Biointerface Research in Applied Chemistry
Open-Access Journal (ISSN: 2069-5837)

Article
Volume 13, Issue 6, 2023, 574
https://doi.org/10.33263/BRIAC136.574

The Protective Effects of Misoprostol Against Amikacin-Induced Liver Injury

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Received: 27.10.2022; Accepted: 5.01.2023; Published: 26.03.2023

Abstract: Amikacin is one of the aminoglycoside antibiotics widely used in intensive care units for pediatric and adult patients, especially for serious infections such as life-threatening Gram-negative infections. This study investigated whether misoprostol, which has antioxidant and antiapoptotic properties, had a beneficial effect on liver damage caused by amikacin administration in rats. Misoprostol treatment increased serum high-density lipoprotein levels and significantly decreased alkaline phosphatase, alanine transaminase, aspartate transaminase, total cholesterol, low-density lipoprotein, direct bilirubin, total bilirubin, and triglyceride levels. Cytochrome P450 2B1 and Cytochrome P450 2B2 gene expressions induced by amikacin treatment were ameliorated by misoprostol. Liver weights increased by the phospholipidosis effect of amikacin were improved by misoprostol treatment. A decrease in malondialdehyde levels and an increase in glutathione activities were detected in the amikacin+misoprostol group compared to the amikacin group. Oxidative damage caused by amikacin was ameliorated by misoprostol treatment due to its antiapoptotic and antioxidative effects. Treatment with misoprostol significantly reduced Caspase 3 and Tumor Necrosis Factor-alpha levels compared to amikacin. Our results showed that misoprostol could serve as a new therapeutic target to prevent amikacin-induced hepatotoxicity by inhibiting reactive oxygen species generation and modulating apoptosis.

Keywords: Amikacin; apoptosis; CYP2B1; CYP2B2; misoprostol; oxidative stress

1. Introduction

Aminoglycosides (AG) are one of the most effective antibiotics developed in the 1940s to treat a broad spectrum of bacterial infections [1, 2]. AGs were isolated from *Micromonospora purpurea*, and *Streptomyces griseus*, and several natural and synthetic AGs were subsequently developed, such as gentamicin (GC), amikacin (AK), and tobramycin (TM) [3]. AGs bind to the 30S subunit of the bacterial ribosome, inhibiting the synthesis of proteins necessary for bacterial growth [4]. As the use of AGs becomes more widespread, the toxic effects of these agents, especially ototoxicity and nephrotoxicity, are more common. The development of multidrug resistance, especially among bacteria, has led clinicians to use AGs to treat serious infections. Studies have shown that one of the mechanisms underlying the toxic effect of AK causes apoptosis by generating reactive oxygen radical species (ROS) [5]. In a study on hepatotoxicity of AK, primary and secondary micro cholestasis which are associated with the detachment of mitochondrial cristae and phospholipid aggregations, were observed,

and an increase in blood urea nitrogen (BUN) and aspartate transaminase (AST) was reported [6].

The liver is a crucial center for numerous physiological processes, including nutrient metabolism, endocrine control, blood volume regulation, lipid and cholesterol homeostasis, immune support, and the disintegration of xenobiotic compounds and many drugs [7]. The liver becomes an important target for the emergence of toxicity of xenobiotics and drugs [8]due to its complex metabolism and relationship with the gastrointestinal tract [9]. The imbalance between detoxification processes and the formation of toxic metabolites during drug metabolism affects the degree of hepatotoxicity [9]. Liver diseases due to drugs are increasing day by day. As a result, morbidity and mortality increase, and quality of life gradually decreases. Therefore, there is a need for natural protective agents to reduce the toxic effects of drugs. The liver is the main organ involved in drug metabolism, and Cytochrome P-450s (CYP)s are predominantly involved in drug metabolism [10, 11].

According to the general view, the most important cause of drug-induced liver injury is hepatotoxicity of parent compounds by metabolization by CYPs. The resulting reactive metabolites lead to hepatocyte apoptosis and mitochondrial dysfunction [12]. Therefore, CYP enzymes have an important role in investigations regarding drug-induced hepatotoxicity. CYPs, consisting of a large family of hemoproteins, are phase I metabolic enzymes with essential roles in the biotransformation of fatty acids, various xenobiotics, steroids [13, 14], dietary chemicals, and endogenous molecules [15]. This study investigated the effect of AK and MP on CYP2B1 and CYP2B2 gene expressions. Cytochrome P450 2B1, encoded by CYP2B1, and cytochrome P450 2B2, which CYP2B2 encodes, can be induced in rat liver [16]. However, the molecular mechanisms underlying the increased expression of cytochrome P450 genes, which play a key enzyme role in drug metabolism, have not yet been clarified [17]. In particular, the induction mechanism of the CYP2B subfamily is not fully understood [18].

Many agents have been tried to prevent toxicity from AK, but no effective and safe agent has been found, and it is still used despite serious side effects. This study used Misoprostol (MP) to reduce or prevent AK-induced hepatotoxicity. MP is an antioxidant Prostaglandin E1 (PGE1) analog, and in recent years, it has had antioxidant properties and properties such as reactive oxygen species scavenger [19] and antiapoptotic [20], or cytoprotective effects [20, 21]. In a study, it was reported that hepatotoxicity caused by drugs is closely related to enzyme induction, and liver function tests alone are not sufficient to determine drug-induced hepatotoxicity [22]. Therefore, in this study, biochemical and oxidative stress parameters, gene expressions, and histopathological and immunochemical studies were considered together within the framework of possibilities to understand AK-induced hepatotoxicity. Also, there are no data showing the protective effect of MP on hepatic toxicity caused by AK. This experimental study was designed to reveal whether MP has antioxidant and antiapoptotic properties and curative and preventive effects against AK-induced ROS formation.

2. Materials and Methods

2.1. Chemicals.

AK was supplied as Amikozit Flacon (0.5g/2ml; Eczacıbaşı, Istanbul, Turkey). Misoprostol was available as Cytotec® (0.2 mg; Ali Raif, Istanbul, Turkey). All other chemicals used in the study were bought from Sigma Aldrich.

2.2. Animals.

Male Sprague-Dawley rats (n = 24; weight: 220-270 g; age: 3-4 months) were obtained from Adıyaman University Experimental Animals Research Center. All rats were in standard cages at 22 ± 2 °C, 60% ± 5% humidity, and 12 h/12 h light/dark cycle. Animals were given standard rat chow and water throughout the experiment. All animal experiments conducted during this study were approved by Adıyaman University Animal Experiments Ethics Committee (Approval ID: 2021/024).

2.3. Experimental design.

24 rats were acclimated for one week and then equally divided into four groups (n = 6/group):

(1) Control group: Intraperitoneal (i.p.) saline for six days;

(2) AK Group: 1.2 g/kg AK i.p. single dose [23];

(3) MP Group: 0.2 mg/kg/day MP [24] orally for six days;

(4) AK+MP Group: 1.2 g/kg AK i.p. single dose + 0.2 mg/kg/day MP orally for six days, three days before AK, and three days after were continued.

After the last MP administration, all rats were fasted for 12 hours and weighed. Samples of blood were taken from the abdominal aorta of anesthetized rats and centrifuged at 5000 rpm for 15 minutes at room temperature (RT) to collect their serum, then stored at 4°C. The liver of each rat was rapidly removed and washed, then divided into three parts. One portion was stocked at -86°C to measure gene expression levels and the other portion to measure oxidative stress biomarkers. The third part was fixed in 10% neutral formalin solution until histological and immunohistochemical analysis.

2.4. Biochemistry.

Serum biomarkers of liver function are alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), cholesterol (CH), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), direct bilirubin (DB) and total bilirubin (TB) levels and were measured by Abbott Labs Architect C16000 systems (Abbott GmbH & Co, Germany) and commercial Abbott kits were used. Serum activities of liver function parameters were determined using the spectrophotometric method in the wavelength range of 340-380 nm [25, 26].

2.5. Biomarkers of oxidative stress.

Malondialdehyde (MDA) as a lipid oxidation product forms thiobarbituric acid reagents (TBARS), which can be easily measured by the double-boiled spectroscopic thiobarbituric acid (TBA) reactivity method and can react with two equivalents of TBA to give a pink adduct [27]. MDA was reacted with TBA at pH 2-3 and 99 °C for 20 minutes. The reaction was finally measured by spectrophotometer (UV mini-1240; Shimadzu, Tokyo, Japan) at 532 nm.

Glutathione (GSH) was measured according to the method described by Elman [28]. After 5,5′-dithiobis 2-nitrobenzoic acid (DTNB) was added to the sample, a yellow-green color was formed at pH 8.9 as a result of the reaction between glutathione and DTNB in the medium. The absorbance was measured at 412 nm with a spectrophotometer, and the amount of reduced glutathione was determined.

2.6. RNA extraction, cDNA synthesis, and quantitative PCR analysis.

Fresh frozen rat livers stored at -86 °C were processed for RNA extraction using AccuZol™ Total RNA Extraction Solution (Bioneer, K-3090) under RNAse-free conditions according to the manufacturer's instructions. After isolation, the absorbance values of RNAs were measured at 230 nm and 260/280 nm with a NanoDrop spectrophotometer (Denovix DS-11). For q-PCR, the first 5 µg of total RNA was reverse transcripted using AccuPower® RT PreMix (Bioneer K-2041) using appropriate controls to ensure no genomic DNA contamination.

qRT-PCR was used for the determination of the expression level of CYP2B1 and CYP2B2 conducted following the instructions of the AccuPower GreenStar qPCR PreMix (Bioneer, Cat No: K-6210) using the ExiCyclerTM96 qRT- PCR system (Bioneer). The GAPDH gene was amplified as an internal control. The primers that were used in this study were selected from previously published studies. Primers were for CYP2B1 forward, 5'-AACCCTTGATGACCGCAGTAAA-3', and reverse, 5'-TGTGGTACTCCAATAGGGACAAGATC-3' [29], CYP2B2 forward 5′-GGACACTGAAAAAGAGTGAAGCTTT-3' and reverse 5′-AATGCCTTCGCCAAGACAAA-3' [30], GAPDH forward, 5'-CAACTCCCTCAAGATTGTCAGCAA-3' and reverse, 5'-GGCATGGACTGTGGTCATGA-3' [25]. The PCR conditions were as follows: 95 °C for 1 min, followed by 45 cycles at 95 °C for 5 sec, and 55 °C for 40 seconds. The $2^{\wedge-\Delta\Delta Ct}$ method was used to calculate the relative mRNA expression.

2.7. Histochemical analyses.

The liver tissue samples were washed in water for 12 hours and then fixed in a 10% neutral formalin solution. The samples were dried by passing through different concentrations of ethanol (50-60-70-80-90-100%). It was transparentized in xylol and embedded in paraffin. 3-4 µm thick sections were taken from the paraffin blocks with a microtome (Leica SM2000R, Germany) and stained with Hematoxylin-Eosin (H-E) and coated with entellan. During the analysis, each sample's tissue structural features and cellular components were carefully identified. Then, a semiquantitative analysis regarding the histopathological findings was calculated to make statistical comparisons among the groups. All groups analyzed by photomicroscopy were evaluated, taking into account the scoring made by Refaiy [31].

Histopathological findings were graded and evaluated by a semiquantitative method using a photomicroscope as follows:

0 (-), negative score: No structural changes,

1 (+), 1 positive score: Light structural changes,

2 (++), 2 positive scores: Middle structural changes,

3 (+++), 3 positive scores: Serious structural changes.

In the evaluation, 10 different areas were scanned for each section at 20X magnification under the objective of all groups. The results were statistically analyzed.

2.8. Immunohistochemical analyzes.

Tissues of 3–4µm thicknesses were collected and stained with CAS-3 primary ab (rabbit Caspase 3 antibody, Abcam, Cambridge, USA) and TNF-α primary ab (rabbit anti-TNF- α antibody, Abcam, Cambridge, USA) and covered with entellan. Prepared liver tissue

samples were analyzed and evaluated. Observed receptor densities were determined through semiquantitative assessment [31]. Five different sections were examined and analyzed in each sample. Then, points from 0 to 3 (-, +, ++, +++) were given according to the intensity of staining;

0 (-), negative score: absence of staining,

1 (+), 1 positive score: slight staining,

2 (++), 2 positive scores: medium staining,

3 (+++), 3 positive scores: intense staining.

In the evaluation, 10 different areas were scanned for each section at 20X magnification under the objective of all groups. The results were statistically analyzed.

3. Statistical analysis

Statistical analyzes were performed using Statistical Package 25.0 (SPSS, Chicago, IL, USA) and GraphPad Prism, version 9 software (GraphPad Software Inc.; La Jolla, CA). Normality was evaluated with the Shapiro-Wilk test. Groups were compared using a paired sample t-test at the beginning and end of the study. Data obtained from within-group comparisons for parametric values in genetic parameters were analyzed by one-way ANOVA after LSD for liver weight data and biochemical parameters. Mann-Whitney U test for histopathological and immunohistochemical analyzes in SPSS 25.0 software Gibson-Corley et al. [32]. Differences were evaluated as significant for $p<0.05$. Values were appropriately expressed as mean ± standard deviation (SD) in at least triplicate.

4. Results

4.1. Weight gain/loss effects of AK and MP.

The total body weight of all rats was weighed daily from the beginning to the end of the experiment. Body weight measurements over 7 days ranged from 221.00 g to 263.66 g. Final body weight was higher for the MP, AK+MP group than the AK group. The body weight of the AK group was significantly less than the control, MP, and AK+MP groups ($p< 0.05$).

At the end of the 7th day, the livers taken from the sacrificed rats were weighed and proportioned according to their body weights (Table 1; Figure 1). Considering the fresh liver weight and fresh liver weight/body weight ratio in the AK group, a statistically significant increase was observed compared to the control, MP, and AK+MP groups ($p<0.05$) (Table 1; Figure 1).

Table 1. Comparison of fresh liver weight (g) and fresh liver weight/body weight ratio of the study population.

	STUDY GROUPS				
	Control	**AK**	**MP**	**AK+MP**	**p-value**
Fresh liver weight (g)	10.60 ± 0.67^{b}	$11.99 \pm 0.45^{a,c,d}$	10.71 ± 0.54^{b}	9.93 ± 0.59^{b}	0,000
Fresh liver weight/body weight ratio (g)	$0.040 \pm 0.002^{b,c,d}$	$0.048 \pm 0.001^{a,c,d}$	$0.041 \pm 0.002^{a,b,d}$	$0.039 \pm 0.002^{a,b,c}$	0,000

Effect of AK and MP on liver weights in rats in a 6-day experiment. Each group represents the mean ± SD for six rats. a: Significant from Control; b: Significant from AK; c: Significant from MP; d: Significant from AK+MP. $p<0.05$. Abbreviations: AK: amikacin; MP: misoprostol; AK: 1.2 g/kg; MP: 0.2 mg/kg/day; AK+MP: 1.2 g/kg AK + 0.2 mg/kg/day MP.

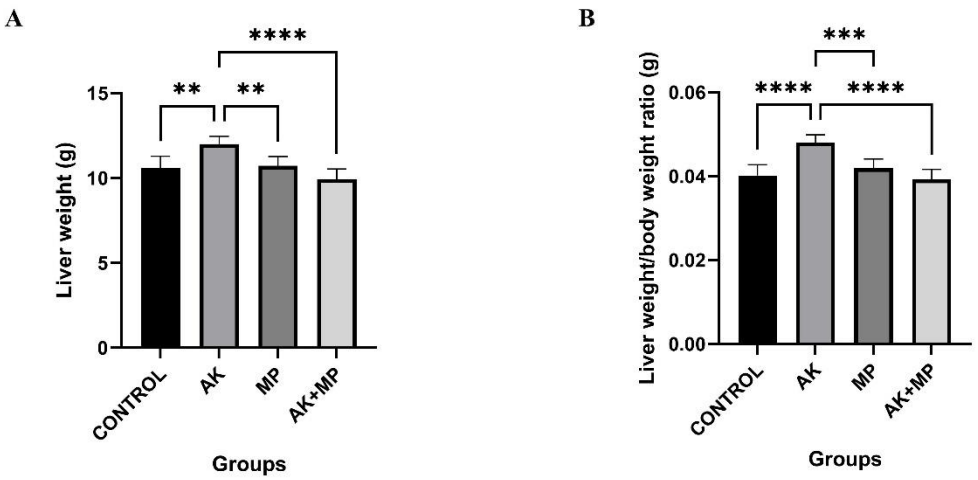

Figure 1. Effect of AK and MP on liver weights in rats in a 6-day experiment. Each group represents the mean ± SD for six rats. Abbreviations: AK: amikacin; MP: misoprostol; AK: 1.2 g/kg; MP: 0.2 mg/kg/day; AK+MP: 1.2 g/kg AK + 0.2 mg/kg/day MP. **** p<0.0001, *** p<0.001, ** p<0.01.

4.2. Biochemistry results.

The effects of AK and MP on biochemical parameters are summarized in Table 2 and Figure 2. Alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), total cholesterol (CH), low-density lipoprotein (LDL), direct bilirubin (DB), total bilirubin (TB), triglyceride (TG) levels AK group was significantly higher when compared to the control, MP and AK+MP groups. High-density lipoprotein (HDL) levels were significantly decreased in the AK group compared to the AK+MP group (Table 2, Figure 2).

Table 2. Comparison of serum biochemical biomarkers of the experimental groups.

	STUDY GROUPS				
Biochemical parameters (Mean±SD)	**Control**	**AK**	**MP**	**AK+MP**	**p value**
ALP (U/L)	429.40±29.71 [b]	492.80±17.88 [a,c,d]	427.60±14.53 [b,d]	431.60±14,17 [a,b,c]	0.000
ALT (U/L)	74.33±1.75 [b]	81.66±5.16 [a,c,d]	72.66±2.42 [b]	73.16±4.62 [b]	0.002
AST (U/L)	129.16±9.02 [b]	155.83±6.08 [a,c,d]	129.66±9.64 [b]	126.50±8.47 [b]	0.000
CH (mg/dL)	51.00±5.93 [b]	59.16±1.94 [a,c,d]	52.66±3.50 [b]	53.00±1.09 [b]	0.005
HDL (mg/dL)	32.50±1.87 [b]	27.16±2.13 [a,c,d]	34.00±2.89 [b]	37.16±3.31 [b]	0.000
LDL (mg/dL)	14.83±1.16 [b]	17.66±1.50 [a,c,d]	14.83±1.72 [b]	15.16±1.47 [b]	0.008
DB (mg/dL)	0,001±0.004[b]	0.030±0,008 [a,c,d]	0.003±0.005 [b]	0.005±0.005 [b]	0.000
TB (mg/dL)	0.101±0.004[b]	0.130±0.009 [a,c,d]	0.103±0.005 [b]	0.105±0.005 [b]	0.000
TG (mg/dL)	25.00±5.89 [b]	35.66±5.12 [a,c,d]	27.50±2.73 [b]	28.50±2.16 [b]	0.002

Effect of AK and MP on markers of liver function in rats in a 6-day experiment. Each group represents the mean ± SD for six rats. a: Significant from Control; b: Significant from AK; c: Significant from MP; d: Significant from AK+MP. p<0.05. Abbreviations: AK: amikacin; MP: misoprostol; ALP: alkaline phosphatase; ALT: alanine transaminase; AST: aspartate transaminase; CH: total cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein; DB: direct bilirubin; TB: total bilirubin; TG: triglycerides. AK: 1.2 g/kg; MP: 0.2 mg/kg/day; AK+MP: 1.2 g/kg AK + 0.2 mg/kg/day MP.

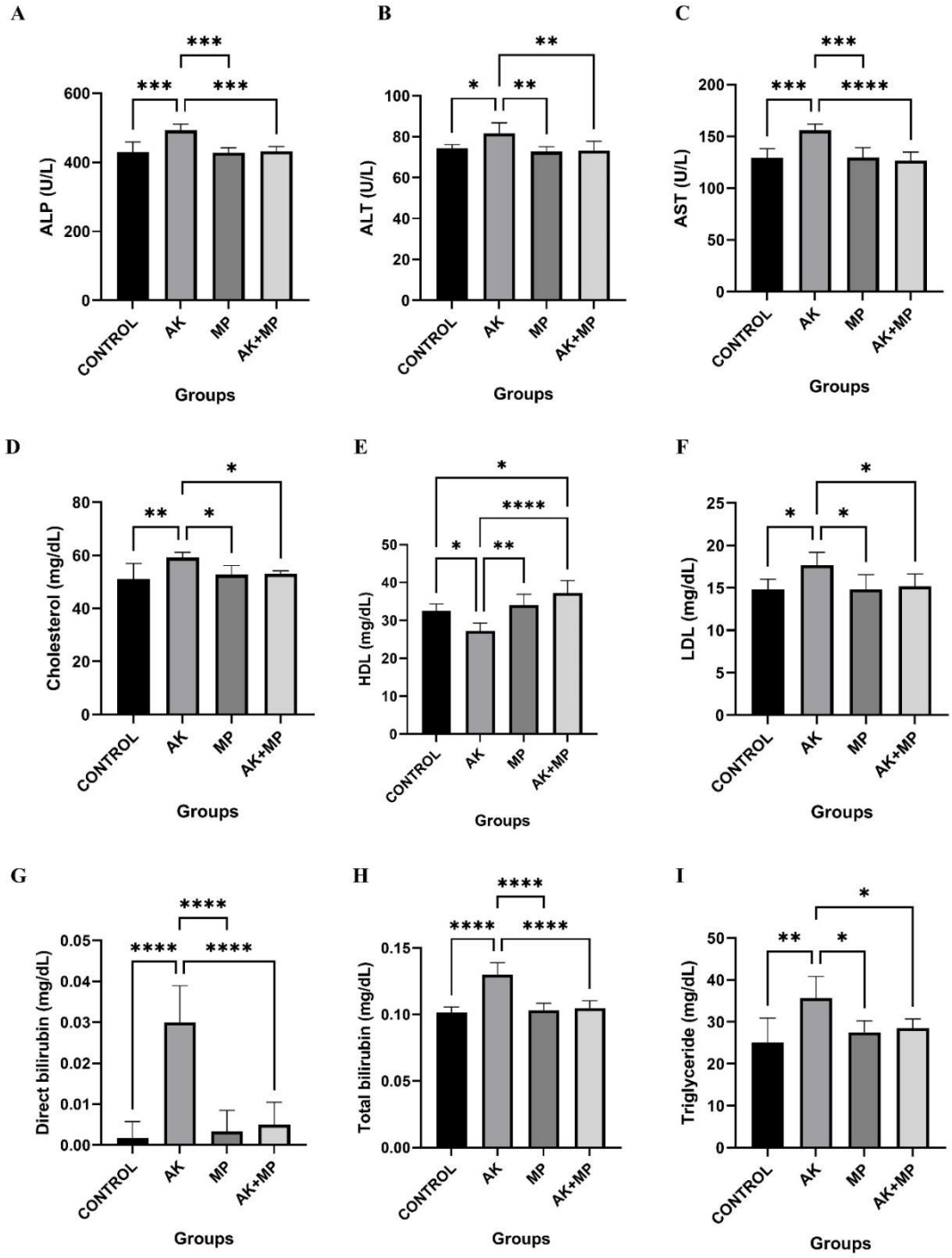

Figure 2. Effects of AK and MP on liver function markers in rats. Alkaline phosphatase **(A),** alanine transaminase **(B),** aspartate transaminase **(C),** cholesterol **(D):** high-density lipoprotein **(E)**, low-density lipoprotein **(F)**, direct bilirubin **(G),** total bilirubin **(H)** and triglycerides (TG) levels were determined. Each group represents the mean ± SD for six rats. Abbreviations: AK: amikacin; MP: misoprostol; ALP: alkaline phosphatase; ALT: alanine transaminase; AST: aspartate transaminase; CH: total cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein; DB: direct bilirubin; TB: total bilirubin; TG: triglycerides AK: 1.2 g/kg; MP: 0.2 mg/kg/day; AK+MP: 1.2 g/kg AK + 0.2 mg/kg/day MP. **** $p<0.0001$, *** $p<0.001$, ** $p<0.01$, * $p<0.05$.

4.3. Oxidative stress biomarkers.

The AK group exhibited a significantly higher level of MDA and significantly lower GSH activities when compared to the control, MP, and AK+MP groups. MP treatments caused a significant decrease in the level of MDA produced by AK. Additionally, MP therapy

significantly increased the GSH activities, which were decreased by AK (Table 3, Figure 3) ($p< 0.05$).

Table 3. Comparison of liver tissue oxidative stress biomarkers of the experimental groups.

	STUDY GROUPS				
Liver tissue oxidative stress biomarkers	**Control**	**AK**	**MP**	**AK+MP**	**p-value**
GSH	412.28±26,46[b]	328.64±20.64[a,c,d]	384.81±38.44[b]	374.72±14.74[b]	0.000
MDA	31,51±4.07 [b]	48.19±10.48[a,c,d]	34.48±6.44[b]	36.73±0.76[b]	0.001

Effects of AK and MP on liver tissue oxidative stress biomarkers. Each group represents the mean ± SD for six rats. AK increased MDA and reduced GSH. MP reduced MDA and increased GSH. a: Significant from Control; b: Significant from AK; c: Significant from MP; d: Significant from AK+MP. $p<0.05$. Abbreviations: AK: amikacin; MP: misoprostol; MDA: malondialdehyde; GSH: glutathione. AK: 1.2 g/kg; MP: 0.2 mg/kg/day; AK+MP: 1.2 g/kg AK + 0.2 mg/kg/day MP.

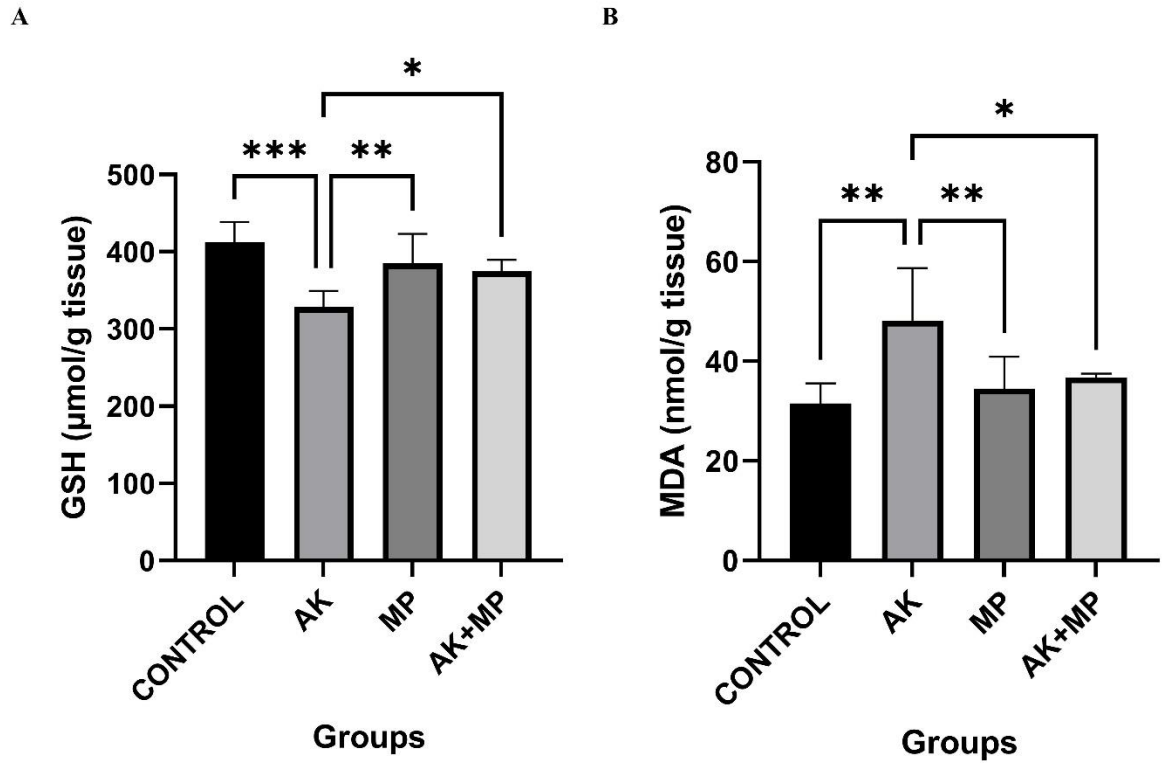

Figure 3. Effects of AK and MP on liver lipid oxidation and antioxidant profile of rats after six days. Each group represents the mean ± SD for six rats. Abbreviations: AK: amikacin; MP: misoprostol; MDA: malondialdehyde; GSH: glutathione. AK: 1.2 g/kg; MP: 0.2 mg/kg/day; AK+MP: 1.2 g/kg AK + 0.2 mg/kg/day MP. *** $p<0.001$, ** $p<0.01$, * $p<0.05$.

4.4. Effects of AK and MP on the expression of CYP2B1 and CYP2B2 gene.

In the current study, the expression of CYP2B1 and CYP2B2 mRNAs was determined by real-time reverse transcriptase-polymerase chain reaction (RT-PCR). Figure 4 shows the effects of MP treatment against AK on CYP2B1/2 mRNA expression in all study groups and control. CYP2B1 and CYP2B2 gene expressions significantly increased in the AK group compared to the control group. The AK+MP group had significantly lower CYP2B1 and CYP2B2 gene expression levels than the AK group ($p<0.0001$, and $p<0.01$, respectively) (Figure 4).

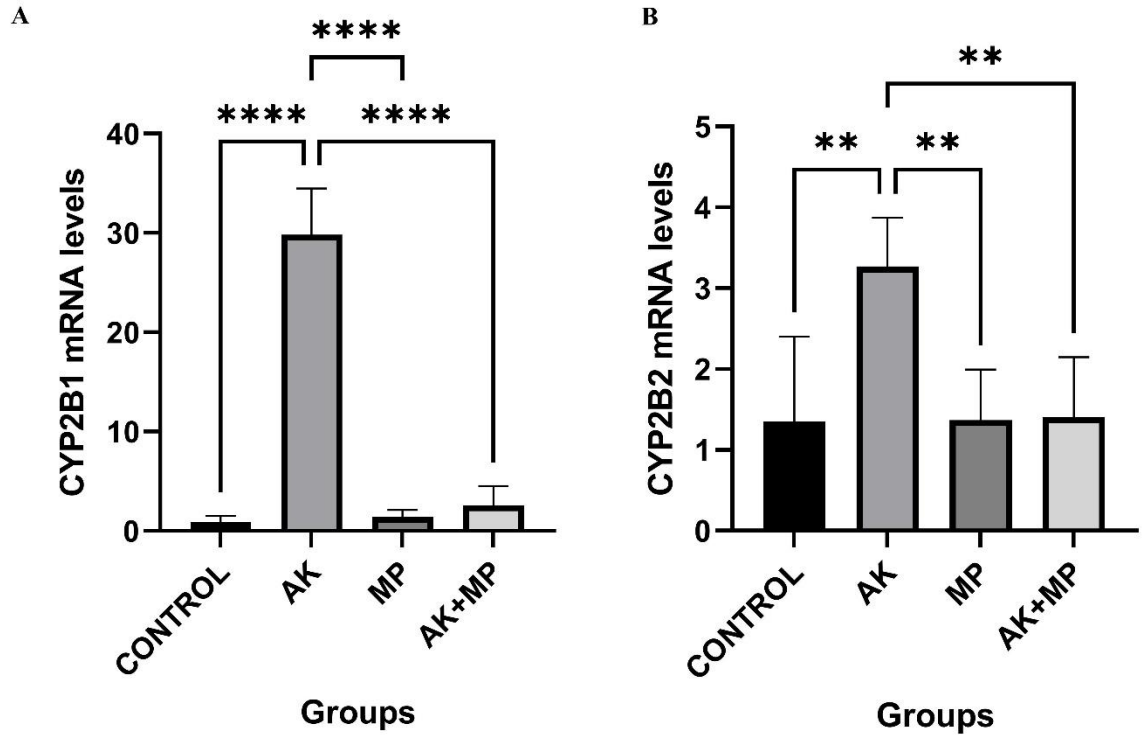

Figure 4. Effects of AK and MP on CYP2B1 and CYP2B2 mRNA expression in Spraque-Dawley rats. CYP2B1 and CYP2B2 mRNA expressions were determined by quantitative PCR. Data are presented as CYP2B1 and CYP2B2 mRNA expressions relative to the expression of the GAPDH housekeeping gene. AK increased CYP2B1 and CYP2B2 mRNA expression. MP reduced CYP2B1 and CYP2B2 mRNA expression. Each group represents the mean ± SD for six rats. Abbreviations: CYP2B1: cytochrome P450 (CYP) 2B1; CYP2B2: cytochrome P450 (CYP) 2B2. AK: amikacin; MP: misoprostol; AK: 1.2 g/kg; MP: 0.2 mg/kg/day; AK+MP: 1.2 g/kg AK + 0.2 mg/kg/day MP. **** $p<0.0001$, ** $p<0.01$.

4.5. Histochemical results.

The histological structures were normal in the control and MP groups (Table 4; Figure 5: A-C).

Table 4. Average score of histopathological findings between all groups.

Groups	Mononuclear Cell Infiltration	Hemorrhagic Areas	Vascular Congestions	Vacuolar - Granular Degeneration	Sinusoidal Dilatation
1 (CONTROL)	-/+	-	-	-/+	-/+
2 (AK)	+++	+++	++	+++	+++
3 (MP)	-/+	-	-	-/+	-/+
4 (AK+MP)	++	++	+	++	++
The significance status obtained as a result of the comparison of the groups					
1-2	p = 0.000	p = 0.000	p = 0.000	p = 0.001	p = 0.001
1-3	p = 0.87	p = 0.98	p = 0.79	p = 0.94	p = 0.97
1-4	p = 0.001	p = 0.000	p = 0.000	p = 0.001	p = 0.001
2-3	p = 0.003	p = 0.002	p = 0.001	p = 0.000	p = 0.002
2-4	p = 0.008	p = 0.001	p = 0.001	p = 0.000	p = 0.000
3-4	p = 0.007	p = 0.008	p = 0.000	p = 0.004	p = 0.003

$p<0.05$ were considered statistically significant. The relationships between groups and the results of histopathological markers are assessed by the Mann-Whitney U test. (-), negative score: No structural changes; (+), 1 positive score: Light structural changes; (++), 2 positive score: Middle structural changes; (+++), 3 positive score: Serious structural changes. Abbreviations: AK: amikacin; MP: misoprostol; AK: 1.2 g/kg; MP: 0.2 mg/kg/day; AK+MP: 1.2 g/kg AK + 0.2 mg/kg/day MP.

When the AK and AK+MP groups were compared to the control and MP groups, significantly increased histopathological findings, sinusoidal and tubular dilatation, mononuclear cell infiltration, vascular occlusion in hepatocytes, pycnotic nuclei, and hemorrhagic areas, granular and vacuolar degeneration were observed ($p<0.05$), (Table 4; Figure 5: B-D). Histopathological changes were significantly decreased in the AK+MP group when compared to the AK group ($p<0.05$) (Table 4; Figure 5: D).

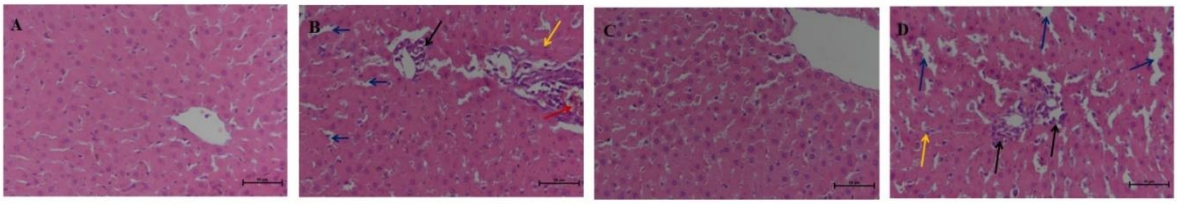

Figure 5. Histopathological findings in liver tissues belonging to control and experimental groups: A, Control group; normal histological structure of the liver, B, AK group; pyknotic nucleus in hepatocyte (yellow arrow), mononuclear cell infiltration (black arrow), sinusoidal dilatation (blue arrows), hemorrhagic (red arrow), C, MP group; normal histological structure, D, AK+MP group; mild histopathological findings, H-E, x20. Abbreviations: AK: amikacin; MP: misoprostol; AK: 1.2 g/kg; MP: 0.2 mg/kg/day; AK+MP: 1.2 g/kg AK + 0.2 mg/kg/day MP.

4.6. Immunohistochemical results.

CAS-3 and TNF-α receptors in the centrilobular areas of the liver sections of the control and MP groups were observed to stain very slightly (Table 5; Figure: 6A-A1, C-C1). On the other hand, more intense staining was detected in AK and AK+MP groups when compared to control and MP groups ($p<0.05$) (Table 5; Figure 6: B-B1, C-D1).

The staining intensity of the receptors was highest in AK and moderated in AK+MP, whereas it was less and the same in control and MP groups ($p<0.05$) (Table 5; Figure 6). When stainings were compared, CAS-3 staining was more intense than TNF-α staining.

Table 5. CAS-3 and TNF-α marking average degrees between all groups

Groups	CAS-3 Staining Degree	TNF-α Staining Degree
1 (CONTROL)	-/+	-/+
2 (AK)	+++	++
3 (MP)	-/+	-/+
4 (AK+MP)	+/++	+/++
The significance status obtained as a result of the comparison of the groups		
1-2	p = 0.002	p = 0.003
1-3	p = 0.127	p = 0.135
1-4	p = 0.004	p = 0.005
2-3	p = 0.003	p = 0.004
2-4	p = 0.002	p = 0.004
3-4	p = 0.007	p = 0.006

$p<0.05$ were considered statistically significant. The relationships between groups and the results of immunohistochemical markers are assessed by the Mann-Whitney U test. (-), negative score: No staining; (+), 1 positive score: Light staining; (++), 2 positive score: Middle staining; (+++), 3 positive score: Serious staining. Abbreviations: AK: amikacin; MP: misoprostol; CAS-3: Caspase 3; TNF-α: Tumor Necrosis Factor-alpha. AK: 1.2 g/kg; MP: 0.2 mg/kg/day; AK+MP: 1.2 g/kg AK + 0.2 mg/kg/day MP.

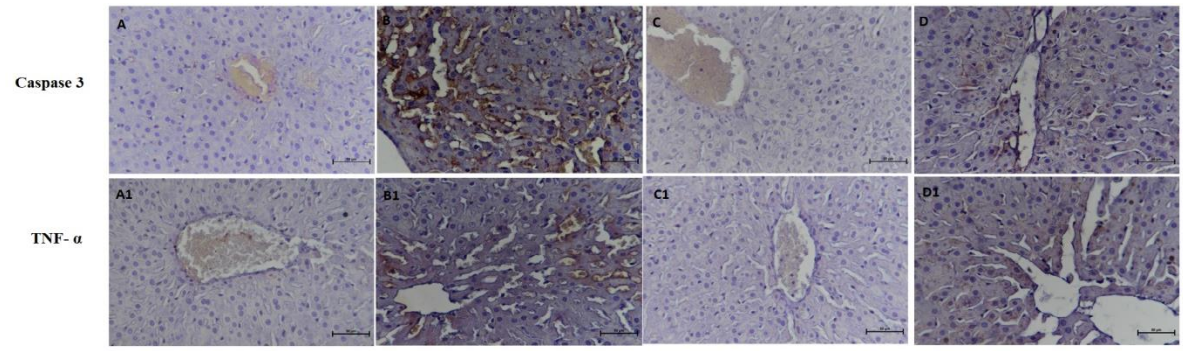

Figure 6. CAS-3 and TNF-α immune stainings in liver tissues belonging to control and experimental groups. A-A1, control group; no positive staining, B-B1, AK group; intensive positive staining, C-C1, MP group; no positive staining, D-D1, AK+MP group; mild positive stainings, A-B-C-D; Caspase 3, A1-B1-C1-D1; TNF-α, immün satining, x20. Abbreviations: AK: amikacin; MP: misoprostol; CAS-3: Caspase 3; TNF-α: Tumor Necrosis Factor-alpha. AK: 1.2 g/kg; MP: 0.2 mg/kg/day; AK+MP: 1.2 g/kg AK + 0.2 mg/kg/day MP.

5. Discussion

Aminoglycoside (AG) antibiotics are widely used in many life-threatening infectious diseases [33]. AGs activate cell death pathways by producing reactive oxygen species (ROS), which induces apoptosis [34]. It is known that AGs, a group of antibiotics, produce ROS [35]. The use of amikacin (AK), an AG, often results in toxicity due to the overproduction of ROS [36]. The molecular basis of hepatotoxicity is mainly due to oxidative stress and inflammation, ROS production, impaired mitochondrial function, and apoptosis [37, 38]. This study linked AK-induced liver injury to many mechanisms, including oxidative stress, apoptosis, and inflammation. ROS primarily affects cellular structures: DNA, hepatocytic proteins, and lipids [39]. The imbalance between the cell's antioxidant capacity and ROS production is defined as oxidative stress [40, 41]. ROS is an agent that causes cell death in many different developmental and pathological conditions [42, 43]. ROS are produced in the endoplasmic reticulum and mitochondria of hepatocytes by cytochrome P450 enzymes [39].

In this study, AK treatment increased CYP2B1 and CYP2B2 mRNA expression levels. It has been found that treatment with MP improves CYP2B1 and CYP2B2 mRNA expression levels. Cytochrome P450 (CYP) 2B1 and Cytochrome P450 (CYP) 2B2 are markedly induced by the administration of phenobarbital (PB), drugs, and other hydrophobic compounds, and the molecular mechanisms controlling both basal and induced transcription of these genes are not yet known. [44]. Cytochrome P-450 (P-450) enzymes, which play a crucial role in the elimination of foreign compounds such as the CYP2B1 isoform, also contribute to the production of toxic intermediates, especially ROS, which can cause cellular damage when overproduced [45, 46]. A study found that overexpression of CYP2B1 significantly increased ROS generation, cytotoxicity, cell death, and collapse of the actin cytoskeleton [47]. In another study, induction of CYP2B1 in rat liver resulted in the production of hydroxyl radicals. As a result, active oxygen produced by oxidized genomic DNA was shown to increase oxidative stress, which may contribute to tumor initiation and progression [48]. A previous study reported that doxorubicin-mediated induction of CYP2B1 and CYP2B2 causes cardiotoxicity [49]. An experimental study reported that rats were exposed to octamethylcyclotetrasiloxane by inhalation, inducing cytochrome P450 2B1/2 and 3A in the liver and causing hepatomegaly and sustained hypertrophy [50]. Studies have reported that reactive intermediates formed during the metabolism of toxic substances by CYP2B1, CYP2E1, and CYP1A1/2 isoenzymes often covalently bind to tissue macromolecules and cause tissue damage [51, 52]. Consistent

with the results of this study, this study demonstrates that AK treatment increased ROS formation by inducing CYP2B1 and CYP2B2 mRNA expression levels, and as a result, it causes liver tissue damage.

The serum ALP, ALT, AST, CH, LDL, HDL, direct bilirubin, total bilirubin, and TG levels were also measured to assess liver damage. The AK group increased AST and ALT levels, consistent with a previous study on liver toxicity [53]. In this study, a significant improvement was observed in the levels of biochemical biomarkers indicating liver damage in the AK+MP group. A significant increase was found in serum ALP, ALT, AST, CH, direct bilirubin, total bilirubin, LDL, and TG levels. A significant decrease was seen in HDL concentration in the AK group. However, the increased ALP, ALT, AST, CH, direct bilirubin, total bilirubin, LDL, TG, and HDL levels after AK application in rats showed improvement as a result of MP treatment, consistent with previous studies [54]. A study reported that treatment with MP protected against CCl_4-induced hepatocellular necrosis and decreased ALT, AST, and ALP levels [55]. According to the findings of this study, it is thought that ROS should be cleared and antioxidant levels should increase in order best to prevent liver damage from the toxic effect of AK. It has been reported that natural products, medicinal plant extracts, and some chemical substances have a mitigating or preventive effect against AG-induced toxicity [56] and hepatotoxicity [57, 58]. According to the results that were obtained in this study, it is thought that MP with its antioxidant properties may be useful in reversing the liver damage caused by AK. MP, a prostaglandin E1 (PGE1) analog, has received great attention in recent years as a reactive oxygen species (ROS) scavenger [55, 59].

ROS alters the functions of lipids, nucleic acids, and proteins, and oxidative stress occurs when the balance between antioxidative defense and ROS production is disrupted [60-62]. In this study, malondialdehyde (MDA) and Glutathione (GSH) levels, which are oxidative stress markers, were studied [63]. GSH is important in many cellular processes, especially apoptosis, cell differentiation, and proliferation [64, 65]. GSH deficiency or a decrease in its ratio causes increased susceptibility to oxidative stress, which plays a role in cancer progression [64]. GSH can protect cells against oxidative stress damage by scavenging ROS [66]. MDA is one of the most frequently measured oxidative stress biomarkers, lipid peroxidation [67]. Cell death occurs at the end of the lipid peroxidation process, and its end product is MDA, which acts as an indicator of oxidative damage [68]. Excessive MDA produced as a result of tissue damage combines with free amino groups of proteins and can alter their biological properties [69]. The AK group increased MDA levels and decreased GSH levels, consistent with a previous study on liver toxicity [53]. On the other hand, the MP group decreased MDA levels and increased GSH levels [70]. Studies show that GSH has an important role in detoxifying xenobiotics and drug metabolism and the inflammatory response and repair systems against oxidative stress by supporting anti-inflammatory and antioxidant activities [71, 72].

In this study, weight gain/loss was statistically significant in the control and other groups according to the body weight measurements made for 7 days. It was observed that fresh liver weight and fresh liver weight/body ratio increased statistically in the AK group compared to the control, MP, and AK+MP groups. Consistent with the results of this study, studies in rats have reported that AK causes weight gain in organs due to its phospholipidosis feature [73, 74]. A study showed that AG antibiotics induce phospholipidosis as a result of excessive phospholipid accumulation in lysosome-derived vesicles [75]. This study observed inflammatory reactions and histopathological changes because AK causes phospholipidosis in the liver. Per the findings of this study, previous studies have reported that organs affected by

phospholipidosis show inflammatory reactions and histopathological changes [76]. Treatment with MP prevented phospholipidosis by improving liver damage.

Gene expressions were consistent with the findings of this study on biochemical and oxidative stress and with the histological and immunochemical results as well. According to the histological results of this study, normal liver histology was observed in the control and MP groups. Significant histopathological findings, including vascular occlusion in hepatocytes, sinusoidal and tubular dilatation, pycnotic nuclei, hemorrhagic areas, mononuclear cell infiltration, and vacuolar and granular degeneration, were observed in the AK group. Histological structural changes were significantly reduced in the AK+MP group compared to the AK group, which is consistent with the findings of previous studies [70]. This study showed that the decrease in histological findings in rats treated with MP was achieved by normalized liver enzymes, serum levels of biochemical and lipid profiles, and findings associated with oxidative stress and inflammation. Thus, administration of MP may protect the liver tissue from the AK's toxic effects and strengthen the structural integrity as well as the functional integrity of the liver.

This study measured Caspase-3 (CAS-3) and Tumor Necrosis Factor-alpha (TNF-α) levels to comprehend the relationship between AK and apoptosis and inflammation. The findings of this study showed that AK mediates apoptosis and inflammation by ROS production. CAS-3 is a protease that plays a crucial role in apoptosis [77], cytokine maturation, cell growth, and differentiation [78]. According to the findings, as a result of the antiapoptotic property of MP, CAS-3 in the centrilobular regions of liver sections stained more intensely in the AK group than in the AK+MP group. A study showed that AK significantly reduced the number of viable cells in the liver compared to the control group [79]. According to these findings, it can be said that MP reduces AK-induced apoptosis by inhibiting CAS-3. In a study conducted in line with the results of this study, it was reported that MP protects liver cells from apoptosis with its antiapoptotic property in hepatic ischemia-reperfusion injury[80]. In another study, it was reported that apoptosis, necrosis, and proinflammatory cytokines activated by ROS were improved as a result of ischemia/reperfusion treatment with MP [21].

TNF-α, produced by macrophages and monocytes in acute inflammation, is an inflammatory cytokine and has an important role in various signaling events causing necrosis or apoptosis [81, 82]. High TNF-α expression has been found to be associated with septic shock, diabetes, tumorigenesis, rheumatoid arthritis, cardiovascular diseases, and inflammatory bowel disease [83]. Oxidative stress can activate TNF-α gene expression, and TNF-α plays an important role in many events, from inflammation to apoptosis [84, 85]. Oxidative stress mediates some of the effects of TNF-α [86]. In this study, it was found that TNF-α was significantly higher in the AK group. As a result of the anti-inflammatory properties of MP, liver TNF-α was significantly lower in the AK+MP group than the AK group, consistent with the findings of previous studies [70]. Another study reported that MP inhibited TNF-α and IL-1β production and increased IL-6 production [87].

According to the findings of this study, AK damages cell components such as lipids, proteins, and DNA by causing the formation of ROS. On the other hand, this caused tissue damage, resulting in a decrease in GSH and an increase in MDA. At the same time, this situation was also seen in the serum biochemical parameters of liver damage in the findings of this study. In fact, antioxidants such as MP neutralize free radicals and protect cells from damage. However, due to the increase in the free radical level compared to the antioxidant level, oxidative damage occurs, and oxidative stress occurs as a result [88, 89]. Oxidative stress,

on the other hand, causes apoptosis and inflammatory processes by increasing CAS-3 and TNF-α levels, as in the findings of this study, which in turn causes hepatotoxicity and ultimately causes many diseases.

This study has some limitations regarding the budget. To understand the molecular mechanism of MP's effect on AK-induced liver injury, more parameters related to liver injuries, such as protein expression levels and oxidant/antioxidant system indicators, can be examined. Therefore, these issues should be the target of new research in the future.

6. Conclusions

According to our findings, it is thought that antioxidants such as MP can be used to prevent the negative effects and oxidative damage caused by AK, whose use is limited due to its toxic effects, although its use is widespread. Although this study showed the beneficial effects of MP against AK-induced liver injury, more clinical studies are needed to confirm these results.

Funding

This research received no external funding.

Acknowledgments

This paper has no acknowledgment.

Conflicts of Interest

The authors declare no conflict of interest.

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