BİNGÖL UNIVERSITY

Turkish Journal of Nature and Science

e-ISSN: 2149-6366
www.dergipark.gov.tr/tdfd

Research Article

Volume 15, Issue 2, Page 244-254, 2026

Received: 24.08.2025
Accepted: 09.06.2026
Published: 30.06.2026

https://doi.org/10.46810/tdfd.1771398

Protective Effects of Polydatin in Methotrexate-Induced Liver Toxicity in Rats; Multi-Pathway Analysis

Selman GENCER[1*], Özge KANDEMİR[2], Nurhan AKARAS[3], Hasan ŞİMŞEK[4]

[1]Aksaray University, Medicine Faculty, Internal Diseases Department, Aksaray, Türkiye
[2]Aksaray University, Aksaray Technical Sciences Vocational School, Food Processing Department, Aksaray, Türkiye
[3]Aksaray University, Medicine Faculty, Histology and Embryology Department, Aksaray, Türkiye
[4]Aksaray University, Medicine Faculty, Physiology Department, Aksaray, Türkiye

**Corresponding author: selman.gencer@aksaray.edu.tr*

Abstract

This study evaluated polydatin's protective effects against methotrexate (MTX)-induced hepatotoxicity in rats. Thirty-five *Wistar albino* rats were divided into (n=7): Control, Polydatin-only (100 mg/kg), MTX-only (20 mg/kg), MTX + Polydatin (50 mg/kg), and MTX + Polydatin (100 mg/kg). Treatments lasted 7 days. MTX administration significantly increased serum AST/ALT levels, hepatic malondialdehyde (MDA), and pro-inflammatory/apoptotic markers (NF-κB, TNF-α, Bax, Caspase-3 mRNA), while decreasing anti-apoptotic Bcl-2 and tissue-repair signals (Wnt3 mRNA). Co-treatment with polydatin dose-dependently reversed these effects: the 100 mg/kg dose notably normalized AST/ALT and MDA levels more effectively than 50 mg/kg ($p<0.05$), while both doses comparably suppressed TNF-α, Bax, and Caspase-3. Polydatin (100 mg/kg) significantly elevated Bcl-2 and Wnt3 expression versus lower-dose treatment ($p<0.05$). Antioxidant defenses (SOD, CAT, GPx, GSH) were also restored in a dose-responsive manner. Histopathology confirmed severe inflammation and cellular damage in MTX groups, whereas polydatin groups showed near-normal liver architecture. Findings demonstrate polydatin's multi-target protection against MTX induced liver injury through mitigating oxidative stress, inflammation, apoptosis, and enhancing regeneration, supporting its adjunctive therapeutic potential.

Keywords

Apoptosis, Hepatotoxicity, Methotrexate, Oxidative stress, Polydatin

Author(s) publishing with the journal retain(s) the copyright to their work licensed under the CC BY-NC-ND 4.0.
https://creativecommons.org/licenses/by-nc-nd/4.0/

BİNGÖL ÜNİVERSİTESİ

Türk Doğa ve Fen Dergisi

e-ISSN: 2149-6366
www.dergipark.gov.tr/tdfd

Araştırma Makalesi

Cilt 15, Sayı 2, Sayfa 244-254, 2026

Alınış: 24.08.2025
Kabul: 09.06.2026
Basım: 30.06.2026

https://doi.org/10.46810/tdfd.1771398

Sıçanlarda Metotreksat ile İndüklenen Karaciğer Toksisitesinde Polidatinin Koruyucu Etkileri; Çoklu Yolak Analizi

Selman GENCER[1*], Özge KANDEMİR[2], Nurhan AKARAS[3], Hasan ŞİMŞEK[4]

[1]Aksaray Üniversitesi, Tıp Fakültesi, İç Hastalıkları Bölümü, Aksaray, Türkiye
[2]Aksaray Üniversitesi, Teknik Bilimler Meslek Yüksekokulu, Gıda İşleme Bölümü, Bingöl, Türkiye
[3]Aksaray Üniversitesi, Tıp Fakültesi, Histoloji ve Embriyoloji Anabilim Dalı, Aksaray, Türkiye
[4]Aksaray Üniversitesi, Tıp Fakültesi, Fizyoloji Anabilim Dalı, Aksaray, Türkiye

**Sorumlu yazar: selman.gencer@aksaray.edu.tr*

Özet

Bu çalışma, sıçanlarda metotreksat (MTX) kaynaklı hepatotoksisiteye karşı polidatinin koruyucu etkilerini değerlendirdi. Otuz beş *Wistar albino* sıçan (n=7) şu gruplara ayrıldı: Kontrol, polidatin (100 mg/kg), MTX (20 mg/kg), MTX + polidatin (50 mg/kg) ve MTX + polidatin (100 mg/kg). Tedaviler 7 gün boyunca uygulandı. MTX uygulaması, serum AST/ALT düzeylerini, karaciğer malondialdehitini (MDA) ve proinflamatuar/apoptotik belirteçleri (NF-κB, TNF-α, Bax, Caspase-3 mRNA) önemli ölçüde artırırken, anti-apoptotik Bcl-2 ve doku onarım sinyallerini (Wnt3 mRNA) azalttı. Polidatin ile birlikte uygulanan tedavi, bu etkileri doza bağlı olarak tersine çevirdi: 100 mg/kg dozu, AST/ALT ve MDA seviyelerini 50 mg/kg dozuna göre belirgin şekilde daha etkili bir şekilde normalleştirdi ($p<0.05$), her iki doz da TNF-α, Bax ve Caspase-3'ü karşılaştırılabilir şekilde baskıladı. Polidatin (100 mg/kg), düşük doz tedavisine kıyasla Bcl-2 ve Wnt3 ekspresyonunu önemli ölçüde artırdı ($p<0.05$). Antioksidan savunmalar (SOD, CAT, GPx, GSH) da doza bağlı bir şekilde restore edildi. Histopatoloji, MTX gruplarında şiddetli inflamasyon ve hücresel hasarı doğrularken, polidatin gruplarında karaciğer mimarisi neredeyse normaldi. Bulgular, polidatinin oksidatif stresi, inflamasyonu, apoptozu hafifleterek ve rejenerasyonu artırarak MTX kaynaklı karaciğer hasarına karşı çok yönlü koruma sağladığını ve yardımcı tedavi potansiyelini desteklediğini göstermektedir.

Anahtar kelimeler

Apoptozis, Hepatotoksisite, Metotreksat, Oksidatif stres, Polidatin

1. INTRODUCTION

Methotrexate (MTX) can be used in oncological diseases such as acute lymphoblastic leukemia, non-Hodgkin's lymphoma, breast cancer, lung cancer, osteosarcoma, choriocarcinoma, as well as rheumatological diseases such as rheumatoid arthritis (RA), psoriatic arthritis, systemic lupus erythematosus (SLE) [1–3]. MTX is a folate antagonist that competently inhibits the enzyme dihydrofolate reductase (DHFR), which is responsible for the conversion of dihydrofolate to tetrahydrofolate (THF) [4,5]. THF is essential in the production of purines and thymidylates, which are critical for DNA/RNA synthesis [6,7]. Decreased levels of THF disrupt DNA synthesis and cell division, especially in fast-proliferating cells (cancer cells, immune cells) [8]. At low doses, used in autoimmune diseases, it increases the release of adenosine. Adenosine suppresses neutrophil/macrophage activity as well as having anti-inflammatory effects [9]. TNF-α reduces the production of cytokines such as IL-6, inhibits T-cell proliferation [10]. Common side effects include mouth ulcers, bone marrow suppression, liver toxicity, nausea [11]. More serious side effects are lung fibrosis, teratogenicity, kidney toxicity [11]. Due to these side effects, its clinical use is restricted.

On the other hand, the research of substances that can be used naturally against the side effects caused by the use of chemotherapeutics such as MTX has been the focus of interest.

Polydatin (Poly) is a polyphenol glycoside that is a glucose molecule attached to the resveratrol molecule [12,13]. It is a compound that is naturally found in some medicinal plants and has anti-inflammatory and antioxidant properties [14,15]. It shows protective and therapeutic effects in metabolic diseases such as liver diseases (alcohol or fatty liver, fibrosis, hepatitis), obesity, diabetes, hyperlipidemia and gout. Reduces oxidative stress and inflammation, improves insulin resistance [15,16]. Antioxidant, anti-apoptotic and anti-inflammatory effects of Poly have been reported in the literature [14,16].

In this study, it was aimed to investigate the mechanisms of prevention or reversal of inflammation, oxidative stress, apoptosis caused by MTX on the liver by Poly.

2. MATERIAL AND METHOD

2.1. Research and Publication Ethics

Animal studies were approved by Necmettin Erbakan University Animal Experiments Local Ethics Committee. Approval No/ Date: 207000 / 2024

2.2. Drugs and Chemicals

MTX (Koçak Farma, ≥99% purity) and Poly (Sigma, U.S.A., 97% purity) literature was used to determine the dose [7,16].

2.3. Groups and Experiment Protocol

In this study, 35 Wistar albino male rats aged 10–12 weeks and weighing between 220–250 g were included. The animals were obtained from Necmettin Erbakan University KONUDAM Experimental Medicine Application and Research Center. During the experiment, the animals were housed in standard laboratory animal cages under controlled environmental conditions of 24 ± 1 °C temperature, 12-hour light / 12-hour dark cycle, and 45 ± 5 % humidity. Throughout the experiment, the animals had unlimited access to standard feed and water.
Groups (n=7);
1. Control: Rats were given a single dose of intraperitoneal (i.p.) saline on the first day and oral saline for 7 days after 30 minutes.
2. Polydatin (Poly): rats were given 100 mg/kg Poly orally for 7 days.
3. Methotrexate (MTX): Rats were given a single dose of 20 mg/kg MTX i.p. on the first day.
4. Methotrexate + Polydatin50 (MTX+Poly50): Rats were given a single dose of 20 mg/kg MTX i.p. on the first day and continued with 50 mg/kg/oral Poly for 7 days.
5. Methotrexate + Polydatin100 (MTX+Poly100): Rats were given a single dose of 20 mg/kg MTX i.p. on the first day and continued with 100 mg/kg/oral Poly for 7 days.
Poly was administered 30 minutes after the MTX treatment.

2.4. Tissue Collection

24 hours after the last administration (day 8), the animals were decapitated under light sevoflurane anesthesia and liver and blood samples were collected. Blood samples were transferred to vacuum tubes without anticoagulants for biochemical analysis, centrifuged at +4°C at 3,000 rpm for 10 minutes, separated serums and stored in a deep freezer at -20°C until biochemical analyzes were performed. Some of the liver tissues were taken for biochemical analysis and stored at -20 °C until the analyzes were performed. The other part was left in a 10% formaldehyde solution to be used in histological analysis.

2.5. Liver function markers

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were determined in accordance with the manufacturer's procedures with the commercial kit to determine liver function.

2.6. Lipid Peroxidation Analysis

In order to determine the lipid peroxidation level, the absorbance of the color released as a result of the reaction of malondialdehyde (MDA) and thiobarbituric acid from liver tissues was measured at a wavelength of 532 nm. For analysis, tissues were homogenized with a homogenizer in a 1.15% potassium chloride (KCl) solution. Homogenates were then centrifuged at 4°C and 1,000×g for 15 minutes to obtain the supernatant, which was used to determine MDA levels. The method developed by Placer et al. was used [17].

2.7. Analysis of Anti-oxidant Markers

Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities for antioxidant status in liver tissues were analyzed with commercial ELISA kits. Glutathione (GSH) levels were higher than those of Sedlak and Lindsay. [18] measured according to the method he determined. In determining the total protein

content of liver tissues, which is necessary to calculate enzyme activities, Lowry et al. [19] method was used.

2.8. RT-PCR analysis

At the end of the experiment, mRNA transcription levels of genes listed in Table 1 were analyzed by the RT-PCR method in liver tissues obtained. RNA isolation was performed using commercially available QIAzol Lysis Reagent (Qiagen, 79306). Isolated total RNA was converted to cDNA with OneScript Plus cDNA Synthesis Kit (ABM, G236, Richmond, Canada). Then, the PCR mixture was prepared by adding BlasTaq™ 2X qPCR MasterMix (ABM, G891, Richmond, Canada) with primer sequences, and the reaction started. The procedures were carried out in appropriate temperature cycles in the Rotor-Gene Q (Qiagen) device according to the protocol specified by the manufacturer. Gene expressions obtained from the analysis were normalized with the β-Actin reference gene and evaluated using the $2^{-\Delta\Delta CT}$ method[20].

Table 1. Primer sequences

Gene	Sequences (5'-3')	Length (bp)	Accession No
NF-kB	F:AGTCCCGCCCCTTCTAAAAC R:CAATGGCCTCTGTGTAGCCC	106	NM_0012767 11.1
TNF-a	F:CTCGAGTGACAAGCCCGTAG R:ATCTGCTGGTACCACCAGTT	139	NM_012675.3
Caspase -3	F:ACTGGAATGTCAGCTCGCAA R:GCAGTAGTCGCCTCTGAAGA	270	NM_012922.2
Bax	F:TTTCATCCAGGATCGAGCAG R:AATCATCCTCTGCAGCTCCA	154	NM_017059.2
Bcl-2	F:GACTTTGCAGAGATGTCCAG R:TCAGGTACTCAGTCATCCAC	214	NM_016993.2
Wnt3A	F:GTCACAACAATGAGGCTGG R:AGGTCTTCACTTCGCAACTG	96	NM_0011077 05
Lrp5	F:CTTCATCCACCGTGCCAACC R:CCAGTAGAGTGTGTCCCCAG	106	NM_0011063 21
B-Actin	F:CAGCCTTCCTTCTTGGGTAT R:AGCTCAGTAACAGTCCGCCT	360	NM_031144.3

2.9. Histopathological Analysis

At the end of the experiment, liver samples taken from rats were kept in a 10% neutral buffered formalin solution for 72 hours for fixative purposes. Liver tissues that were washed under running water for 1 night to free them from formalin were first dehydrated in alcohols of increasing grade and then cleared of xylene. Then, 5 µm thick sections were taken from the paraffin-embedded tissues and stained with hematoxylin & eosin (H&E) for histopathological evaluation. Evaluation was performed using a binocular Olympus Cx43 light microscope (Olympus Inc., Tokyo, Japan) and an EP50 camera (Olympus Inc., Tokyo, Japan) to obtain images from stained preparations. A semi-quantitative histopathological scoring was performed by evaluating pyknosis, sinusoidal dilatation, vascular congestion, and mononuclear cell infiltration in liver tissue. Histopathological changes were examined under a light microscope using a blinded method and scored as absent (0), mild (1), moderate (2), or severe (3) according to their severity.

2.10. Statistical Analysis

Data were analyzed using IBM SPSS software and presented as mean ± standard deviation (SD). Data were analyzed using one-way ANOVA with Tukey's post hoc tests for multiple comparisons. $p<0.05$ was considered statistically significant.

3. RESULTS

3.1. Liver function Findings

A statistically significant increase was observed in the MTX group compared to the control group in the serum activities of AST and ALT, which are biomarkers of hepatocyte damage. However, Poly treatment co-administered with MTX suppressed this enzyme elevation in a dose-dependent manner. In particular, in the MTX+Poly100 group, there was a significantly higher decrease in AST and ALT activities compared to the MTX+Poly50 group, and this difference was statistically significant ($p < 0.05$) (Figure 1).

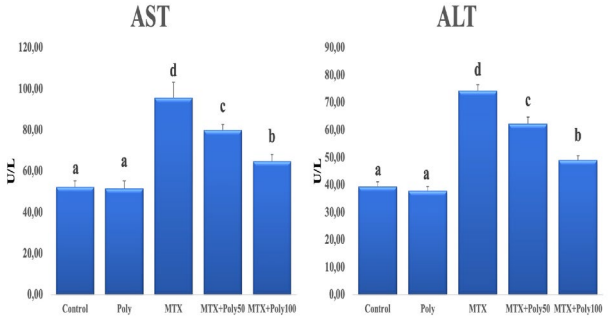


Figure 1. Effects of MTX and Poly on serum AST and serum ALT levels. Values are given as mean ± SD. Different letters indicate statistical difference: a, b, c, d $p<0.05$

3.2. Oxidative Stress Findings

A statistically significant increase was found in the MTX group compared to the control group in hepatic MDA levels, which is a critical marker of lipid peroxidation ($p<0.05$). However, in combined treatments with Poly, it has been observed that this oxidative damage triggered by MTX is suppressed in a dose-dependent manner. Especially in the MTX+Poly100 group, the increase in MDA levels was found to be significantly lower compared to the MTX+Poly50 group ($p<0.05$) (Figure 2).

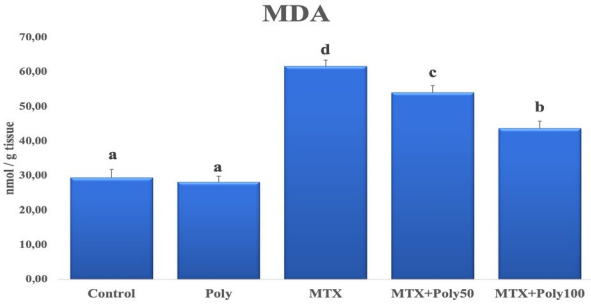


Figure 2. Effects of MTX and Poly on MDA level in rat liver tissues. Values are given as mean ± SD. Different letters indicate statistical difference: a, b, c, d $p<0.05$

A statistically significant increase was observed in CAT, GPx, SOD activities and GSH levels as a compensatory response to oxidative stress in the MTX group compared to the control group ($p<0.05$). However, it was determined that the increase in these antioxidant parameters was

suppressed in a dose-dependent manner in the groups treated with combination therapies with Poly. Especially in the MTX+Poly100 group, the decrease in CAT, GPx, SOD and GSH levels was significantly higher than in the MTX+Poly50 group, and this difference was statistically significant ($p<0.05$) (Figure 3).

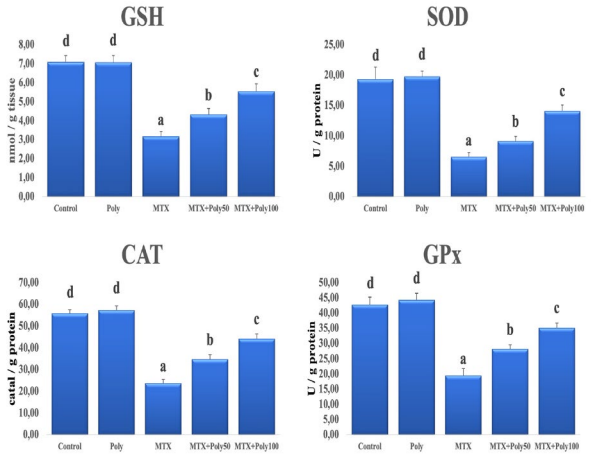


Figure 3. Effects of MTX and Poly on GSH level and SOD, CAT, and GPx activities in rat liver tissues. Values are given as mean ± SD. Different letters indicate statistical difference: a, b, c, d $p<0.05$

3.3. Inflammatory Cytokines Findings

Nuclear factor kappa B (NF-κB) and tumor necrosis factor-alpha (TNF-α) mRNA expression levels, which are critical regulators of the pro-inflammatory signaling cascade, were statistically significantly increased in the MTX treatment group compared to the control group ($p<0.05$). In the groups treated with poly in combination, it was determined that the increase in TNF-α gene expression was suppressed at a similar level at both doses (50 and 100 mg/kg) and there was no significant difference between the groups. In contrast, suppression of NF-κB expression correlated with polydatin dose: NF-κB mRNA levels were statistically significantly lower in the MTX+Poly100 group than in the MTX+Poly50 group ($p<0.05$) (Figure 4).

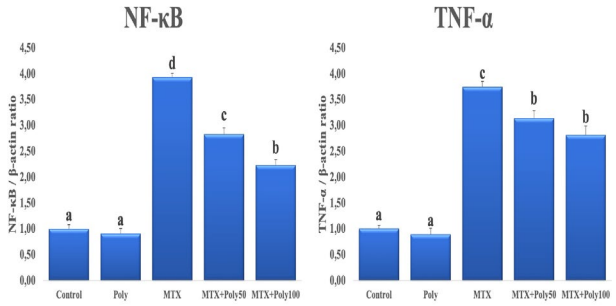


Figure 4. Effects of MTX and Poly on NF-κB and TNF-α mRNA transcription levels in rat liver tissues Values are given as mean ± SD. Different letters indicate statistical difference: a, b, c, d $p<0.05$

3.4. Apoptosis Findings

A statistically significant increase was observed in the mRNA expression levels of pro-apoptotic proteins Bax and caspase-3, which are critical markers of apoptotic pathways, in the MTX group compared to the control group ($p<0.05$), while a significant decrease was observed in the mRNA expression of anti-apoptotic protein Bcl-2 ($p<0.05$). In the groups MTX+Poly50 and MTX+Poly100, the increase in Bax and caspase-3 expression was suppressed at a similar level at both doses and there was no statistical difference between the groups. However, the change in Bcl-2 expression exhibited a dose-dependent profile: decreased Bcl-2 levels in the MTX group showed the most significant increase in the MTX+Poly100 group ($p<0.05$) and were significantly higher than in the MTX+Poly50 group (Figure 5).

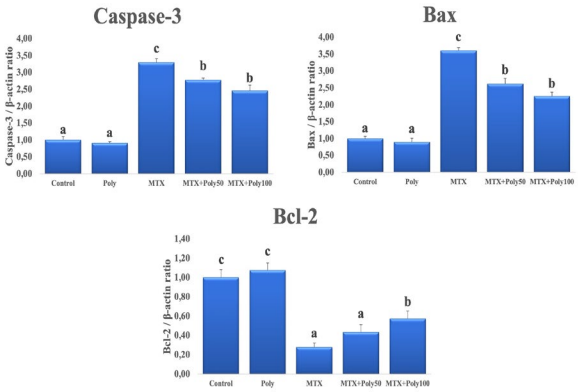


Figure 5. Effects of MTX and Poly on Caspase-3, Bax, and Bcl-2 mRNA transcription levels in rat liver tissues Values are given as mean ± SD. Different letters indicate statistical difference: a, b, c, d $p<0.05$

3.5. Hepatic Fibrosis and Regeneration Analysis

Statistically significant suppression was observed in the activation of Wingless-type MMTV integration site family member 3 (Wnt3) and Low Density Lipoprotein Associated Protein 5 (Lrp5) signaling pathways, which play critical roles in hepatic regeneration and cell survival, in the MTX group ($p<0.05$). On the other hand, in the group treated with high-dose Poly (100 mg/kg), there was a significant increase in Wnt3 mRNA expression levels compared to the control group ($p<0.05$), while no change was detected in Lrp5 expression

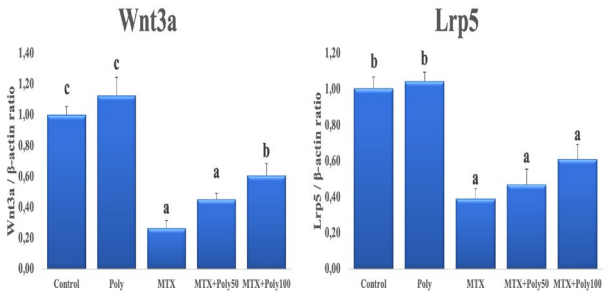


Figure 6. Effects of MTX and Poly on Wnt3A and Lrp5 mRNA transcription levels in rat liver tissues. Values are given as mean ± SD. Different letters indicate statistical difference: a, b, c, d $p<0.05$

3.6. Histopathologic Findings

Histopathological examination images based on H&E staining are presented in Figure 7. Histopathological findings are also presented in Table 2. When the control and Poly groups were examined, hepatocytes, the central vein, and hepatic lobules in the liver tissues exhibited well-preserved histological architecture (Figure 7A, B). In the MTX-treated group, a marked increase in inflammatory cell infiltration was observed, accompanied by hepatocytes with eosinophilic cytoplasm and pyknotic nuclei. In addition, congestion in the central vein and

sinusoids, along with pronounced sinusoidal dilatation, was noted (Figure 7C). In rats treated with MTX and Poly, hepatic lobules closely resembled those in the control group, except for very mild sinusoidal dilation and congestion.

In short, there were very slight pathological differences between the images of MTX+Poly50 and MTX+Poly100 (Figure 7D,E). Histopathological scoring revealed that pyknosis, sinusoidal dilatation, vascular congestion, and mononuclear cell infiltration were minimal or absent in the control and Poly groups. In contrast, the MTX group showed a significant increase in all evaluated parameters, with histopathological changes ranging from moderate to severe ($p < 0.05$). In the treatment groups, both doses of Poly resulted in a significant reduction in pyknosis, sinusoidal dilatation, and mononuclear cell infiltration compared with the MTX group ($p < 0.05$); however, these parameters remained significantly higher than those observed in the control group ($p < 0.05$).

Table 2. Histopathological findings and scores in rat liver tissue.

Parameters	Control	Poly	MTX	MTX+Poly50	MTX+Poly100
Pyknosis,	0,42 ±0,20$^{\#}$	0,28 ±0,18$^{\#}$	2,42 ±0,20*	1,57 ±0,20*$^{\#}$	1,28 ±0,18*$^{\#}$
Sinusoidal dilatation	0,28 ±0,18$^{\#}$	0,14 ±0,14$^{\#}$	2,85 ±0,14*	1,71±0,18*$^{\#}$	1,57±0,20*$^{\#}$
Vascular congestion	0,57 ±0,20$^{\#}$	0,28 ±0,18$^{\#}$	2,57 ±0,20*	1,57 ±0,20*$^{\#}$	1,42 ±0,20*$^{\#}$
Mononuclear cell infiltration	0,14 ±0,14$^{\#}$	0,14 ±0,14$^{\#}$	2,57 ±0,20*	1,28 ±0,18*$^{\#}$	1,28 ±0,18*$^{\#}$
Total score	1,41	0,84	10,41	6,13	5,55

Note: Data are presented as mean ± standard error. *($p<0.05$) compared with control groups, #($p<0.05$) compared with MTX group, +($p<0.05$) compared with MTX+Poly 50 group

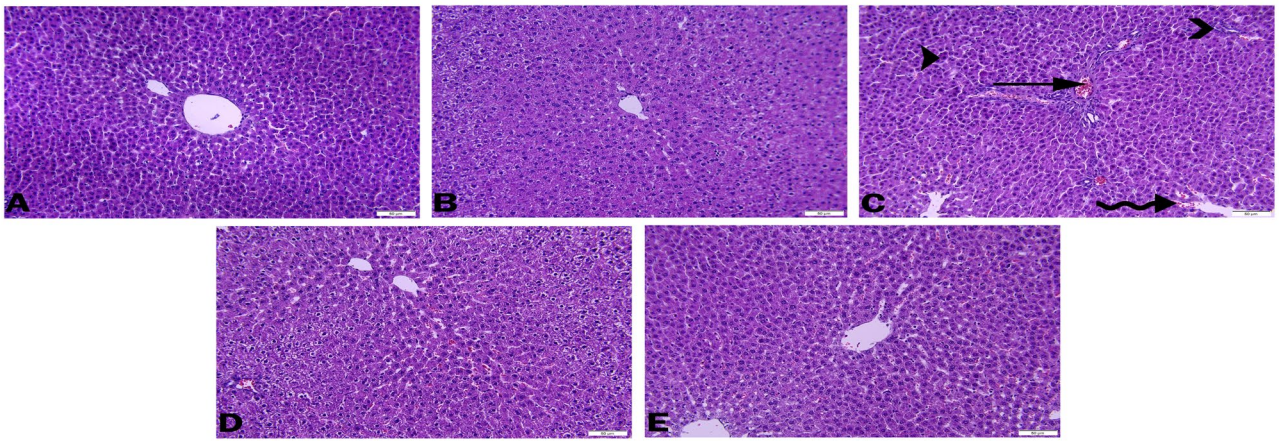


Figure 7. H&E stained liver tissue photomicrographs (x200) of control and different experimental groups. Liver tissues belonging to Control (A) and Poly (B) groups showed normal structure. Liver tissues belonging to the MTX (C) group showed markedly increased inflammatory cells (arrowhead), eosinophilic cytoplasmic hepatocytes (arrowhead), sinusoidal dilatation (curled arrow), and vascular congestion (arrow). MTX+Poly50 (D) and MTX+Poly100 (E) groups showed decreased sinusoids and improvement similar to the Control group, except for dilatation and congestion. Scale bar = 50 µm

4. DISCUSSION AND CONCLUSION

MTX is one of the main drugs in the treatment of many inflammatory and autoimmune diseases, especially rheumatoid arthritis. It is also used as a chemotherapeutic agent in some types of cancer [11,21]. The mechanism of action of MTX is multifaceted and carries both anti-inflammatory and immunosuppressive properties [22,23]. At high doses, it acts as a chemotherapeuthetic, while at low doses, its anti-inflammatory effects are observed [24,25]. Gastrointestinal, hematological, cutaneous and hepatological side effects may be seen in long-term use or at high doses [26,27].

MTX increases the production of reactive oxygen species (ROS) in the liver and triggers lipid peroxidation. This leads to damage to the cell membrane and leakage of liver enzymes into the blood [2,28,29]. As a result of hepatocyte damage, a significant increase in ALT and AST levels is observed, reflecting liver cell necrosis and inflammation. In case of bile duct damage or cholestasis, ALP, GGT and total/direct bilirubin levels may be elevated [30–35]. In this study, increasing doses of Poly administration reversed hepatotoxic effects with anti-oxidant, anti-inflammatory, anti-apoptotic effects.

In this context, understanding the oxidative stress mechanisms underlying MTX-induced hepatotoxicity necessitates a more detailed examination of cellular antioxidant defense systems. Cells have an advanced antioxidant defense system to combat ROS that occurs

during metabolism. The most important enzymatic components of this system are GSH, SOD, CAT [34–36]. SOD converts the superoxide radical (O_2•−) formed inside the cell into hydrogen peroxide (H_2O_2) and oxygen (O_2). This reaction eliminates the harmful effects of the superoxide radical and allows the formation of H_2O_2, which is less reactive [33,37,38]. Catalase breaks down the H_2O_2 produced by SOD into water (H_2O) and O_2. With this, the harmful effects of H_2O_2 accumulating in the cell are prevented and oxidative stress is reduced [39,40]. GSH, another intracellular component of this system, is a powerful antioxidant that is free; It works in conjunction with the enzyme GPx. GPx, organic peroxides and H_2O_2 GSH converts it to alcohol and water using its reduced form [41,42]. In this process, GSH is converted to oxidized form (GSSG). The glutathione system plays a critical role, especially in maintaining intracellular redox balance and eliminating the harmful effects of ROS [37,43–47]. Again, the increased free radical load in the cell causes lipid peroxidation, causing an increase in MDA, a reactive aldehyde [24,28,33,45]. MDA is formed as a result of the oxidative degradation of polyunsaturated fatty acids and triggers events that result in cell and tissue damage, including DNA damage. Its levels are considered an indicator of oxidative stress in the cell [48]. In this study, it was found that increasing Poly doses were found to be effective by activating endogenous antioxidant systems such as CAT, SOD, and GSH [47,49,50] It has been shown to reduce mediated oxidative stress, lower MDA levels, a marker of lipid peroxidation, and thus reverse MTX-induced hepatotoxic damage. While the findings revealed the liver-protective potential of Poly, it suggested that it should be evaluated as an adjuvant therapy in clinical practice.

Building upon these findings related to oxidative stress and antioxidant defense mechanisms, it is also important to consider the contribution of inflammatory signaling pathways to MTX-induced hepatotoxicity. While the anti-inflammatory effect of MTX predominates at low doses, a pro-inflammatory response and tissue damage may be seen in some tissues or at high doses [51–54]. In general, high doses of MTX can activate pro-inflammatory signaling pathways in the liver, leading to chronic inflammation and ultimately tissue damage. One of these pathways of action is NF-κB activation. MTX activates the IκB kinase (IKK) complex in the cell, resulting in phosphorylation and degradation of IκBα [29,55–57]. The released NF-κB (p50/p65) crosses the nucleus and enhances the transcription of pro-inflammatory cytokines such as TNF-α, IL-6, IL-1β. These cytokines trigger neutrophil infiltration and hepatic stellate cell activation in the liver, paving the way for fibrosis [54,56,58,59]. Poly, which has high bioavailability thanks to its stilbenoid structure and glycosyl group to this pathway, prevents IκBα degradation by inhibiting IKK phosphorylation. The nuclear translocation of NF-κB is blocked, thereby reducing the synthesis of TNF-α, IL-6 and IL-1β [60,61]. In this study, it was found that Poly was used in MTX-induced hepatic inflammation; It was found that it blocked NF-κB translocation by inhibiting IKK-mediated IκBα degradation and accordingly significantly reduced the synthesis of pro-inflammatory mediators such as TNF-α, IL-6 and IL-1β.

In addition to the modulation of inflammatory signaling pathways, the regulation of apoptosis-related mechanisms represents another critical aspect of MTX-induced hepatotoxicity that warrants consideration. MTX acts by inhibiting cell proliferation at low doses, while at high doses it can trigger pathways to apoptosis [62]. This effect increases mitochondrial membrane permeability, resulting in Bax/Bak oligomerization. It increases the expression of pro-apoptotic Bax and Bak while lowers the levels of anti-apoptotic Bcl-2 and Bcl-xL [63–66]. This increases the permeability of the outer membrane of the mitochondrial membrane. Cytocrim c activates caspase-9 to form the apoptotic proteasome complex (apoptosome) [52,67]. The activated caspase-9 initiates apoptosis by cleaving effector caspases (caspas-3/7) [68]. Poly inhibits cytochrome c leakage by stabilizing mitochondrial membrane permeability, as well as inhibiting the translocation of Bax/Bak to mitochondria by increasing Bcl-2 and Bcl-xL expression. At the same time, it shows an anti-apoptotic effect by suppressing the activation of caspase-9 and caspase-3 [29,53,69]. In this study, Poly decreased Bax levels while increasing Bcl-2 levels, and its antiapoptotic effect was more effective at higher doses. It increased the level of Bcl-2 more with increasing doses. In this study, Poly was shown to prevent MTX-induced apoptotic damage by mitochondrial protective mechanisms. Poly inhibited cytochrome c release by providing mitochondrial membrane stabilization, regulated Bcl-2/Bax balance in an anti-apoptotic direction, and suppressed the cascade cascade. In particular, dose-dependent increase in Bcl-2 expression is one of the main mechanisms of Poly's anti-apoptotic effect. These findings suggest that Poly may be evaluated as a potential protective agent against the hepatotoxic effects of MTX.

Beyond its effects on apoptotic pathways, MTX-induced hepatotoxicity also involves disruptions in cellular proliferation and regeneration mechanisms, particularly through modulation of the Wnt/β-catenin signaling pathway. Another effect of high-dose MTX is that it suppresses the transcription of Wnt3 ligand and LRP5 receptors, inhibiting the stabilization of β-catenin [70]. Wnt3 is a ligand of the canonical Wnt signaling pathway that binds to the frizzled receptor and LRP5/6 co-receptor on the cell surface to provide β-catenin stabilization [71,72]. It activates β-catenin accumulation and target gene transcription (e.g., c-Myc, Cyclin D1) by interacting with Lrp 5 and Wnt3. Supports cell proliferation, stem cell regeneration and tissue repair [73]. MTX's suppression of this mechanism eventually causes the increase and destruction of β-catenin degradation. As a result, it leads to impaired cell renewal and tissue repair. Activation of Wnt-3A and Lrp5 signaling pathways and increased levels of mRNA transcription are recognized as molecular markers of chronic damage. Wnt3 and LRP5/6 accelerate liver regeneration by increasing mRNA and protein levels, promoting β-Catenin stabilization, ultimately hepatocyte proliferation and extracellular matrix remodeling [74,75]. The present study reveals that Poly activates the MTX-suppressed Wnt3/LRP5 signaling

pathway in a dose-dependent manner. Polydatin, which prevents β-catenin degradation by increasing Wnt ligand-receptor expression, stimulated the transcription of proliferative genes such as cyclin D1 and c-Myc, thereby supporting liver regeneration processes.

Histopathological findings showed significant inflammation, sinusoidal congestion and hepatocyte damage in the MTX group, which were significantly reduced in the Poly treatment groups. The fact that there were only minimal histological differences between the MTX+Poly 50 and MTX+Poly100 groups suggests that Poly will have an effective hepatoprotective effect even at low doses.

Although antioxidant levels were investigated in the present study, one of its limitations is the lack of study of the Poly-associated Nrf2/HO-1 (ARE) pathway.

This study revealed that Poly exhibits multifaceted antioxidant, anti-inflammatory, anti-apoptotic, and anti-fibrotic potential, as well as protective mechanisms against MTX-induced hepatotoxicity. Histopathological findings confirm the functional results of these molecular mechanisms and show that especially high-dose Poly maintains liver histology at a near-normal level. This comprehensive effect profile suggests that Poly may be evaluated as a hepatoprotective adjuvant in patients receiving MTX therapy.

Acknowledgement

None.

REFERENCES

[1] Alqarni A, Zeidler M. "How does methotrexate work?." Biochemical Society Transactions 48.2 (2020): 559-567.

[2] Chan E, Cronstein B. Methotrexate—how does it really work? Nat Rev Rheumatol. 2010 Mar 1;6:175–8. doi:10.1038/nrrheum.2010.5

[3] Cronstein B. Low-Dose Methotrexate: A Mainstay in the Treatment of Rheumatoid Arthritis. Pharmacol Rev. 2005 Jun 1;57:163–72. doi:10.1124/pr.57.2.3

[4] Bedoui Y, Guillot X, Selambarom J, Guiraud P, Giry C, Jaffar-Bandjee M, at al. Methotrexate an Old Drug with New Tricks. Int J Mol Sci [Internet]. 2019 Oct 1;20.

[5] Cronstein B, Aune T. Methotrexate and its mechanisms of action in inflammatory arthritis. Nat Rev Rheumatol. 2020 Feb 17;16:145–54. doi:10.1038/s41584-020-0373-9

[6] Cronstein B, Naime D, Ostad E. The antiinflammatory mechanism of methotrexate. Increased adenosine release at inflamed sites diminishes leukocyte accumulation in an in vivo model of inflammation. J Clin Invest. 1993 Dec 1;92 6:2675–82. doi:10.1172/JCI116884

[7] Maksimovic V, Pavlović-Popović Z, Vukmirović S, Cvejić J, Mooranian A, Al-Salami H, at al. Molecular mechanism of action and pharmacokinetic properties of methotrexate. Mol Biol Rep. 2020 May 15;47:4699–708.

[8] Kandemir FM, Kucukler S, Caglayan C, Gur C, Batil AA, Gülçin İ. Therapeutic effects of silymarin and naringin on methotrexate-induced nephrotoxicity in rats: Biochemical evaluation of anti-inflammatory, antiapoptotic, and antiautophagic properties. J Food Biochem. 2017;41(5):e12398.

[9] Li Z, Chen X, Liu G, Li J, Zhang J, Cao Y, at al. Antioxidant activity and mechanism of resveratrol and polydatin isolated from mulberry (Morus alba L.). Molecules. 2021;26(24):1–18.

[10] Deminice R, Da Silva RP, Lamarre SG, Kelly KB, Jacobs RL, Brosnan ME, at al. Betaine supplementation prevents fatty liver induced by a high-fat diet: Effects on one-carbon metabolism. Amino Acids. 2015 Jan 11;47(4):839–46.

[11] Khan ZA, Tripathi R, Mishra B. Methotrexate: A detailed review on drug delivery and clinical aspects. Expert Opin Drug Deliv. 2012 Feb;9(2):151–69. doi:10.1517/17425247.2012.642362 PubMed PMID: 22251428.

[12] Cheng K, Niu J, Zheng X, Qiao Y, Zhang J, Guo R, Dong G, Song Z, Huang J, Wang J. Aflatoxin-B1-exposure-induced hepatic injury could be alleviated by polydatin through reducing oxidative stress, inhibiting inflammation and improving mitophagy. Toxics. 2023;11(4):309.

[13] Stochino Loi E, Pontis A, Cofelice V, Pirarba S, Fais MF, Daniilidis A, Melis I, Paoletti AM, Angioni S. Effect of ultramicronized-palmitoylethanolamide and co-micronized palmitoylethanolamide/polydatin on chronic pelvic pain and quality of life in endometriosis patients: an open-label pilot study. Int J Womens Health. 2019;443–9.

[14] Tang D, Zhang Q, Duan H, Ye X, Liu J, Peng W, Wu C. Polydatin: a critical promising natural agent for liver protection via antioxidative stress. Oxid Med Cell Longev. 2022;2022(1):9218738.

[15] Ye G, Zhao Y, Zhu J, Zhang Z, Wang Q, Jiang X, Wang Z. Synergistic Effect of Polydatin and Polygonatum sibiricum Polysaccharides in Combating Atherosclerosis via Suppressing TLR4-Mediated NF-κB Activation in ApoE-Deficient Mice. Evidence-Based Complement Altern Med. 2022;2022(1):3885153.

[16] Ahmad P, Alvi SS, Iqbal D, Khan MS. Insights into pharmacological mechanisms of polydatin in targeting risk factors-mediated atherosclerosis. Life Sci. 2020;254:117756.

[17] Placer ZA, Cushman LL, Johnson BC. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Anal Biochem. 1966;16(2):359–64.

[18] Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968;25:192–205.

[19] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol

reagent. [Internet]. 1951 [cited 2025 Apr 23]. Available from: https://www.cabidigitallibrary.org/doi/full/10.5555/19511404458

[20] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2- ΔΔCT method. methods. 2001;25(4):402–8.

[21] Segal R, Yaron M, Tartakovsky B. Methotrexate: mechanism of action in rheumatoid arthritis. Semin Arthritis Rheum. 1990 Dec 1;20 3:190–200. doi:10.1016/0049-0172(90)90060-S

[22] Koźmiński P, Halik P, Chesori R, Gniazdowska E. Overview of Dual-Acting Drug Methotrexate in Different Neurological Diseases, Autoimmune Pathologies and Cancers. Int J Mol Sci. 2020 May 1;21. doi:10.3390/ijms21103483

[23] El-Esawy FM, Ahmed IA, El-Fallah AA, Salem RM. Methotrexate mechanism of action in plaque psoriasis: something new in the old view. J Clin Aesthetic Dermatol. 2022;15(8):42.

[24] Zhao Z, Hua Z, Luo X, Li Y, Yu L, Li M, Lu C, Zhao T, Liu Y. Application and pharmacological mechanism of methotrexate in rheumatoid arthritis. Biomed Pharmacother Biomedecine Pharmacother. 2022 Jun 1;150:113074. doi:10.1016/j.biopha.2022.113074

[25] Aboubakr EM, Ibrahim ARN, Ali FEM, Mourad AAE, Ahmad AM, Hofni A. Fasudil Ameliorates Methotrexate-Induced Hepatotoxicity by Modulation of Redox-Sensitive Signals. Pharmaceuticals. 2022 Nov 19;15(11):1436. doi:10.3390/ph15111436

[26] Friedman B, Cronstein B. Methotrexate mechanism in treatment of rheumatoid arthritis. Joint Bone Spine. 2019 May;86(3):301–7. doi:10.1016/j.jbspin.2018.07.004

[27] Kalantari E, Zolbanin NM, Ghasemnejad-Berenji M. Protective effects of empagliflozin on methotrexate induced hepatotoxicity in rats. Biomed Pharmacother. 2024 Jan;170:115953. doi:10.1016/j.biopha.2023.115953

[28] Erişir M, Benzer F, Kandemir FM. Changes in the rate of lipid peroxidation in plasma and selected blood antioxidants before and during pregnancy in ewes. Acta Vet Brno. 2009;78(2):237–42.

[29] Kandemir O, Kucukler S, Comakli S, Gur C, Ileriturk M. Docetaxel-induced liver and kidney toxicity in rats can be alleviated by suppressing oxidative stress, endoplasmic reticulum stress, inflammation, apoptosis and autophagy signaling pathways after Silymarin treatment. Food Chem Toxicol. 2025;196:115202.

[30] Khafaga AF, El-Sayed YS. Spirulina ameliorates methotrexate hepatotoxicity via antioxidant, immune stimulation, and proinflammatory cytokines and apoptotic proteins modulation. Life Sci. 2018 Mar;196:9–17. doi:10.1016/j.lfs.2018.01.010

[31] Kalantari H, Asadmasjedi N, Abyaz MR, Mahdavinia M, Mohammadtaghvaei N. Protective effect of inulin on methotrexate- induced liver toxicity in mice. Biomed Pharmacother. 2019 Feb;110:943–50. doi:10.1016/j.biopha.2018.11.144

[32] El Masri AER, Tobler C, Willemijn B, Von Bueren AO, Ansari M, Samer CF. Case report: Hepatotoxicity and nephrotoxicity induced by methotrexate in a paediatric patient, what is the role of precision medicine in 2023? Front Pharmacol. 2023 May 2;14:1130548. doi:10.3389/fphar.2023.1130548

[33] Eriten B, Caglayan C, Gür C, Küçükler S, Diril H. Hepatoprotective effects of zingerone on sodium arsenite-induced hepatotoxicity in rats: Modulating the levels of caspase-3/Bax/Bcl-2, NLRP3/NF-κB/TNF-α and ATF6/IRE1/PERK/GRP78 signaling pathways. Biochem Biophys Res Commun. 2024 Sep 17;725:150258. doi:10.1016/j.bbrc.2024.150258

[34] Erzincan R, Caglayan C, Kandemir FM, İzol E, Gür C, İleritürk M. Hepatoprotective Effects of Royal Jelly Against Vincristine-Induced Hepatotoxicity in Rats: A Biochemical and Molecular Study. Life. 2025 Mar;15(3):459. doi:10.3390/life15030459

[35] Kandemir O, Kucukler S, Comakli S, Gur C, İleriturk M. Docetaxel-induced liver and kidney toxicity in rats can be alleviated by suppressing oxidative stress, endoplasmic reticulum stress, inflammation, apoptosis and autophagy signaling pathways after Silymarin treatment. Food Chem Toxicol. 2025 Feb 1;196:115202. doi:10.1016/j.fct.2024.115202

[36] Caglayan C, Ekinci İ, Gur C, Ayna A, Bayav İ, Kandemir FM. Protective Effects of Chrysin Against Diclofenac-Induced Nephrotoxicity in Rats via Attenuation of Oxidative Stress, Apoptosis and Endoplasmic Reticulum Stress. J Biochem Mol Toxicol. 2025;39(6):e70373. doi:10.1002/jbt.70373

[37] Gezer A, Üstündağ H, Karadağ Sarı E, Bedir G, Gür C, Mendil AS, Duysak L. β-carotene protects against α-amanitin nephrotoxicity via modulation of oxidative, autophagic, nitric oxide signaling, and polyol pathways in rat kidneys. Food Chem Toxicol. 2024 Nov 1;193:115040. doi:10.1016/j.fct.2024.115040

[38] Cakmak F, Kucukler S, Gur C, Ileriturk M, Gul M, Varisli B. Cardiotoxicity caused by acrylamide in rats can be alleviated as a result of suppression of oxidative stress, endoplasmic reticulum stress, inflammation, and apoptosis by morin treatment. Iran J Basic Med Sci. 2025;28(3):376–84. doi:10.22038/ijbms.2024.81490.17634 PubMed PMID: 39906618; PubMed Central PMCID: PMC11790200.

[39] Verhoeven F, Prati C, Chouk M, Demougeot C, Wendling D. Methotrexate and cardiovascular risk in rheumatic diseases: A comprehensive review. Expert Rev Clin Pharmacol. 2021;14(9):1105–12.

[40] Tavazzi B, Lazzarino G, Di Pierro D, Giardina B. Malondialdehyde production and ascorbate decrease are associated to the reperfusion of the isolated postischemic rat heart. Free Radic Biol Med. 1992;13(1):75–8.

[41] Welbat JU, Naewla S, Pannangrong W, Sirichoat A, Aranarochana A, Wigmore P. Neuroprotective effects of hesperidin against methotrexate-induced changes in neurogenesis and oxidative stress in the adult rat. Biochem Pharmacol. 2020 Aug;178:114083. doi:10.1016/j.bcp.2020.114083

[42] Felemban SG, Aldubayan MA, Alhowail AH, Almami IS. Vitamin B17 Ameliorates Methotrexate-Induced Reproductive Toxicity, Oxidative Stress, and Testicular Injury in Male Rats. Mendoza-Núñez VM, editor. Oxid Med Cell Longev. 2020 Oct 27;2020:1–11. doi:10.1155/2020/4372719

[43] Abdulwahhab RQ, Ali Alabdali SM. Study of the protective effects of cyanocobalamin on methotrexate induced nephrotoxicity in rats. F1000Research. 2022 Oct 31;11:1012. doi:10.12688/f1000research.124081.2

[44] Öz MA, Şimşek H, Özdemir S, Küçükler S, Kandemir Ö, Mutlu H, Yazıcı R, Kandemir FM. Nephroprotective Effects of Vanillic Acid on Bortezomib-Induced Renal Injury: Role of Oxidative Stress, Mitochondrial Dysfunction, Apoptosis and miR-204/Nephrin/Podocin/CD2AP Axis. Basic Clin Pharmacol Toxicol. 2026 Apr;138(4):e70223. doi:10.1111/bcpt.70223

[45] Çelik H, Kucukler S, Çomaklı S, Caglayan C, Özdemir S, Yardım A, Karaman M, Kandemir FM. Neuroprotective effect of chrysin on isoniazid-induced neurotoxicity via suppression of oxidative stress, inflammation and apoptosis in rats. Neurotoxicology. 2020;81:197–208.

[46] Aygörmez S, Küçükler S, Çomaklı S, Şimşek H, Kandemir Ö, Kandemir FM, Maraşlı Ş. Protective Role of Polydatin Against Vancomycin-Induced Lung Toxicity via Oxidative Stress, Inflammation, Endoplasmic Reticulum Stress, Apoptosis, and Ferroptosis Pathways. J Appl Toxicol. 2026 Apr 10;jat.70203. doi:10.1002/jat.70203

[47] Akay M, Küçükler S, Özdemir S, Çomaklı S, Kandemir Ö, Şimşek H, Mutlu H, Kandemir FM. Rutin attenuates colistin-induced nephrotoxicity through modulation of inflammation, oxidative stress, and miRNA-Associated pathways: An experimental study. Food Biosci. 2026 Apr;78:108527. doi:10.1016/j.fbio.2026.108527

[48] Wang SC, Huang YC, Hsiao CC, Sheen JM, Huang LT, Lo WS, Hsieh HY, Chen YC. Melatonin protects against methotrexate hepatotoxicity in young rats: Impact of PI3K/Akt/mTOR signaling. J Biochem Mol Toxicol. 2023;37(5):e23323.

[49] Alqarni AM, Zeidler MP. How does methotrexate work? Biochem Soc Trans. 2020;48(2):559–67.

[50] Öz MA, Şimşek H, Özdemir S, Kandemir Ö, Küçükler S, Mutlu H, Yazıcı R, Kandemir FM. Sinapic acid attenuates colistin-driven nephrotoxicity by targeting the miRNA-21/SIRT1/NF-κB pathway and facilitating tubular repair and inflammation in a rat model. Toxicol Appl Pharmacol. 2026 Feb;507:117708. doi:10.1016/j.taap.2026.117708

[51] Şener G, Ekşioğlu-Demiralp E, Çetiner M, Ercan F, Şirvanci S, Gedik N, Yeğen BÇ. L-Carnitine ameliorates methotrexate-induced oxidative organ injury and inhibits leukocyte death. Cell Biol Toxicol. 2006 Jan;22(1):47–60. doi:10.1007/S10565-006-0025-0 PubMed PMID: 16463019.

[52] Çakir T, Özkan E, Dulundu E, Topaloğlu Ü, Şehirli AÖ, Ercan F, Şener E, Şener G. Caffeic acid phenethyl ester (CAPE) prevents methotrexate-induced hepatorenal oxidative injury in rats. J Pharm Pharmacol. 2011 Dec;63(12):1566–71. doi:10.1111/J.2042-7158.2011.01359.X PubMed PMID: 22060287.

[53] Yılmaz S, Küçükler S, Şimşek H, Aygörmez S, Kandemir FM. Naringin protects against colistin-induced sciatic nerve damage by reducing oxidative stress, apoptosis and inflammation damage. Deney Ve Klin Tıp Derg. 2024;41(1):53–9.

[54] Gür C, Kandemir Ö, Kandemir FM. Evaluation of the effects of chrysin on diclofenac-induced cardiotoxicity in rats by the markers of oxidative stress, endoplasmic reticulum stress and apoptosis. kvj. 2022 Jun;15(2):151–60. doi:10.30607/kvj.1062346

[55] Ali YA, Ahmed AA, Abd El-Raouf OM, Elkhoely A, Gad AM. Polydatin combats methotrexate-induced pulmonary fibrosis in rats: Involvement of biochemical and histopathological assessment. J Biochem Mol Toxicol. 2022;36(5):e23019.

[56] Akcılar R, Akcılar A, Koçak C, Koçak FE, Bayat Z, Şimşek H, Şahin S, Savran B. Effects of Ukrain on intestinal apoptosis caused by ischemia-reperfusion injury in rats. Int J Clin Exp Med. 2015;8(12):22158.

[57] Öz MA, Kandemir Ö, Özdemir S, Şimşek H, Küçükler S, Yazıcı R, Mutlu H, Kandemir FM. Chrysin attenuates tramadol-driven renal dysfunction via regulation of RNA networks, antioxidant pathways, and ketogenic metabolism. Eur J Pharmacol. 2026 May;1023:178882. doi:10.1016/j.ejphar.2026.178882

[58] Al-khawalde AA marddyah A, Abukhalil MH, Jghef MM, Alfwuaires MA, Alaryani FS, Aladaileh SH, Algefare AI, Karimulla S, Alasmari F, Aldal'in HK, Alanezi AA, Althunibat OY. Punicalagin Protects against the Development of Methotrexate-Induced Hepatotoxicity in Mice via Activating Nrf2 Signaling and Decreasing Oxidative Stress, Inflammation, and Cell Death. Int J Mol Sci. 2022 Oct 15;23(20):12334. doi:10.3390/ijms232012334

[59] Aydin M, Cevik A, Kandemir F, Yuksel M, Apaydin A. Evaluation of hormonal change, biochemical parameters, and histopathological status of uterus in rats exposed to 50-Hz electromagnetic field. Toxicol Ind Health. 2009 Apr;25(3):153–8. doi:10.1177/0748233709102717

[60] Zhao XJ, Yu HW, Yang YZ, Wu WY, Chen TY, Jia KK, Kang LL, Jiao RQ, Kong LD. Polydatin prevents fructose-induced liver inflammation and

lipid deposition through increasing miR-200a to regulate Keap1/Nrf2 pathway. Redox Biol. 2018 Sep 1;18:124–37. doi:10.1016/J.REDOX.2018.07.002 PubMed PMID: 30014902.

[61] Wang HL, Gao JP, Han YL, Xu X, Wu R, Gao Y, Cui XH. Comparative studies of polydatin and resveratrol on mutual transformation and antioxidative effect in vivo. Phytomedicine. 2015 May 15;22(5):553–9. doi:10.1016/J.PHYMED.2015.03.014 PubMed PMID: 25981921.

[62] Uraz S, Tahan V, Aygun C, Eren F, Unluguzel G, Yuksel M, Senturk O, Avsar E, Haklar G, Celikel C, Hulagu S, Tozun N. Role of ursodeoxycholic acid in prevention of methotrexate-induced liver toxicity. Dig Dis Sci. 2008 Apr 13;53(4):1071–7. doi:10.1007/S10620-007-9949-3/FIGURES/7 PubMed PMID: 17934844.

[63] Mazur AJ, Nowak D, Mannherz HG, Malicka-Błaszkiewicz M. Methotrexate induces apoptosis in CaSki and NRK cells and influences the organization of their actin cytoskeleton. Eur J Pharmacol. 2009 Jun 24;613(1–3):24–33. doi:10.1016/J.EJPHAR.2009.04.020 PubMed PMID: 19383496.

[64] Jahovic N, Çevik H, Şehirli AÖ, Yeğen BÇ, Şener G. Melatonin prevents methotrexate-induced hepatorenal oxidative injury in rats. J Pineal Res. 2003 May 1;34(4):282–7. doi:10.1034/J.1600-079X.2003.00043.X PubMed PMID: 12662351.

[65] Erfan OS, Hany |, Sonpol MA, Marwa |, El-Kader A, Hany C. Protective effect of rapamycin against acrylamide-induced hepatotoxicity: The associations between autophagy, apoptosis, and necroptosis. Anat Rec. 2021 Sep 1;304(9):1984–98. doi:10.1002/AR.24587 PubMed PMID: 33480149.

[66] Gencer S, Akaras N, Şimşek H, Gür C, İleritürk M, Küçükler S, Kandemir FM. The Protective Effects of Chrysin on Acrylamide-Induced Hepatotoxicity: Insights Into Oxidative Stress, Inflammation, Apoptosis, Autophagy, and Histological Evaluation in Rats. J Biochem Mol Toxicol. 2025;39(6):e70334. doi:10.1002/jbt.70334

[67] Hemeida RAM, Mohafez OM. Curcumin attenuates methotraxate-induced hepatic oxidative damage in rats. J Egypt Natl Cancer Inst. 2008 Jun 1;20(2):141–8. PubMed PMID: 20029470.

[68] Araya LE, Soni IV, Hardy JA, Julien O. Deorphanizing Caspase-3 and Caspase-9 Substrates In and Out of Apoptosis with Deep Substrate Profiling. ACS Chem Biol. 2021 Nov 19;16(11):2280–96. doi:10.1021/acschembio.1c00456

[69] Anson F, Thayumanavan S, Hardy JA. Exogenous Introduction of Initiator and Executioner Caspases Results in Different Apoptotic Outcomes. JACS Au. 2021 Aug 23;1(8):1240–56. doi:10.1021/jacsau.1c00261

[70] Georgiou KR, King TJ, Scherer MA, Zhou H, Foster BK, Xian CJ. Attenuated Wnt/β-catenin signalling mediates methotrexate chemotherapy-induced bone loss and marrow adiposity in rats. Bone. 2012 Jun;50(6):1223–33. doi:10.1016/j.bone.2012.03.027

[71] Fenderico N, Van Scherpenzeel R, Goldflam M, Proverbio D, Jordens I, Kralj T, Stryeck S, Bass T, Hermans G, Ullman C, Aastrup T, Gros P, Maurice M. Anti-LRP5/6 VHHs promote differentiation of Wnt-hypersensitive intestinal stem cells. Nat Commun. 2019. doi:10.2210/PDB6H15/PDB

[72] González-Sancho J, Aguilera O, García J, Pendás-Franco N, Peña C, Cal S, Herreros A, Bonilla F, Muñoz A. The Wnt antagonist DICKKOPF-1 gene is a downstream target of β-catenin/TCF and is downregulated in human colon cancer. Oncogene. 2005 Feb 3;24:1098–103. doi:10.1038/sj.onc.1208303

[73] Qin K, Yu M, Fan J, Wang H, Zhao P, Zhao G, Zeng W, Chen C, Wang Y, Wang A, Schwartz Z, Hong J, Song L, Wagstaff W, Haydon RC, Luu HH, Ho SH, Strelzow J, Reid RR, He TC, Shi LL. Canonical and noncanonical Wnt signaling: Multilayered mediators, signaling mechanisms and major signaling crosstalk. Genes Dis. 2024 Jan;11(1):103–34. doi:10.1016/j.gendis.2023.01.030

[74] Duspara K, Bojanic K, Pejic JI, Kuna L, Kolaric TO, Nincevic V, Smolic R, Vcev A, Glasnovic M, Curcic IB, Smolic M. Targeting the Wnt Signaling Pathway in Liver Fibrosis for Drug Options: An Update. J Clin Transl Hepatol. 2021 Sep 13;000(000):000–000. doi:10.14218/JCTH.2021.00065

[75] Rutt L, Orlicky D, McCullough R. Investigating the role of Wnt3a and Wnt5a as critical factors of hepatic stellate cell activation in acute toxicant-induced liver injury. Cell Biol Toxicol. 2024 Dec 21;41. doi:10.1007/s10565-024-09956-4