (Anwar and Mohamed, 2015; El-Bahr, 2015; Karimi et al., 2019; Khalil et al., 2020; Lu et al., 2020). *Nigella sativa* seeds (NSS) is a dietary favorable candidate because of their wide range of safety, efficacy, and availability (Yimer et al., 2019). The healing powers of *Nigella sativa* were referred to by the Prophet Muhammad [Peace be Upon Him (PBUH)]: "In this Black seed a cure for every disease except death" (Al-Bukhari). The hypoglycemic, hypolipidemic, antioxidant, and anti-apoptotic properties of NSS (Abd-Elkareem et al., 2020; Abou Khalil et al., 2017; Ali and Blunden, 2003; El-Gindy et al., 2019; Hosseinian et al., 2018; Kotb et al., 2018) provide strong rationality in blocking the

multifaceted targets of MSG.

The presence of a variety of bioactive constituents in NSS gives a strong driving force for many scientists to validate their historically claimed usage in combating hepatic dysfunction (Beheshti et al., 2018). Thymoquinone (TQ), one of the principal volatile oils in NSS, is supposed to protect against chemical toxicants *via* stimulating hepatic detoxification machinery and cellular proliferation, scavenging reactive oxidants, up-regulating enzymatic antioxidants, and counteracting apoptosis (Elbarbry et al., 2012; Ince et al., 2012; Ismail et al., 2010; Tabeshpour et al., 2019). The current study aims to investigate the possible protective effect of NSS against MSG-induced hepatocellular damage using an experimental rat model.

## 2. Materials and methods

### 2.1. Identification of phytochemical constituents

*Nigella sativa* seeds were purchased from Imtenan Heath Shop Company, Obour City, Egypt. Phytochemical constituents of NSS were determined using gas chromatography/mass spectrophotometry in the previously published protocol (Abd-Elkareem et al., 2021). The gas chromatography/mass spectrophotometry of NSS showed the presence of 26 active phytochemical constituents. The major constituents were 9, 12-octadecadienoic acid, hexadecanoic acid, and TQ (1.5%) (Abd-Elkareem et al., 2021).

### 2.2. Animals and experimental groups

Fifteen adult Wistar albino rats aged 2-3 months (233.8 ± 9.01 gm in weight) were obtained from the Animal House, Faculty of Medicine, Assiut University, Assiut, Egypt. They were housed at room temperature in polypropylene cages and were exposed to a natural 12 h light/dark cycle with free access to standard laboratory chow and water. The experimental procedures in this study were conducted following the internationally accepted principles for the Care and Use of Laboratory Animals and were approved by the Medical Ethics Committee, Faculty of Medicine, Assiut University (Approval Number: 17300469). After one week of acclimatization, rats were randomly divided into three groups (five rats each): the control group received no treatment, MSG group supplemented with MSG (Morgan Chemical industry, Egypt, purity 99%, NH2. CH(COOH). CH2. CH2.COONa.H2O) in the diet at a dose of 30 g/kg feed for 21 days as this dose is known to impair hepatic functions (Kazmi et al., 2017). MSG + NSS group administrated MSG at the same previous dose together with NSS, after finely crushed by an electric mill mixer, and added to the diet as a powder at a dose of 30 g/kg feed for the same period. This dose provides cytoprotection against chemotoxic substances (Abd- Elkareem et al., 2020; Yousif et al., 2016).

### 2.3. Sample collection

At the end of the experiment, the blood samples were collected immediately from the retro-orbital sinus using microcapillary tubes by an experienced laboratory technician. Blood samples were collected, centrifuged at 3000 rpm for 15 min to obtain serum, and kept at −20 °C until measurement of liver function parameters and lipid peroxides (LPO) level. Rats were euthanized by cervical dislocation for liver specimen collection. The liver was carefully dissected out and quickly fixed in 10% neutral buffered formalin to be used in the histological, histochemical, and immunohistochemical examination.

### 2.4. Biochemical measurements

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, total cholesterol (TC), triglycerides (TG), total protein (TP), albumin, total bilirubin, urea, uric acid, and creatinine were assessed according to the manufacturer's instructions using commercial colorimetric kits provided by Egyptian Company for Biotechnology, Cairo, Egypt. Serum direct (conjugated) bilirubin was calculated by subtraction of indirect bilirubin from total bilirubin (O'Malley et al., 2015). Serum alkaline phosphatase (ALP) was measured by a kinetic method using a commercial kit (catalog number: 1051, Vitro Scient Co., Cairo, Egypt). Serum LPO was estimated according to the method of (Ohkawa et al., 1979). The biochemical parameters were measured using a spectrophotometer (S1200, Unico, USA).

### 2.5. Histological examination

The formalin-fixed livers were dehydrated in ascending grades of ethanol, cleared in methyl benzoate, and embedded in paraffin wax. Paraffin sections at 5 μm in thickness were cut and stained with the following histological stains:

- Haematoxylin and Eosin for general histological examination of the liver (Bancroft and Gamble, 2008).
- Periodic acid Schiff (PAS) technique for demonstration of glycogen (Bancroft and Gamble, 2008).
- Crossmon's trichrome technique for staining collagen fibers (Crossmon, 1937).

### 2.6. Immunohistochemistry of glutathione reductase and superoxide dismutase 2 in the liver

For immunohistochemical detection of glutathione reductase (GR) and superoxide dismutase 2 (SOD2) in the liver, we used polyclonal anti-glutathione reductase and anti-superoxide dismutase 2 antibodies, respectively (Chongqing Biospes Co., Ltd, China) and Power-Stain™ 1.0 Poly horseradish peroxidase (HRP) DAB Kit (Genemed Biotechnologies, Inc, 458 Carlton Ct. South San Francisco, CA 94080, USA). The protocol was used as the previously published protocol (Abd-Elkareem et al., 2021).

### 2.7. TUNEL assay

Detection and quantification of apoptosis were carried out using *In Situ* Cell Death Detection Kit, Fluorescein (Sigma-Aldrich). TUNEL technology was based on labeling of DNA strand breaks which formed during apoptosis as a result of cleavage of genomic DNA. Sections (3–5 μm) of paraffin-embedded tissues were dewaxed in xylene and rehydrated through a graded series of ethanol and double-distilled water. Then the slides were rinsed in PBS (pH 7.4) (three times for 5 min each time). The slides were placed in a jar containing 100 ml 0.1 M citrate buffer (pH 6.0) and heated to near boiling (95-98 °C) in a water bath for 30 min followed by cooling for 20 min at room temperature. Sections were then rinsed in PBS at a pH of 7.4 (three times for 1 min each time). TUNEL reaction mixture (500 μl) was prepared by adding 50 μl of enzyme solution to 450 μl of label solution. Then mixed well to equilibrate components. Slides were rinsed three times with PBS at 15 to 25 °C and excess fluid was drained off. Then drops of TUNEL reaction mixture were added to the samples and the slides were incubated overnight in a humidified atmosphere at 37 °C in the dark. Slides were rinsed three times with PBS and directly analyzed under a fluorescence microscope.

All staining slides were examined by an Olympus BX51 microscope and the photographs were taken by an Olympus DP72 camera adapted into the microscope.

### 2.8. Statistical analysis

Data were expressed as the mean ± standard error of the mean (SEM). Statistical differences between groups were identified by one-way analysis of variance (ANOVA) followed by Duncan post-test. All statistical analyses were carried out using SPSS for Windows software, version 16.0. (SPSS, Inc., Chicago, IL, USA). A probability (p) value of <

0.05 was considered statistically significant.

## 3. Results

### 3.1. Effect of NSS on the liver function parameters and LPO in MSG challenged rats

As shown in Table 1, the impairment in the liver functions by MSG was evident by a significant elevation in serum ALP and ALT activities and TC and TG levels in comparison with the control group. Except for ALT activity, NSS was effective in returning all the above-mentioned parameters towards the control levels. MSG induced hyperglycemia which significantly subsided to the control level by dietary supplementation of NSS. On the other hand, there were no significant changes in serum AST activity, and total and direct bilirubin, TP, and albumin levels between all experimental groups. MSG-supplemented rats were characterized by an oxidant/antioxidant imbalance manifested by a significant elevation in serum LPO level which was significantly reduced to be comparable to the normal by dietary administration of NSS.

### 3.2. Effect of NSS on the histopathological features of liver in MSG-challenged rats

Histopathological examination of the liver of the control group showed a typical hepatic lobular architecture consisting of tightly packed plates of large polygonal hepatocytes with prominent round nuclei and eosinophilic cytoplasm and separated by hepatic sinusoids. Numerous hepatocytes were binucleated (Fig. 1a and b). The portal triad showed no change and consisted of branches of the hepatic artery, portal vein, and bile duct (Fig. 1c). Livers of the MSG group exhibited a focal area of necrosis characterized by increased cytoplasmic acidophilia and pyknotic nuclei (Fig. 1d and e). Sporadic hepatocytes showed degeneration and necrosis with loss of nuclei (Fig. 1f and g). The liver parenchyma also showed moderate multiple focal lymphoid cell aggregations (Fig. 1h). Additionally, there were mild multiple lymphoid cell aggregations in the portal area (Fig. 1i). Occasionally, periportal hemorrhage was observed (Fig. 1j). Mild to moderate diffuse congestion of the hepatic sinusoids was noticed along with mild Kupffer cell activation (Fig. 1k and l). On the other hand, NSS treatment alleviated these changes and restored the normal structure of the hepatic parenchyma and portal area. Hepatocyte degeneration and necrosis as well as the aggregation of lymphoid cells in the parenchyma and portal area were markedly regressed. In addition, congestion of hepatic sinusoids was markedly reduced (Fig. 1m-o).

### 3.3. Effect of NSS on glycogen and collagen deposition in the liver of MSG-challenged rats

Most of the hepatocytes in the liver sections of the control group exhibited cytoplasmic PAS-positive material (Fig. 2a and b). However, the PAS-positive material was severely reduced particularly in the periportal cells in the MSG group (Fig. 2e and f). However, the group treated with NSS showed noticeable restoration of the cytoplasmic PAS-positive material (Fig. 3i and j). Furthermore, the collagen was normal in amount and distribution in the liver of the control group (Fig. 2c and d). However, thick bundles of collagen fibers were seen around the central vein and in the periportal area in the MSG group (Fig. 2g and h), which were markedly improved in the liver of MSG-intoxicated rats treated with NSS (Fig. 2k and l).

### 3.4. Effect of NSS on immuno-expression of GR and SOD2 in the liver of MSG-challenged rats

Glutathione reductase and superoxide dismutase2 immunohistochemical investigation in the liver of the MSG group showed positive GR and SOD2 immuno-expression in some hepatocytes. Whereas GR and SOD2 immunolocalization in the MSG + NSS group showed a slight GR and SOD2 immunostaining in the hepatocytes and were nearly similar to the control group (Fig. 3).

### 3.5. Effect of NSS on the number of apoptotic cells in the liver of MSG-challenged rats

Using TUNEL assay in the paraffin sections of the liver, we observed that the MSG group showed a significant increase in the number of apoptotic cells compared to the control group. While the MSG + NSS group showed a significant decrease in the number of apoptotic cells which nearly returned to normal (Fig. 4a and b).
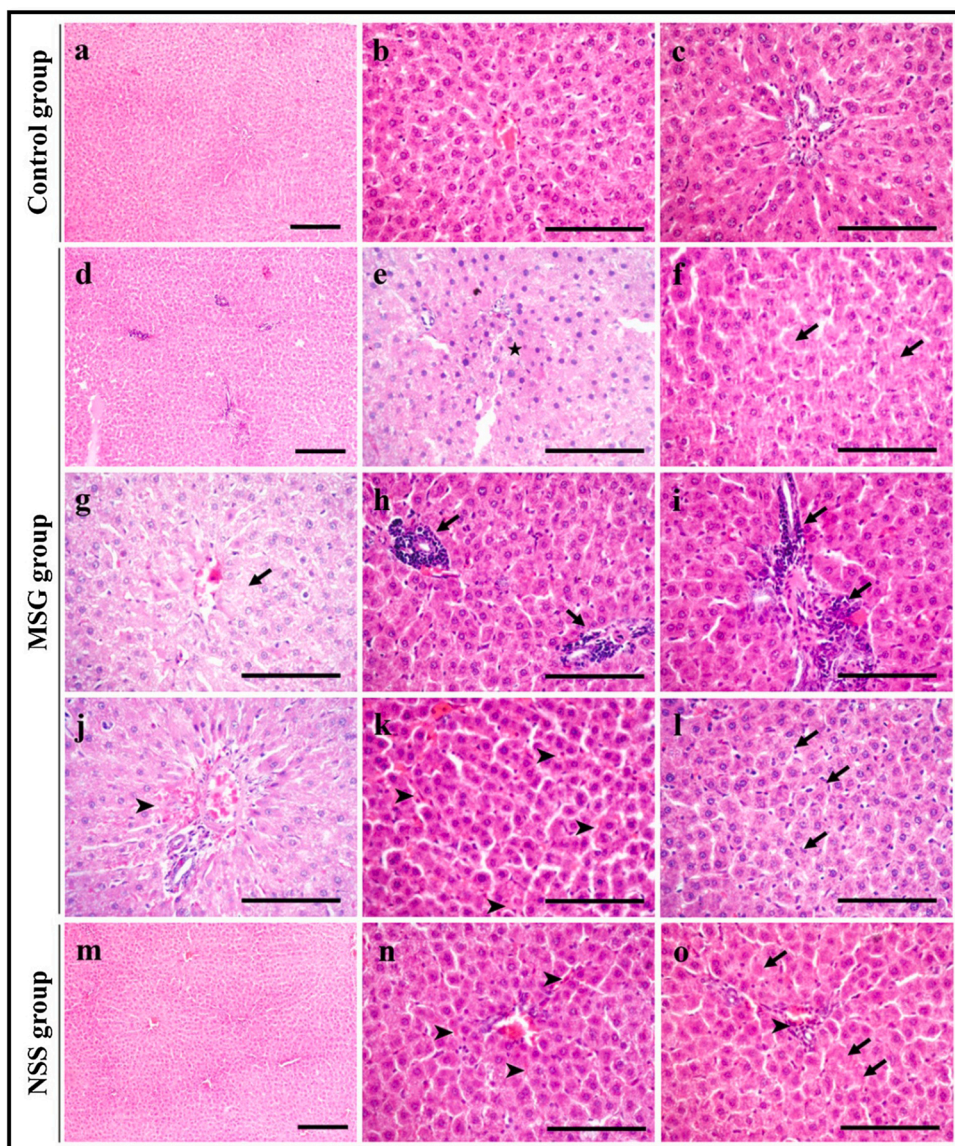
The overall histopathological changes induced by SMG was of mild to moderate compared to the control.

## 4. Discussion

Monosodium glutamate intake was associated with multiple deleterious effects. The determination of agents with a strong protective role against their health hazards with the potential natural occurrence and broad safety window represents a field of growing interest. In the present study, MSG induced hepatic dysfunction, redox imbalance, and apoptosis. Alternatively, NSS dietary intervention effectively mitigated these disturbances via its antioxidant and anti-apoptotic properties.

The marked elevation in serum ALP and ALT activities following the MSG challenge is consistent with a previous report (Eid et al., 2019) and supports the degenerative changes in hepatocytes in our findings.

**Table 1**

Effect of NSS on the liver function parameters and lipid peroxides in serum of rats with MSG-induced hepatic dysfunction

| Group Parameter | Control | MSG | MSG + NSS | P value |
|---|---|---|---|---|
| ALP activity (U/L) | $190.250 \pm 5.030^b$ | $250.520 \pm 9.264^a$ | $177.975 \pm 9.373^b$ | 0.000 |
| ALT activity (U/L) | $22.457 \pm 2.850^b$ | $36.156 \pm 5.927^a$ | $39.073 \pm 4.190^a$ | 0.033 |
| AST activity (U/L) | $122.810 \pm 10.23$ | $136.660 \pm 3.072$ | $134.850 \pm 2.286$ | 0.258 |
| Glucose level (mg/dL) | $92.233 \pm 11.017^b$ | $152.730 \pm 23.239^a$ | $100.590 \pm 11.308^b$ | 0.024 |
| TC level (mg/dL) | $37.323 \pm 4.010^b$ | $51.698 \pm 2.363^a$ | $34.935 \pm 3.286^b$ | 0.003 |
| TG level (mg/dL) | $50.917 \pm 3.390^b$ | $66.192 \pm 5.854^a$ | $53.555 \pm 2.208^b$ | 0.042 |
| Total bilirubin level (mg/dL) | $0.192 \pm 0.052$ | $0.200 \pm 0.037$ | $0.168 \pm 0.030$ | 0.851 |
| Direct bilirubin level (mg/dL) | $0.062 \pm 0.010$ | $0.067 \pm 0.020$ | $0.058 \pm 0.009$ | 0.912 |
| TP level (g/dL) | $3.126 \pm 0.350$ | $3.117 \pm 0.337$ | $2.387 \pm 0.307$ | 0.207 |
| Albumin level (g/dL) | $2.015 \pm 0.310$ | $2.037 \pm 0.345$ | $1.666 \pm 0.260$ | 0.636 |
| LPO level (nmol/mL) | $1.284 \pm 0.095^b$ | $1.937 \pm 0.283^a$ | $1.079 \pm 0.078^b$ | 0.006 |

MSG: monosodium glutamate; NSS: *Nigella sativa* seeds; ALP: alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; TC: total cholesterol; TG: triglycerides; TP: total protein; LPO: lipid peroxides.

Results are expressed as the mean $\pm$ SEM of 5 rats per group. [a,b] Different letters indicate significant differences between groups at $p < 0.05$ (one-way ANOVA followed by Duncan's post-test).

**Fig. 1.** Histopathological changes of the liver in control-, MSG-, and NSS-treated groups. (a-c) Liver from the control group showed a normal arrangement of the hepatic cells (a), central vein (b), and the portal area containing branches of the hepatic artery, portal vein, and bile duct (c). (d-l) In the MSG-treated group, the liver sections showed a focal area of necrosis (star) (e) and degenerated and necrotic hepatocytes (arrows) (f and g). Focal lymphoid cell aggregations in the hepatic parenchyma (arrows) (h) and portal area (arrows) (i). The periportal area showed hemorrhage (arrowhead) (j). The hepatic sinusoids were congested (arrowheads) (k) and the kuppfer cells were mildly activated (arrows) (l). Note that numerous hepatocytes were still normal and healthy, and some were compensatory hypertrophied (hp) in (Fig. k). (m-o) Liver from the NSS-treated group showed a normal arrangement of the hepatic cords in the parenchyma with minimally congested hepatic sinusoids (arrowheads) (m and n), a few degenerated and necrotic hepatocytes (arrows) (o), and a few lymphoid cell infiltrations in the portal area (arrowhead) (o). Hematoxylin and eosin stain. Scale bars in panels a, d, and m = 200 μm and panels b, c, e-l, n, and o = 50 μm.
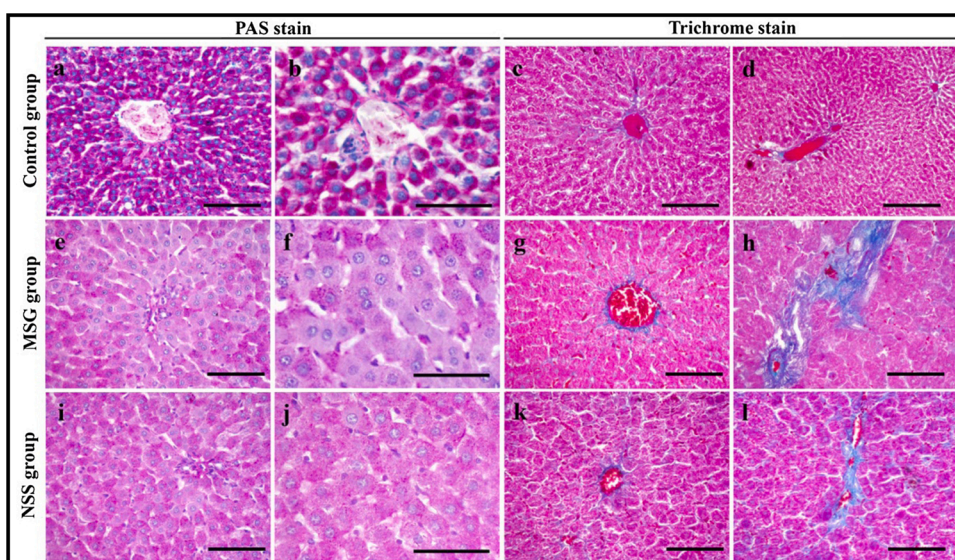


**Fig. 2.** Evaluation of the glycogen and collagen fibers in the liver using PAS and Crossmon's trichrome stains, respectively. (a-d) Liver from the control group showed normal cytoplasmic PAS-positive material (a and b) and normal collagen amount and distribution (c and d). (e-h) The liver from the MSG-treated group showed decreased PAS-positive material in the periportal area (e and f) and dense collagen fibers (g and h). (i-l) The NSS-treated group showed improved PAS-positive material (i and j) and collagen fibers amount (k and l). PAS and Crossmon's trichrome stain. Scale bars in panels a, c, d, e, g, h, i, k, and l = 100 μM and panels b, f, and j = 50 μm.
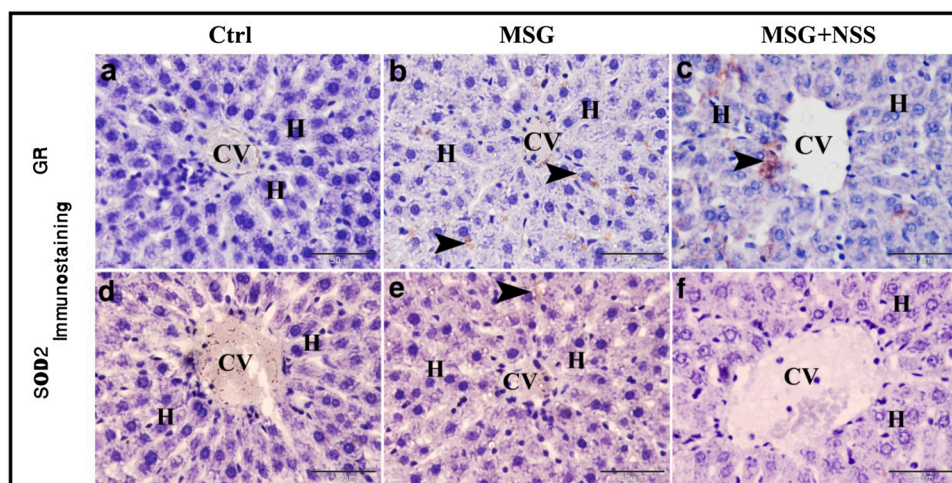
**Fig. 3.** Photomicrograph of GR (a-c) and SOD2 (d-f) immunostaining in the liver showing the protective effect of NSS on MSG-induced hepatic damages. a: Control group showing negative GR immunostaining in the hepatocytes (H). b: MSG group showing positive GR immuno-expression (arrowheads) in some hepatocytes (H). c: MSG + NSS group showing slight GR immunostaining (arrowheads) in the hepatocytes (H). Note the central vein (CV) from which the hepatic plates were radiated. d: Control group showing negative SOD2 immunostaining in the hepatocytes (H). e: MSG group showing positive SOD2 immuno-expression (arrowheads) in some hepatocytes (H). f: MSG + NSS group showing slight SOD2 immunostaining (arrowheads) in the hepatocytes (H). Note the central vein (CV) from which the hepatic plates were radiated. Original magnification, ×400, scale bar = 50 μm.
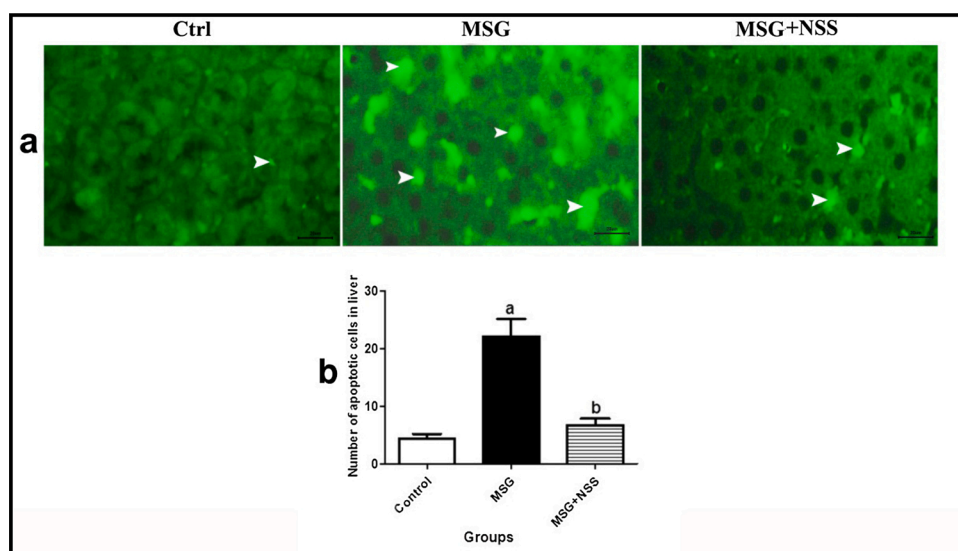


**Fig. 4.** Fluorescent Photomicrograph of TUNEL assay in paraffin sections of liver showing the protective effect of NSS on MSG-induced hepatic damages. a: Liver in the control group (Ctrl) group showed few numbers of apoptotic cells (arrowhead), the liver in MSG group showed a high number of apoptotic cells (arrowheads), the liver in MSG + NSS group showed few numbers of apoptotic cells (arrowheads). Scale bars in a = 20 μm and in b&c = 50 μm. b: Morphometric analysis of the number of apoptotic cells in the liver.
[a] significantly different from the control group
[b] Significantly different from the MSG group

Dietary administration of NSS reversed the negative influences of MSG on ALP activity in agreement with the findings of(Beheshti et al., 2018), probably due to stabilizing of the physicochemical characters of the cell membrane by attenuating the degree of peroxidative stress, secondary to the enhancement of the antioxidant reservoir. The ability of some bioactive components in NSS to scavenge free radicals and up-regulate the expression of enzymatic antioxidants and cytoprotective proteins strengthens this assumption (Ismail et al., 2010; Kundu et al., 2013; Samarghandian et al., 2016). Squalene in methotrexate-challenged rats displayed a marked hepatoprotective effect by counteracting oxidative damage (Sumi et al., 2020). The protective effects of TQ have been confirmed in other models of liver toxicity (Nili-Ahmadabadi et al., 2018; Zeinvand-Lorestani et al., 2018). TQ can suppress the covalent binding of free radical products to intracellular macromolecules such as lipids (Mansour, 2000). In addition, the administration of TQ effectively enhances glutathione transferase and quinone reductase activity (Nagi and Almakki, 2009).

The MSG-induced hyperglycemia in our experimental model may be attributed to the reduction of skeletal muscle GLUT 2 content, pancreatic β-cell mass and insulin sensitivity, and activation of glucose-6-phosphate dehydrogenase (Araujo et al., 2017; Boonnate et al., 2015; Quines et al., 2019). On the other hand, NSS succeeded in normalizing the glucose level similar to a previous study (El Rabey et al., 2017). The hypoglycemic effect of NSS is due to its insulinotropic action (Fararh et al., 2002) along with down-regulation of gluconeogenic enzymes and reduction of intestinal glucose absorption (Meddah et al., 2009).

The hypercholesterolemia and hypertriglyceridemia in the MSG group are in accordance with previous findings (Shukry et al., 2020). MSG could increase the activity of 3-hydroxy-3- methylglutaryl-CoA reductase (Ibegbulem et al., 2016), a rate-limiting enzyme in cholesterol biosynthesis, shifting the glucose metabolism towards lipogenesis. The hypolipidemic effect of NSS in our study is matched with that observed in another chemotoxic-induced hepato-renal dysfunction model (Al-Seeni et al., 2018). Inhibition of intestinal cholesterol absorption and hepatic cholesterol biosynthesis, as well as up-regulation of low-density lipoprotein receptors (Asgary et al., 2015), could be involved in the hypolipidemic effects of NSS. TQ-rich fraction extracted from *Nigella sativa* and TQ up-regulate the transcript level of the low-density-lipoprotein receptor and down-regulate 3-hydroxy-3-methylglutaryl-coenzyme A reductase in rats (Al-Naqeep et al., 2009).

Increasing in a mitochondrial proton gradient (Sharma, 2015), up-regulation of α-ketoglutarate dehydrogenase activity (Tretter and Adam-Vizi, 2005), and persistent activation of glutamate receptors (Lan et al., 2001) secondary to exposure to MSG play role in inducing oxidative stress. This disturbance in redox homeostasis could in turn exert a compensatory regulatory response by up-regulating the

antioxidant defensive mechanism (Chen et al., 2011). This is confirmed in our study by positive GR and SOD2 immuno-expression in the hepatocytes, an outcome which is similar to that found in the liver of astaxanthin-challenged rats (Monmeesil et al., 2019).

It appears that oxidative stress caused by MSG exposure induced free radical scavengers such as SOD2 and GR to protect the cells from damage by reactive oxygen species. However, these adaptive mechanisms seemed to be insufficient to protect against hepatocellular damage in our study because some hepatocytes were lysed and released their intracellular enzymes. In our study, the beneficial role of NSS in ameliorating the redox disturbance driven by MSG was exemplified by the normalization of LPO similar to previous reports (Mady et al., 2016). Limitation of reactive oxygen species formation (Karimi et al., 2019) is the main mechanistic tool by which NS protects against the adverse effects of feed additives. Scavenging free radicals by TQ and squalene could underlie the suppression of lipid peroxidation (Elsherbiny and El-Sherbiny, 2014; Ibrahim et al., 2020). Squalene enriched-herbal treatment shows a promising dual antioxidant action both by enhancing antioxidant capacity and quenching the reactive free radicals (Ibrahim et al., 2020). Squalene increased the activities of glutathione peroxidase, catalase, and superoxide dismutase when administrated along with 3-methylcholanthrene in a rat model (Suriyakalaa et al., 2018). TQ effectively improved the plasma and liver antioxidant capacity and up-regulated the expression of liver antioxidant genes in hypercholesterolemic rat model (Ismail et al., 2010).

Reducing the activity of xanthine oxidase by farnesol plays a central role against cigarette smoke extract-induced redox imbalance in the prostate of rats (Lateef et al., 2013). The down-regulation of SOD2 and GR in the hepatic tissue following NSS consumption points to an improvement in the status of oxidative stress, as the perturbation in redox homeostasis induces up-regulation of endogenous antioxidant defenses mediated by activation of redox-sensitive transcription factors and its downstream signaling pathways, causing an increase in the overall capacity to antagonize the harmful effects of oxidative damage (Done and Traustadóttir, 2016). Therefore, the weak immunostaining of SOD2 and GR reflects the free-radical quenching activity of NSS (Butt et al., 2018).

The histopathological lesions observed in the liver of the MSG group are consistent with those previously found (Eid et al., 2019; Elbassuoni et al., 2018). The obvious improvement in the hepatic histo-architecture of MSG-challenged rats following NSS administration is on the same line with other investigators (Eshami et al., 2015). The active phytochemical ingredients of NSS such as TQ, thymol, and α-hederin are fundamental players in the hepato-protection against harmful agents by inhibition of iron-mediated lipid peroxidation, nuclear factor kappa B, cyclooxygenase, and lipoxygenase (Tabassum et al., 2018). TQ stimulates cell division and proliferation leading to enhanced regeneration after tissue damage (Kanter, 2011).

The pro-apoptotic effect of MSG on the hepatic tissue is in harmony with that observed in the testicular tissue and hippocampus of rats (Anbarkeh et al., 2019). Inconsistent with a previous study (Al-Gayyar et al., 2016), concurrent administration of NSS provided benefits in preventing the development of MSG-induced apoptosis which can be explained based on the antioxidant and anti-inflammatory properties of NSS (Hosseinian et al., 2018). TQ is responsible for the anti-apoptotic activity of NSS by decreasing malondialdehyde and down-regulating mitogen-activated protein kinase pathway, caspase 3, and heat shock proteins (Öztürk et al., 2020; Tabeshpour et al., 2019). Stabilization of the cell membrane (Micera et al., 2020) and inhibition of ataxia telangiectasia mutated kinase-dependent signaling pathway (Tatewaki et al., 2016) are possible mechanistic pathways by which squalene blocks the action of pro-apoptotic inducers. Linolenic acid (octadecadienoic acid) rescued Bcl-2 expression, inhibited Bax translocation to mitochondria and suppressed caspase-3 activity (Carotenuto et al., 2016).

## 5. Conclusion

Monosodium glutamate-challenged rats were characterized by hepatic dysfunction and redox imbalance along with increased programmed cell death. The negative consequences of MSG consumption have been partially overcome by the nutritional inclusion of NSS by restoring the redox potential and ameliorating the histopathological deteriorations and apoptosis in the liver. These outcomes are of major importance in paving the road towards the incorporation of NSS as a candidate strategy against MSG-related abnormalities, and opening interesting possibilities for studying its effectiveness in fighting the other side effects of MSG.

## Declarations Ethical approval

The experimental procedures in this study were conducted in accordance with the internationally accepted principles for the Care and Use of Laboratory Animals and were approved by the Medical Ethics Committee, Faculty of Medicine, Assiut University (Approval Number: 17300469).

## Consent to participate

Not applicable.

## Consent to publish

Not applicable.

## Authors contributions

MA and MS performed the histopathological examination. MA and MAMA carried out the experimental design. NSA performed the biochemical measurements and statistical analysis and was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

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## Declaration of Competing Interest

The authors declare that they have no competing interests.

## Availability of data and materials

Not applicable.

## Declaration of Competing Interest

The authors report no declarations of interest.

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