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The Effects of Apocynin on Monosodium Glutamate Induced Liver Damage of Rats

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ABSTRACT

Monosodium glutamate (MSG) is found in refined foods. Apocynin (APO) is a selective NADPH oxidase (NOX) inhibitor. The aim of this experimental study was to investigate possible effects of MSG and the curative effects of APO in rats. Twenty-eight male Sprague-Dawley rats were randomly divided into four groups (Normal control, APO, MSG and MSG + APO, n:7 for each group). The MSG and MSG + APO groups received 120 mg/kg MSG solution orally for 28 consecutive days. The APO and MSG + APO groups received 25 mg/kg APO solution orally for 5 days until the end of the experiment. At the end of the experiment, all rats were sacrificed and liver tissue and blood samples were taken for histological, ultrastructural, and biochemical analyses. In the MSG group, vacuolization and loss in glycogen content in the hepatocytes, leukocyte infiltration and fibrosis in the liver parenchyme and portal triads, were observed. Terminal deoxynucleotidyl transferase dUTP (TUNEL)-positivity and NADPH oxidase (NOX)-2-positivity were higher in the MSG group compared with the other experimental groups. The concentrations of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total bilirubin, malondialdehyde (MDA), and myeloperoxidase (MPO) were higher, whereas albumin, glutathione (GSH), and superoxide (SOD) levels were lower in the MSG group. All these data has been reversed in MSG + APO group. The histological and biochemical criteria indicated the prominent ameliorating effect of APO on MSG -induced liver injury.

1. Introduction

Monosodium glutamate (MSG), which has a unique flavour-enhancing property, is a salt form of nonessential glutamic acid [1]. An essential component of proteins and peptides in many tissues is the amino acid glutamate, which is produced by the body itself in significant quantities. Glutamate also has a significant influence on human metabolism. Naturally occurring glutamic acid in food does not affect metabolism. Industrially produced synthetic glutamic acid may have toxic effects [2]. While the average daily intake of MSG in developed countries is estimated to be 0.3–1.0 g/day, it has been reported to be 0.58 g/day and 10.0 g/day in the United Kingdom

Abbreviations: AST, Aspartate transaminase; DMSO, Dimethyl sulfoxide; FDA, American food and drug administration; GSH, Glutathione; H&E, Hematoxylin and eosin; JNK, c-Jun N-terminal kinase; MAPK, Mitogen-interacting protein kinase; MDA, Malondialdehyde; MSG, Monosodium glutamate; MPO, Myeloperoxidase; NADPH, Nicotinamid adenine dinucleotid phosphate; NOX, NADPH oxidase; PAS, Periodic acid-Schiff reaction; TUNEL, Terminal deoxynucleotidyl transferase dUTP.

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and Germany, respectively. Although FDA (American Food and Drug Administration) reports that MSG is a safe substance, it has been shown in various experimental animal studies that consumption of MSG over a prolonged period of time had adverse effects. These negative effects have been demonstrated in various organs such as the thymus, brain, pancreas, testis, liver, and kidneys, and have been linked to many diseases such as obesity, hypertension, headaches, and neurotoxicity [3–5]. It is known that the consumption of MSG increases generation of reactive oxygen species (ROS), leading to the damage of lipids, proteins and DNA by way of free radicals. Lipid peroxidation is the result of damaged polyunsaturated fatty acids in cell membranes. All these events cause cell death through apoptosis. These radicals are broken down in the liver and excreted by the kidneys [6]. Many studies have shown that ingestion of MSG seriously damages the liver and kidney tissue. In this study, we investigated the effects of oral consumption of MSG on rats when they ingested a moderate dose extrapolated directly from the daily intake in humans. Therefore, a dose of 120 mg/kg was used for the experimental design [7]. As a result, administration of MSG deteriorates liver function and causes fibrosis and vacuolization of hepatocytes [8,9]. It has been reported that the intake of MSG impairs the cellular integrity of the liver and causes defects in membrane permeability and cell volume hemostasis [10].

ROS production is a sequence of reactions that usually begins with the generation of superoxide. ROS (mainly O_2^- and H_2O_2) can affect many liver-specific cells, leading to the development of fibrosis. High production of ROS in hepatocytes causes cell death [11]. ROS is produced as a metabolic by-product, by mitochondria, peroxisomes, cytochrome P-450 and other cellular sources. It is also produced by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase which plays a role in various signalling events such as cell growth, survival and death [12]. NADPH oxidase (NOX) enzymes are the major resources for the oxidative stress in hepatocytes and non-hepatocytes. The main function of these enzymes is to transfer electrons from NADPH to O_2, which forms superoxide and hydrogen peroxide. These enzymes consist of seven transmembrane proteins (NOX 1–5 and Duox 1 and 2). It is emphasised that NOX-mediated ROS production has a very important effect on hepatic fibrogenesis [13].

Apocynin (4-hydroxy-3-methoxyacetophenone) (APO), extracted from the roots of the plant *Apocynum cannabinum*, is an NADPH inhibitor [14]. APO reduces hepatic oxidative damage with the mechanism of cellular renewal of NADPH by reducing the expression of $gp91^{phox}$ (subunit of a NOX) [13]. APO inhibits the release of superoxide via NOX, by preventing the formation of the NOX complex. APO is also known inhibiting intracellular translocation of some critical cytosolic components of the NADPH oxidase complex in the cell membrane [15] and interrupts activation of redox-sensitive transcription factors [16]. *c*-Jun *N*-terminal kinase (JNK), a subfamily of mitogen-interacting protein kinase (MAPK), regulates many important cellular activities, including cell proliferation, differentiation, and apoptosis [17]. Activation of JNK plays an important role in inducing apoptosis in hepatocytes in case of inflammatory liver diseases [18]. Experimental studies have shown that APO not only suppresses oxidative stress by inhibiting NADPH oxidases but also protects the cell from apoptosis by preventing excessive phosphorylation of JNK [19,20].

This study aims to evaluate possible negative effects of MSG (120 mg/kg) and to investigate putative healing effects of APO (25 mg/kg) on liver tissue, by histochemical, immunohistochemical ultrastructural and biochemical procedures.

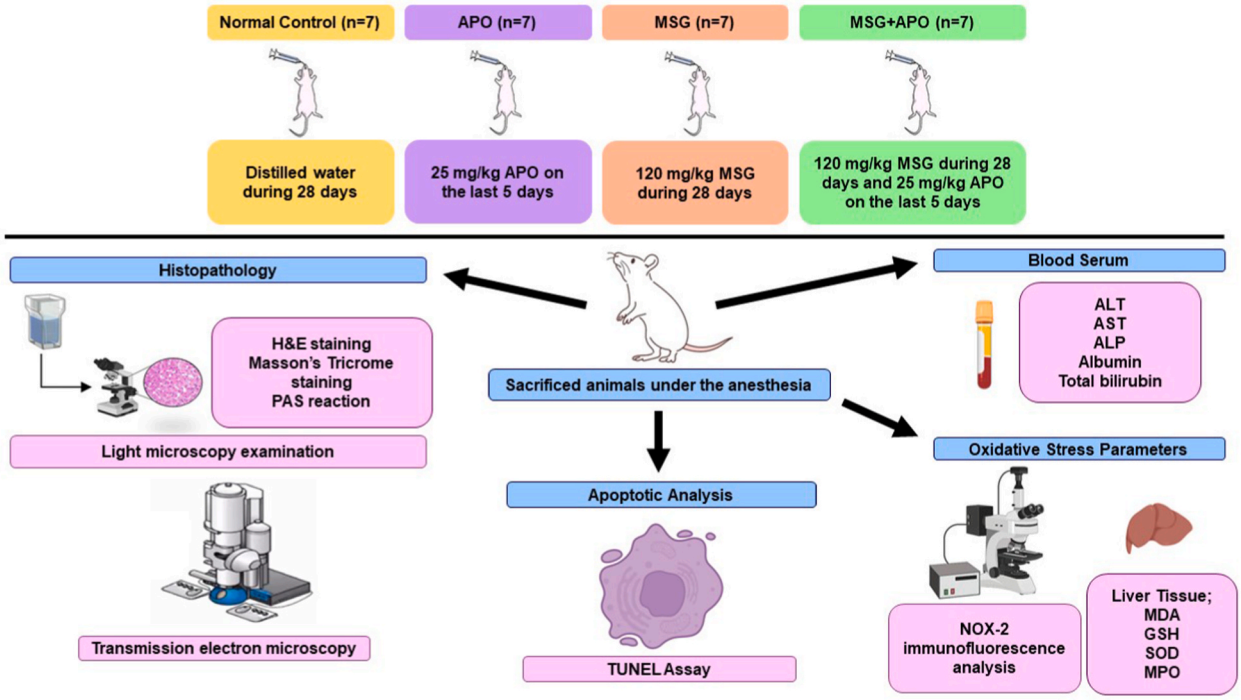

Fig. 1. Experimental design.

2. Methods

2.1. Experimental animals

Eight-week-old Sprague Dawley male rats (200–250 g) were kept in a plastic cage with controlled temperature (21 °C) and light-dark cycle (12:12 h). These rats were fed with standard food and ad libitum water. The experimental study was approved by the Ethics Committee of Acibadem Mehmet Ali Aydinlar University Experimental Animals (HDK-2020/45).

2.2. Experimental design

Power analysis G-Power v3.1.9.2 software was used to determine the number of rats to be used in the experimental design. In experimental studies with quantitative analysis, the power should be at least 80%. According to the power analysis performed in the G-Power program, a total of 28 animals (7 in each experimental group) were calculated for the experimental design with 85% power and 0.4 effect size in all experimental groups and n = 7 for the number of animals in each experimental group. Sprague Dawley male rats (n = 28) were randomly divided into four groups as Normal control, MSG, APO and MSG + APO groups.

Group I: Normal control group; Rats in this group received distilled water by gavage for 28 days.

Group II: APO group; APO solution (25 mg/kg) [21] (Catalogue No.: sc-203321, Santa Cruz, California, U.S.A.) prepared with 8% dimethyl sulfoxide (DMSO) was administered by gavage to the rats in this group for the last 5 days of the experiment.

Group III: MSG group (negative control); MSG solution (120 mg/kg) prepared with distilled water (Catalogue No.: G1626, Sigma Aldrich, Burlington, MA, U.S.A.) was administered by gavage to the rats in the MSG group for 28 days [7].

Group IV: MSG + APO group; MSG solution (120 mg/kg) was administered by gavage to rats in this group for 28 days, and APO solution (25 mg/kg) was administered by gavage during the last 5 days of the experiment [21].

At the end of the experiment, rats were sacrificed under deep anesthesia with ketamine (9 cc/kg) and xylazine (7 cc/kg) anesthesia [22] and liver tissues and blood samples were taken for histochemical, immunohistochemical, transmission electron microscopical and biochemical evaluations (Fig. 1).

2.3. Determination of body weight of the animals

All experimental groups of body weight was calculated weekly and was expressed as grams [23].

2.4. Serum biochemical analysis

Blood samples which were picked up from the heart were kept in a dry vacuum tube for 30 min at room temperature and were centrifuged at 4000 rpm for 15 min. The serum levels of Alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) were determined by using a commercial kit (Biolabo, Maizy, France). Albumin levels were measured using an albumin reagent set (Teco Diagnostics, Albumin Reagent Set, Cat. No: A502-480, Anaheim, CA, U.S.A.). Results were given g/dL. Concentration levels of total bilirubin were determined by a commercial kit (Biolabo-Total Bilirubin, Cat. No: 80,443, Maizy, France) by using spectrophotometry [23].

2.5. Measurement of oxidative stress markers in liver tissue

After decapitation liver tissue samples were washed with phosphate-buffered saline (PBS). 10% tissue homogenate was prepared by homogenising with a cold 150 mM KCl solution [23–25]. Malondialdehyde (MDA) levels were analysed using Ohkawa et al. methods [26]. Results were given nmol/g tissue. Glutathione (GSH) was analysed using Beutler's method [27]. Results were given IU/g protein. Myeloperoxidase (MPO) activity was determined by using a commercial kit (Cat. No: *E*-BC-K074-M, Elabscience, U.S.A.). Results were given nm/mL. Superoxide (SOD) activity was determined by using a commercial kit (Sigma SOD Determination Kit- Cat. No:19,160, Burlington, MA, U.S.A.). Results were given IU/g protein.

2.6. Light microscopical tissue processing

Liver tissue specimens were put in 10% neutral buffered formalin solution for light microscopic evaluation. These samples were dehydrated with increasing ethyl alcohol series, cleared with xylene and incubated in paraffin [23,24]. Paraffin sections (approximately 5 μm) were dyed with hematoxylin and eosin (H&E) and Masson's trichrome Periodic acid-Schiff reaction (PAS) was applied to the sections to evaluate the glycogen distribution within the hepatocytes.

Vacuolization in hepatocytes, inflammatory cell infiltration in the parenchyme and increased connective tissue were evaluated in the sections through scoring semiquantitatively using a scale of 0–3 (0, none; 1, mild; 2, moderate; and 3, severe) for each criterion [28]. The maximum total score for each section was 9. Glycogen content in hepatocytes was calculated quantitatively using the Image J programme (1.44 software, National Institutes of Health, U.S.A).

2.7. Terminal deoxynucleotidyl transferase dUTP (TUNEL) nick end labelling method

TUNEL method was processed according to the manufacturer's protocol (TACS 2 TdT-DAB In Situ Apoptosis Detection Kit, 4810-30-K, Trevigen, Maryland, U.S.A.). Sections were incubated in proteinase K (1:50) and treated with deionized water. The sections were processed in Quenching Solution and processed in 1xTdT Labelling buffer for 5 min. They were covered with Labelling Reaction Mix and incubated for 1 h (at 37 °C). These sections were put in 1xTdT Stop Buffer for 5 min then washed with deionized water. The slides were covered with Strep-HRP solution and incubated 37C for 10 min. Then they were washed with 1xPBS. Liver sections undergo DAB solution incubation, deionized water washing, and methyl green counterstaining. The number of apoptotic cells in each section was divided by the total number of cells to determine the apoptotic index [29].

2.8. NOX-2 immunofluorescence analysis

Cryosections of liver tissue (approximately 8 mm) were microwaved with citrate buffer solution (pH 6.0) and then cooled for 15 min (at room temperature). These slides were treated in blocking solution for 10 min and incubated with *anti*-NOX-2 primary antibody (1:100, Cat. No: NBP2-41291, Novus,Bio-Techne, Minnesota, U.S.A) at overnight (at 4 °C). These sections were cleaned in PBS and incubated with goat anti-rabbit secondary antibody (1:1000 dilution) (Cat. No: A32740, Invitrogen, U.S.A) at room temperature. Liver sections were incubated with 4′-6- diamidino-2-phenylindole (DAPI) at room temperature in the dark environment. These sections were analysed under a fluorescence microscope (Zeiss Axio Scope). The intensity in the sections was calculated using the Image J programme (1.44 software, National Institutes of Health, U.S.A.) [21,30].

2.9. Transmission electron microscopical tissue processing

Liver specimens were fixed with 2.5% glutaraldehyde in PBS (pH 7.2), then postfixed in 1% osmium tetroxide, and processed for transmission electron microscopical analysis [31]. Thin sections (approximately 600 Å thickness) were analysed under a transmission electron microscope (Thermo Scientific TALOS L 120C, The Netherlands).

2.10. Statistical analysis

Data were calculated with one-way ANOVA and Tukey multiple comparison tests with $p < 0.05$ considered significant. Statistical analysis was done using Graph Pad Prism 8.0 (San Diego, CA, USA).

3. Results

3.1. Body weight

All rats gained weight as anticipated but mean body weight was not different among the groups (Fig. 5E).

3.2. Biochemical results

3.2.1. Serum ALT, AST and ALP levels results

ALT level of the MSG group was increased compared to the Normal control and APO groups. ALT level of the MSG + APO group was diminished than the MSG group (Fig. 2A). AST level of the MSG group was increased than the Normal control and APO groups. AST level of the MSG + APO group was lower than the MSG group (Fig. 2B). ALP level of the MSG and the MSG + APO groups were increased than the Normal control group (Fig. 2C).

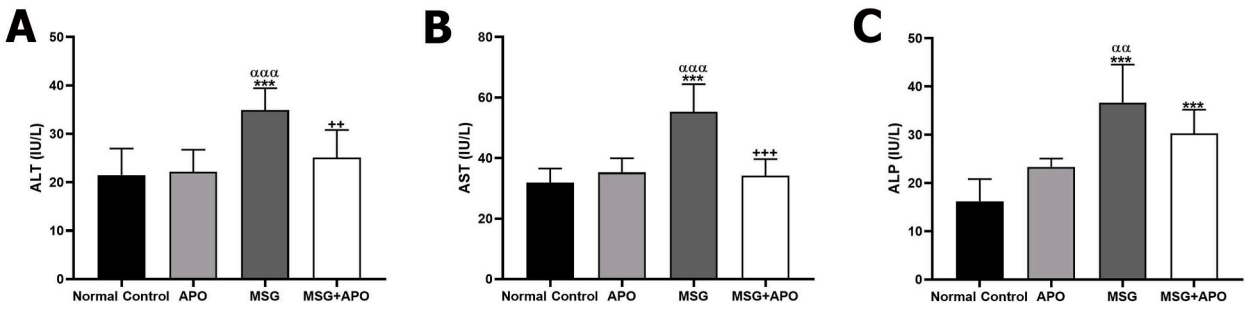

Fig. 2. Graphics of ALT (A), AST (B) and ALP (C) in the experimental groups. ***$p < 0.001$ versus the Normal control group, $^{\alpha\alpha\alpha}p< 0.001$ versus the APO group, $^{++}p<0.01$ versus the MSG group,$^{+++}p<0.001$ versus the MSG group. Values are given ± Standart Deviation.

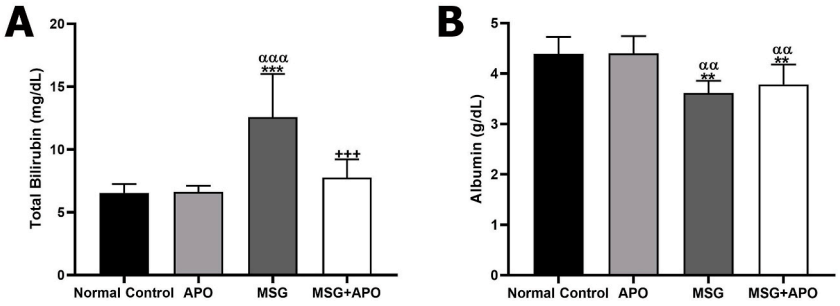

Fig. 3. Graphics of albumin (A) and total bilirubin (B) levels in the experimental groups. **p < 0.01 versus the Normal control group, ***p < 0.001 versus the Normal control group, $^{\alpha\alpha}$p<0.01 versus the APO group, $^{\alpha\alpha\alpha}$p<0.001 versus the APO group, $^{+++}$p<0.001 versus the MSG group. Values are given ± Standart Deviation.

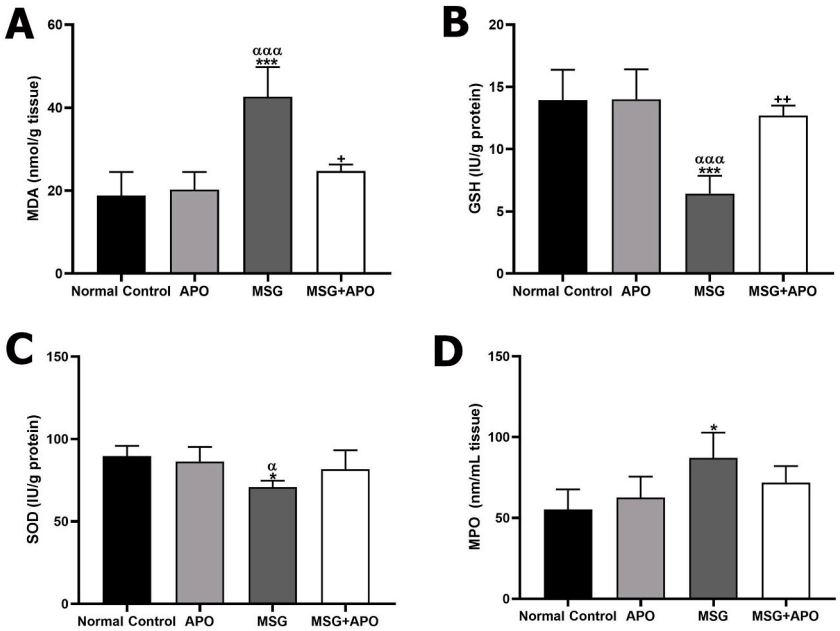

Fig. 4. Graphics of MDA (A), GSH (B), MPO (C) and SOD (D) levels of the experimental animals. ***p < 0.001 versus the Normal control group, *p < 0.05 versus the Normal control group, $^{\alpha\alpha\alpha}$p<0.001 versus the APO group, $^{\alpha}$p<0.05 versus the APO group, $^{++}$p<0.01 versus the MSG group, $^{+}$p<0.05 versus the MSG group. Values are given ± Standart Deviation.

3.2.2. Serum albumin and total bilirubin levels results

Albumin level of the MSG and the MSG + APO groups was lower than that of the Normal control group. The albumin level in the MSG and the MSG + APO groups were decreased than the APO group (Fig. 3A). However, total bilirubin level of the MSG was increased than the Normal control and the APO groups (Fig. 3B).

3.2.3. MDA and GSH levels and MPO and SOD activity results

MDA level in the MSG group was increased than the Normal control and the APO groups while in MSG + APO group it was decreased than the MSG group (Fig. 4A). GSH concentration level in the MSG group was decreased than the Normal control and the APO groups. However, in MSG + APO group GSH level was increased than the MSG group (Fig. 4B). SOD activity of the MSG group was lower than the Normal control and the APO groups (Fig. 4C). MPO activity in the MSG group was increased than the Normal control group (Fig. 4D).

3.3. Histopathological results

The Normal control and APO groups presented a normal liver morphology, with adequate connective tissue distribution and hepatocytes had normal glycogen distribution. In MSG group, vacuolated hepatocytes revealing cellular degeneration, vascular congestion in the sinusoid, leukocyte infiltration and increased connective tissue in the portal triads were detected. MSG group had a

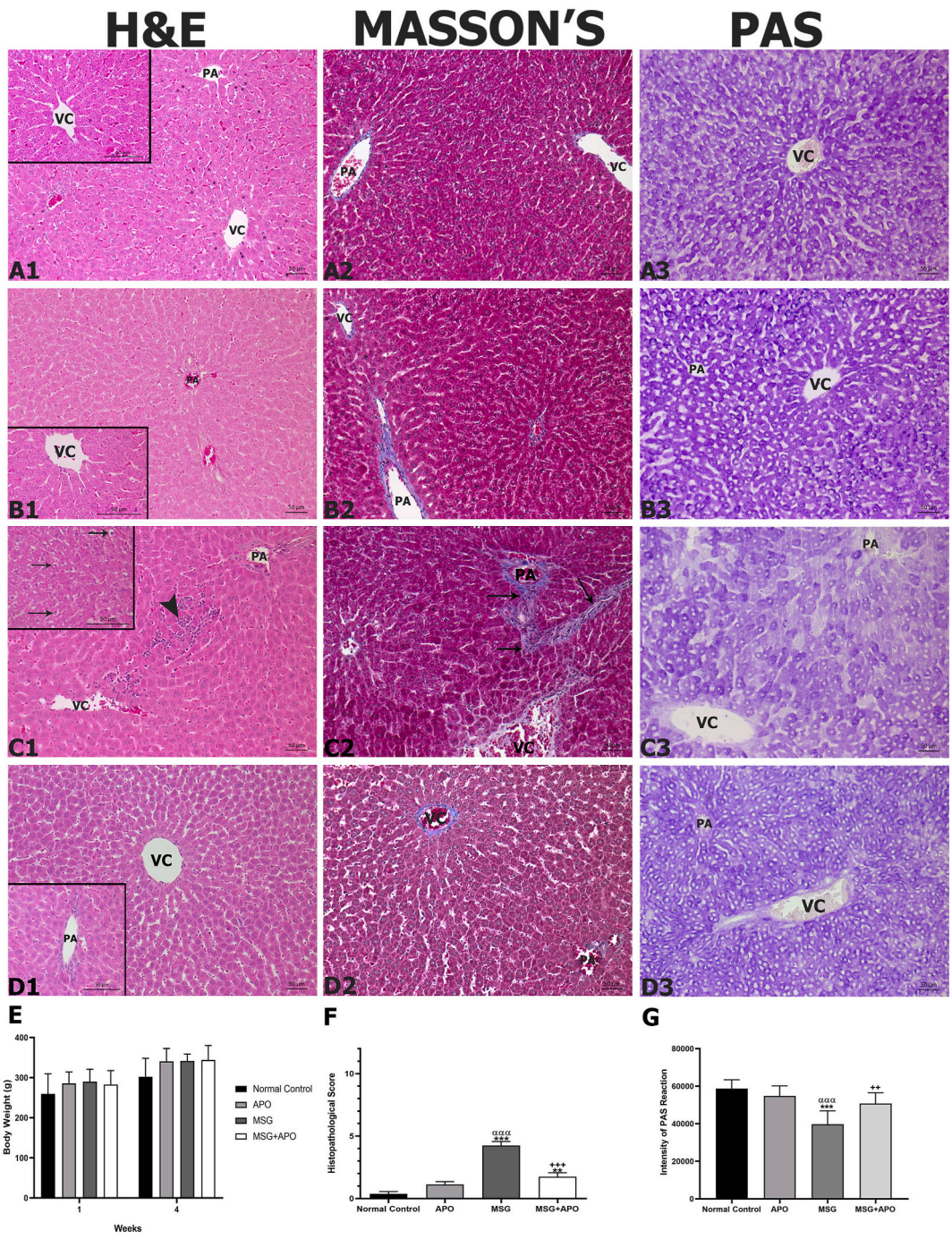

Fig. 5. Micrographs of liver tissue (A–D), graphics of body weight (E), histopathological score (F) and intensity of PAS reaction in the experimental group (G). Normal morphology of liver parenchyma is observed in Normal control (A1) and APO (B1) groups. In the MSG group (C1), leukocyte infiltration in the parenchyma and periportal area (arrowhead) and vacuolization reflecting degeneration of hepatocytes (arrow) are observed. Improved morphology than the MSG group is seen in the MSG + APO group. (D1). Connective tissue is seen in the periportal area of the Normal control (A2), APO (B2) and MSG + APO (D2) groups. Connective tissue is increased in the periportal area of the MSG group (C2). PAS-positive staining is observed in the Normal control (A3), APO (B3) and MSG + APO (D3) groups. It is seen that the MSG (C3) group decreased PAS-positive reaction in many hepatocyte cytoplasm in liver parenchyma. H&E staining (A1-D1), Masson's Tricrom staining (A2—D2), PAS staining (A2-D2). (VC: Vena Centralis, PA:Periportal area).*E*-G) ***$p < 0.001$ versus the Normal control group,**$p < 0.01$ versus the Normal control group,$^{\alpha\alpha\alpha}p<0.001$ versus the APO group, $^{++}p<0.05$ versus the MSG group,$^{+}p<0.05$ versus the MSG group. Values are given ± Standart Deviation.

decreased glycogen distribution. The number of vacuolated hepatocytes was decreased in the liver parenchyme, whereas increased glycogen distribution and normal distribution of the connective tissue were noticed in MSG + APO group. (Fig. 5A–D).

Histopathological scores were increased in the MSG and the MSG + APO compared with the Normal control group. These scores were decreased in the MSG + APO compared with the MSG group (Fig. 5F).

As a reflection of decreased glycogen content, PAS-positivity was disturbed in the MSG group compared with the Normal control and the APO groups. However, the PAS-positive reaction of the parenchyme was higher in MSG + APO than MSG group (Fig. 5G).

3.4. TUNEL results

A few positive cells were observed in the hepatocytes of the Normal control and APO groups (Fig. 6A–D). MSG group presented higher TUNEL-positivity than the Normal control and APO groups, while fewer positive cells were found in the MSG + APO group than MSG group (Fig. 6 E). Numerous TUNEL-positive cells were also observed in the positive control slides (Figure S1).

3.5. NOX-2 immunofluorescence results

In all groups NOX-2-positive reaction was observed in the cytoplasm of hepatocytes as bright red colour, and the nuclei of hepatocytes were dyed with DAPI. A few numbers of NOX-2-positive hepatocytes was observed in the APO group compared with the Normal control group. NOX-2-positive cells were increased in the MSG group. MSG + APO group presented scanty hepatocytes with NOX-2-positivity (Fig. 7A–L). Fluorescence intensity was increased in the MSG group than the Normal control group, while it was higher in the MSG and the MSG + APO groups than the APO group (Fig. 6F). Negative control of NOX -2 immunofluorescence without NOX -2-positive reaction was also applied to the sections (Figure S2).

3.6. Transmission electron microscopical result

Normal ultrastructure of hepatocytes was revealed in the Normal control group and the APO group. MSG group presented vacuolated hepatocytes indicating steatosis with degenerated mitochondria. Deterioration of the microvilli of the bile canaliculi was also observed in the MSG group. The ultrastructure of hepatocytes in the MSG + APO group reflected mild cellular damage with an almost intact tissue structure (Fig. 8A–D).

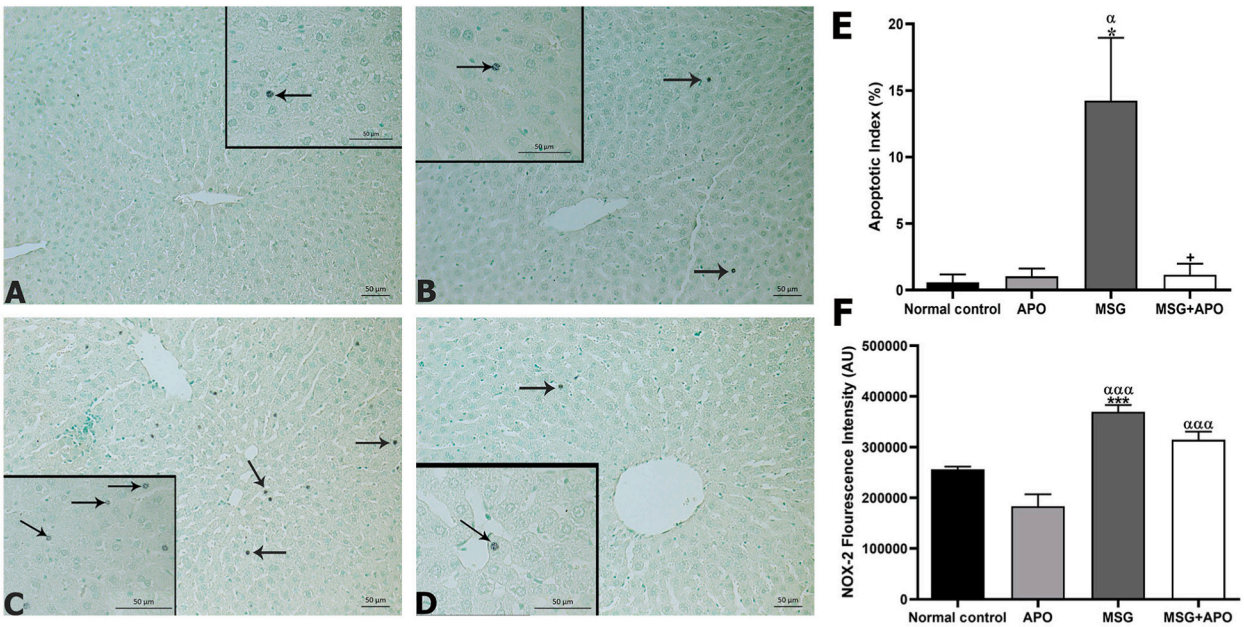

Fig. 6. Representative micrographs of TUNEL stained (A–D), graphics of apoptotic index and NOX-2 fluorescence intensity in the experimental groups. A few TUNEL-positive cells (arrow) are observed in the liver parenchyma of the Normal control (A) and APO (B) groups. An increasing number of TUNEL-positive cells (arrow) are seen in the MSG group (C) and a decreasing number of TUNEL-positive cells (arrow) in the MSG + APO (D) group. ***$p < 0.001$ versus the Normal control group,*$p < 0.01$ versus the Normal control group,$^{ααα}p<0.001$ versus the APO group,$^{α}p<0.05$ versus the APO group,$^{+}p<0.05$ versus the MSG group. Values are given ± Standart Deviation.

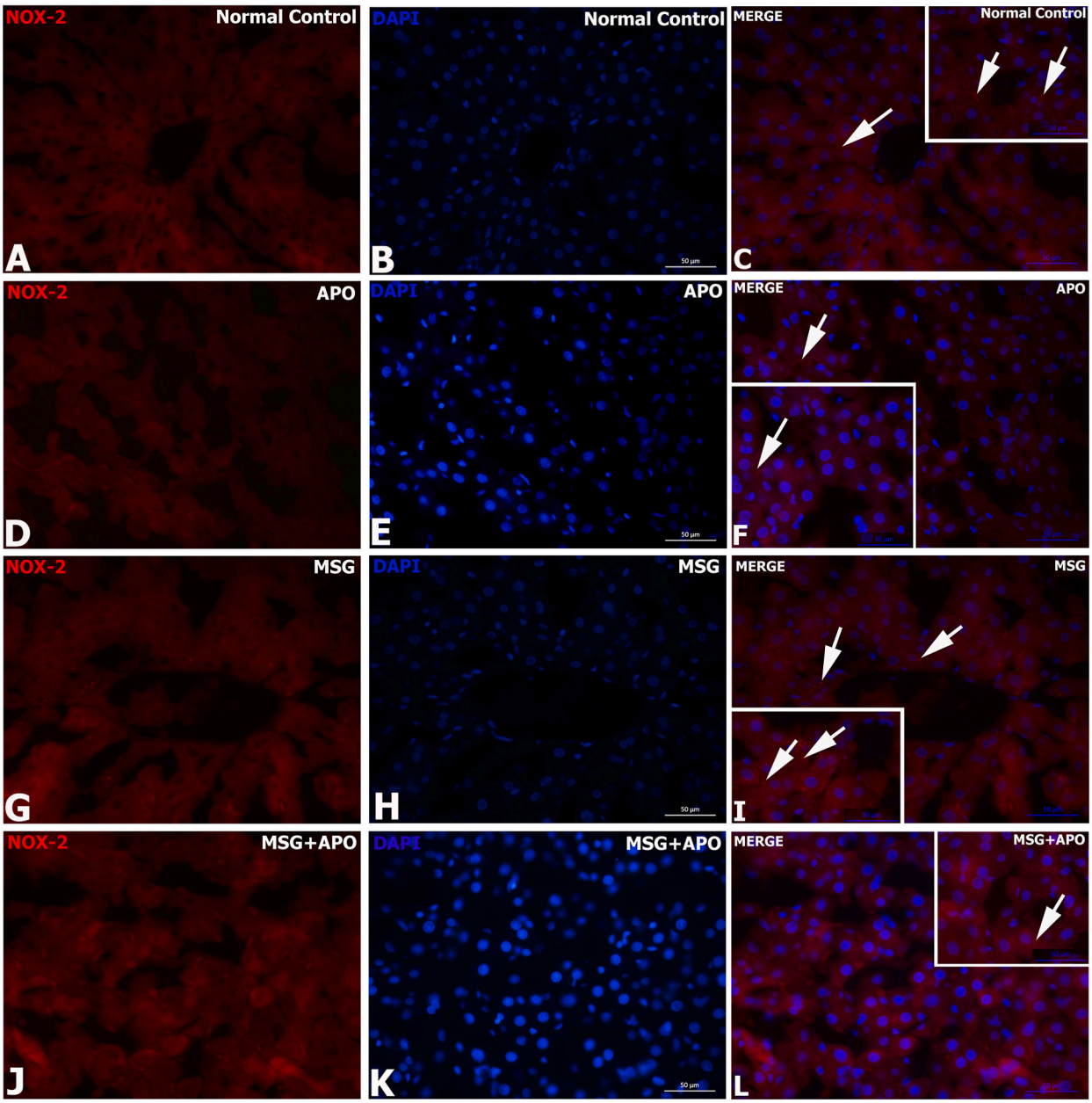

Fig. 7. Representative micrographs of NOX-2 immunoflourescence staining in the experimental groups. NOX-2-positive cells (white arrow) and DAPI are seen bright red and blue colours, respectively in the (A–C) Normal control, (D–F) APO, (G–I) MSG, (J–L) MSG + APO groups. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

In this study, the liver tissue damage caused by MSG and the possible healing effect of APO against this damage were investigated at microscopical and biochemical levels. MSG group had apparently vacuolated hepatocytes indicating steatosis, with leukocyte infiltration and increased collagen fibers in liver parenchyme PAS-positivity of hepatocyte cytoplasm was decreased in MSG group. The number of TUNEL and NOX-2-positive cells were increased in that group. APO administration resulted in a significant improvement of the histopathological damage, as well as biochemical parameters.

It is possible that there is a relationship between the consumption of MSG and the dose of consumption. While some studies have suggested that a low dose of MSG may have an antioxidant effect [32], some authors claimed that chronic treatment of adult rats with MSG might improve acceptance of low-fat diets and increase fluid intake in the elderly sportmen, often with a risk of dehydration [33], Other related studies have emphasised that consumption of MSG may have negative effects both on body/organ weights and tissue

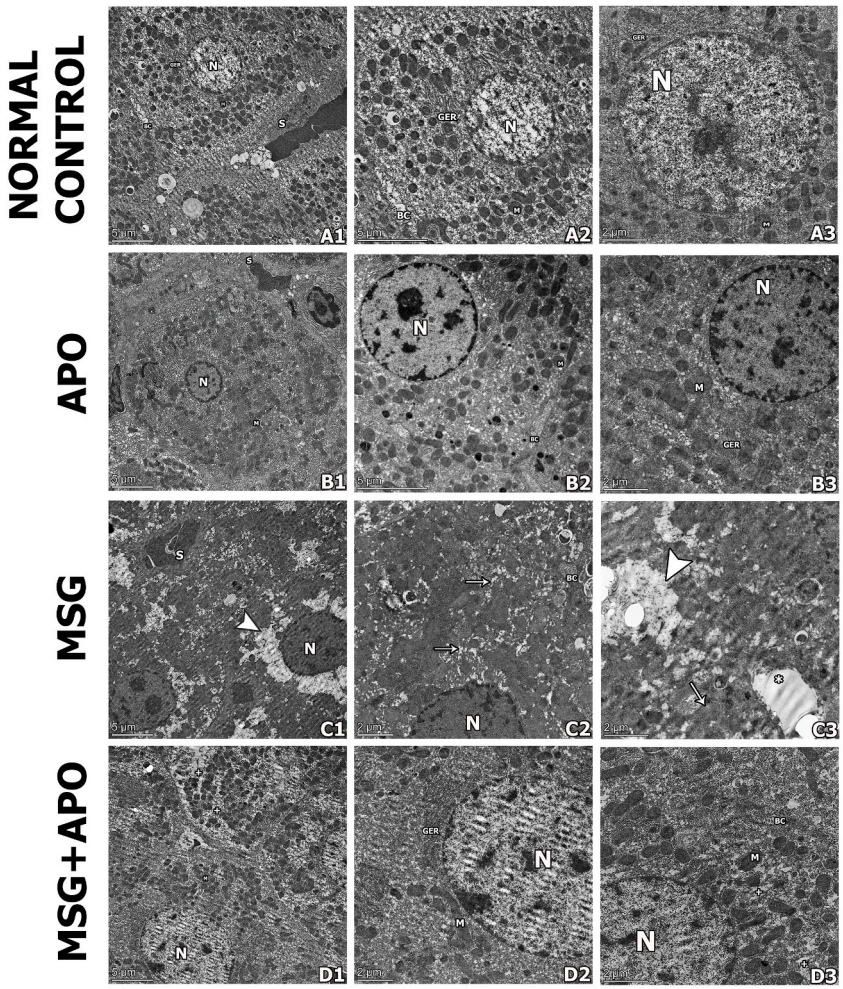

Fig. 8. Transmission electron micrograph of liver tissue. Normal ultrasutructure of the liver parenchyme are seen in the Normal control (A1-3) and APO (B1-3) groups. Vacuolization (arrow), steastosis (arrow head) and deterion of the microvilli of bile canaliculi (asterix) are observed in the MSG group (C1-3). In some hepatocytes, enlarged membranes of the endoplasmic reticulum (plus) indicated a mild degree of degeneration, with a quite regular ultrastructure of the liver parenchyme in the MSG + APO group. (D1-3). N:Nuclei, BC:Bile canaliculi, GER:Granular endoplasmic reticulum, M: Mitochondrion, S:Sinusoid.

[34]. Monosodium glutamate (MSG) in low doses could lead to changes in body weight and liver functions. These changes occur in the liver due to the detoxification ability of hepatocytes for foreign substances ingested by the organism [34]. Oral ingestion of MSG has been shown in studies to be toxic to liver tissue. However, due to the high dosages [8] used in the studies, the observed toxic effects on the digestive system due to MSG consumption were unlikely to be translate to human consumption [8,35]. The daily dose of MSG consumed by humans is estimated to be between 1200 and 3000 mg/kg [7]. The experimental design of this study used a dose of 120 mg/kg body weight of MSG, which was based on an allometric extrapolation of the average daily intake of this substance in humans [36]. Toxic doses of MSG are known to cause liver inflammation, oxidative stress, and hepatocellular damage [37]. MSG overdose is known to increase body weight and fat mass in small rodents [38]. Nehal et al. applied 4 mg/g MSG to experimental animals in their study and concluded that MSG resulted in an increased body weight [39]. Similarly, in this study, although there was no significant weight gain than the other experimental groups, an increase in body weight was observed in the MSG group.

Liver and kidney are responsible for detoxification of unknown components that enter the body [40]. AST is found in high concentrations in various tissues such as heart, liver, skeletal muscle, kidney and pancreas while ALT enzyme is found primarily in liver. Therefore, it is quite a sensitive indicator of hepatocellular damage. Thus, it allows a quantitative evaluation of the damage [41]. MSG is easily separated from sodium and L-glutamate, which are converted to glutamine. Glutamine, which accumulates in hepatocytes, causes cellular damage and, accordingly, increases the levels of ALT and AST enzymes in the blood [40]. ALP is located in the cell membrane and it is increased when cell damage occurs in the liver. Therefore, it is an important marker of liver damage [42].

Bilirubin, a cleavage product of haemoglobin, is used as a specific marker to determine impaired liver function because it reflects uptake, conjugation, and excretion function [43]. In high liver damage, the amount of bilirubin in the plasma increases. Then unconjugated ‘‘indirect’’ bilirubin is produced and is bound to albumin to be transported to the liver. Albumin synthesis is decreased in

liver diseases [44].

GSH is a natural compound formed in the body from the amino acids glutamic acid, cysteine, and glycine. GSH is associated with lipid peroxidation because of its ability to combine with hydrogen peroxide and free radicals that trigger lipid peroxidation [45]. MDA another indicator of lipid peroxidation, causes inactivation by cross-linking with enzymes while increasing lipid peroxidation [43]. SOD is the primary defender in destruction of ROS, and the prevention of liver damage associated with superoxide free radicals [46]. MPO is an enzyme released after phagocyte recruitment and activation and plays an important role in formation of ROS. MPO is used as an indicator of neutrophil infiltration and is highly expressed by neutrophils [47].

Elbassuoni et al. administered orally 35 mg/kg MSG to Sprague–Dawley rats for 2 weeks. As a result of their studies, an increase in ALT, AST, and MDA levels in liver tissue was observed [40]. Farombi and Onyema administered 4 mg/g MSG to albino wistar rats for 10 days. In this study, the levels of SOD were increased and GSH levels in liver tissues were decreased [48]. As a parellel data, we observed increased ALT, AST, ALP, and total bilirubin levels in our current study with a decreased serum albumin level. Results of the biochemical investigations reflected an increase in MDA and MPO, while GSH and SOD were decreased. SOD activation.

Bhattachary et al. reported that MSG, caused deterioted deteriorated hepatocyte cell membrane, and small and pyknotic nuclei. The researchers also reported a prominent increase in vacuolization of hepatocytes and decreased PAS-positive reaction due to glycogen distribution in hepatocytes [49]. Waer and Edress administered 60 mg/100 g MSG orally to rats for two months in their study. They also administered to the rats 100 mg/100 g MSG for one month. In this study, guanidinoethane sulfonic acid (GES) as a therapeutic agent, In MSG group, liver tissue presented, haemorrhagic necrosis, damaged collageneous fibres and inflammation. MSG-induced hepatic parenchymal damage was significantly reduced in animals treated with GES [50]. Ortiz et al. administered 4 mg/g MSG as intraperitoneal injection to rats at the following time intervals as 0, 15, 30 and 45 min. Cytoplasmic damage with numerous vacuoles were apperent in liver cells [51]. In our study, than the Normal control group, enlarged sinusoids, vacuolization in hepatocytes and increased leukocyte infiltration in parenchyme, especially in the periportal area were observed in the MSG group. PAS-positivities of hepatocytes which were decreased in MSG group, were restored in APO and MSG + APO groups.

In liver disease, overactivation of apoptosis can damage hepatocytes, while inhibition of apoptosis can cause proliferation and transformation of hepatocytes [52]. Pavlovic et al. concluded that percentage of apoptotic cells in the MSG group increased significantly in a dose-dependent manner compared with the Normal control group [53]. As a parellel data of our current study, an increase in the number of apoptotic cells and severe degeneration of hepatocytes were observed in the MSG group compared with the Normal control group. Apoptotic cells were decreased in the MSG + APO group.

It is thought that tissue damage caused by the effect of MSG in different organs such as liver, brain, testis and kidney is related to oxidative stress [3,54]. ROS (mainly O_2^- and H_2O_2) can functionally impair many liver-specific cells, leading to the development of fibrosis. High production of ROS in hepatocytes leads to death of these cells. Subsequently, many mediators such as tumor necrosis factor alpha and transforming growth factor beta are released, which enhance the inflammatory and fibrotic response in Kupffer and Ito cells. Pro-fibrogenic stimuli such as alcohol and endotoxin enable the release/expression of biologically active mediators (chemokines, cytokines, adhesion molecules, and ROS) from Kupffer cells [11].

NOX is part of a family of enzymes that produce ROS. APO is the most selective NOX enzyme family inhibitor [55]. The stimulation of antioxidant systems by apocynin may be related to the mechanisms by which the plants protects the tissues damaged by oxidative stress [23]. Apocynin is also known to inhibit intracellular translocation of some critical cytosolic components of the NADPH oxidase complex in the cell membrane [15] and interrupts activation of redox-sensitive transcription factors [16]. Mitazaki et al. administered 240 mg/kg doxycycline to induce acute liver injury. They administered 5 mg/kg APO as a therapeutic agent. At the end of the experiment, researchers concluded that acute liver injury was developed due to the overexpression of NOX while APO reduced the level of intracellular ROS and suppressed production of inflammatory cytokines [15]. In our current study, NOX-2 fluorescence intensity decreased in the APO group whereas an increased fluorescence intensity in the MSG group was detected compared with the APO group.

Atef et al. administered 4 mg/kg MSG orally to animals for 90 days. Transmission electron microscopical examination revealed that the ultrastructure of the liver was normal in the Normal control group whereas pyknotic nuclei, numerous lipid droplets, swollen mitochondria, and numerous erythrocytes in sinusoid due to vascular congestion were observed in the MSG group [56]. In the liver tissue of the MSG group, increased lipid droplets due to steatosis, deterioration of the microvilli structure of bile canaliculi, pyknotic nuclei and deletion of the crista in numerous mitochondria reflected the hepatic parenchymal damage. In the MSG + APO group, all of the deteriorated histopathological criteria were reversed with a mild damage reflecting a regular ultrastructure liver parenchyme.

This study, although proving significant insights into apocynin, might highlight the role of apocynin as a hepatoprotective agent against MSG, due to its antiapoptotic and antioxidant activities. As MSG is a widely used as a flavor enhancer, its toxic effects liver is absolute. The present study could have an impact on the ameliorating effect of apocynin against this harmful effect of MSG. APO could be an effective antioxidant radical scavenger due to its improved antioxidant parameters and liver tissue morphology. Therefore, apocynin could be used as an anti-apoptotic and antioxidant agent, but further experimental and clinical studies are needed to confirm the efficacy of this agent.

5. Conclusion

The possible healing effect of APO against liver tissue damage caused by MSG was investigated in this study at the microscopic and biochemical levels. MSG caused vacuolated hepatocytes indicating steatosis, with leukocyte infiltration and increased collagen fibers in the liver parenchyme. PAS-positivity reflecting glycogen content in the cytoplasm of hepatocytes was decreased in the MSG group. Apoptotic cells and cells with oxidative damage (NOX-2 positive cells) were increased in MSG. Biochemical analysis also revealed a

change both in liver enzymes and oxidative parameters. APO resulted in significant improvement of both histopathological damage and biochemical parameters. APO has a beneficial potential reversing the MSG-induced hepatotoxicity at both biochemical and histopathological levels. To conclude, APO could reverse the liver damage, reduce apoptotic cells, balance liver glycogen content, and balance oxidants and antioxidants to ameliorate morphological and oxidative damage induced by MSG.

Author contribution statement

Begum Sahin: Performed the experiments, contributed reagents, materials, analysis tools or data, wrote the paper.
Merve Acikel Elmas; Serap Arbak: Conceived and designed the experiments, Analysed and interpreted the data, wrote the paper.
Ozlem Bingol Ozakpinar: Performed the experiments, wrote the paper.
Serap Arbak: Conceived and designed the experiments, analyzed and interpreted the data, wrote the paper.

Data availability statement

Data will be made available on request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e17327.

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