Can Alpha-Pinene Prevent Methotrexate-Induced Cardiac and Hepatic Damage?

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Summary

The effects of alpha-pinene (AP), a monoterpenoid, known for its antioxidant, anti-inflammatory, and anti-apoptotic properties, on methotrexate (MTX)-induced cardiac and hepatic damage were investigated in this study. Male Sprague-Dawley rats were divided into Control, Vehicle, AP, MTX, and AP+MTX groups (n=7). AP (50 mg/kg/day, 14 days) was applied subcutaneously in the AP and AP+MTX groups. MTX (20 mg/kg) was injected three days before sacrification. Serum CK-MB, troponin T, ALT, and AST levels, as well as cardiac and hepatic MDA, GSH, caspase-3, and p53 levels, were measured by ELISA. Histological changes in tissues were evaluated by scoring in terms of tissue damage and cellular degeneration parameters after hematoxylin-eosin staining. MTX caused significant increase in serum CK-MB, troponin T, ALT, and AST levels, hepatic and cardiac lipid peroxidation, GSH depletion, and caspase-3 level. However, tissue levels of p53 did not change significantly. MTX-induced histological deterioration was observed in both tissues. These MTX-induced changes were significantly reduced in the AP+MTX group. Present results show that MTX-induced cardiac and hepatic damage is prevented by AP pretreatment. This protection can be attributed to the antioxidant and anti-apoptotic properties of AP. Considering the importance of MTX in cancer treatment, AP appears to have highly promising potential as a cardioprotective and hepatoprotective agent in anti-tumoral therapy.

Key words

MDA • GSH • Caspase-3 • p53 • Oxidative stress • Apoptosis

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Introduction

Methotrexate (MTX) is a chemotherapeutic agent utilized in the treatment of various cancer types, including breast cancer, leukemia, and lymphoma. Beyond its capacity to induce apoptosis, the impact of MTX on folate metabolism is pivotal in suppressing the proliferation of cancer cells. Through the inhibition of tetrahydrofolate synthesis, MTX disrupts DNA and RNA synthesis in the cell, thereby preventing proliferation, particularly in rapidly dividing cells such as hemato-poietic cells, gastrointestinal mucosal cells, along with cancer cells [1]. Approximately 40 years after its discovery in the 1940s, MTX started being used in the treatment of rheumatoid arthritis. Currently, it is also prescribed for the therapy of various autoimmune diseases, including psoriasis, multiple sclerosis, Crohn's disease, and myasthenia gravis [2]. Despite its widespread clinical use, MTX can cause damage to the liver, kidneys, heart, and neurons [3-7]. Different pathological mechanisms have been suggested to explain the MTX toxicity. For example, in MTX-induced hepatic damage, factors such as depletion of hepatic folate stores, increased oxidative and endoplasmic reticulum stresses, apoptotic cell death, accumulation of MTX in

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polyglutamate form, and excessive homocysteine accumulation have been proposed as potential contributors to tissue damage [8-9]. The disruption of hepatocyte membrane integrity caused by MTX results in significant increases in serum liver enzyme levels, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase [10, 11]. MTX treatment, which causes significant damage to cardiomyocytes, results in elevated serum levels of cardiac enzymes, including creatine kinase (CK) and its myocardial band isoenzyme (CK-MB), as well as cardiac proteins like troponin I and troponin T. In the MTX-induced cardiac damage, oxidative stress, mitochondrial damage, inflammatory response, and apoptosis can be considered as the main underlying mechanisms [3, 5]. Ongoing comprehensive research aims to enhance combination therapies that can mitigate the toxic effects of MTX without compromising its chemotherapeutic potential.

Alpha-pinene (AP), a monoterpene, is one of the key metabolites derived from essential oils obtained from coniferous trees [12]. Several *in-vitro* studies have demonstrated that AP influences various biological processes. For instance, it has been reported to exhibit protective effects against oxidative stress, DNA damage, and apoptosis induced by UVA in human epidermal keratinocytes [13], oxidative stress, and cell death induced by aspirin in rat small intestinal epithelial cells [14]. Conversely, AP has been reported to induce apoptotic cell death in cancer cell lines, including murine melanoma and human ovarian cancer cells [15, 16]. Moreover, various studies have highlighted the anti-inflammatory, antimicrobial, and antiparasitic properties of AP [17, 18]. In a limited number of *in-vivo* experiments, AP has demonstrated neuroprotective effects and the potential to alleviate symptoms of neurodegenerative diseases [19-22]. Additionally, protective effects of AP have been reported in cases of gastric mucosal damage [23], testicular damage [24], and cardiac damage [25]. In all these studies utilizing diverse experimental models, the neuroprotective, gastroprotective, testiculoprotective, and cardioprotective effects of AP have been attributed to its antioxidant, anti-apoptotic, and anti-inflammatory properties.

The toxic side effects of MTX are well known to be primarily mediated by oxidative stress, inflammation, and apoptotic cell death. Considering the biological properties of AP, it suggests a strong potential to prevent or alleviate the side effects of MTX. Therefore, this study aimed to investigate the potential protective effects of AP against MTX-induced cardiac and hepatic damage in rats.

Methods

Animals and grouping

Male Sprague Dawley rats aged 2.5-3 months were used, and all procedures were approved by the Local Ethics Committee of Animal Experiments (Approval number: 2023-01/10). The animals were randomly assigned to one of the 5 groups: Control, Vehicle, AP, MTX, and AP+MTX (n=7).

In the Control group, no pretreatment was given to the rats.

In the Vehicle group, animals received subcutaneous (sc) injections of DMSO (0.1 %, the solvent for AP) for 14 consecutive days [25].

In the AP group, animals were given AP (Sigma-Aldrich; 147524) at a dose of 50 mg/kg/day (sc) for 14 days. The dose and duration of AP pretreatment were chosen based on the literature findings [20].

Rats in the MTX group received vehicle (DMSO, 0.1 %, sc) for 14 days. On the 12th day of the experiment, a single dose of MTX (20 mg/kg, sc) injection was performed [26].

In the AP+MTX group, animals received AP injections (50 mg/kg/day, sc) for 14 days, and on the 12th day of the experiment a single dose of MTX injection (20 mg/kg, sc) was performed.

On the 15th day of the study, all rats were anesthetized using ketamine:xylazine (80 mg/kg: 10 mg/kg, sc). After the midline incision, the aorta was exposed, and blood samples were quickly collected from the abdominal aorta. Heart and liver tissue specimens were obtained. After allowing blood samples to stand at room temperature for 30-40 minutes, they were centrifuged at 4000 g for 10 minutes. Serum samples were stored as aliquots at -80°C. One part of the tissue specimens (from the lobus sinister of the liver and the apex of the heart) was placed in 10 % neutral formalin for histological examination, while remaining part was stored at -80°C without any processing for ELISA experiments. The parameters described below were investigated in tissue and serum samples.

Biochemical parameters

Serum levels of cardiac troponin T, CK-MB, ALT, and AST were measured using appropriate commercial ELISA kits (Elabscience E-EL-R0151, Bioassay Technology Laboratory BT-E0311Ra,

BT-E0155Ra, and BT-E0595Ra, respectively).

Evaluation of oxidative stress in tissue

To evaluate oxidative damage in cardiac and hepatic tissues, malondialdehyde (MDA) and reduced glutathione (GSH) levels were measured using suitable commercial ELISA kits (Bioassay Technology Laboratory BT-E0156Ra and BT-EA0113Ra, respectively). Tissue samples were homogenized according to the kit protocol. Measurements were made on the obtained supernatants after centrifugation at 5000 g for 5 minutes. Tissue levels of MDA and GSH were presented relative to total protein level, which was spectrophotometrically determined by Bradford method using the Coomassie reagent (Thermo Scientific; 23200).

Evaluation of apoptosis in tissue

To assess apoptotic damage, the levels of caspase-3 and p53 in heart and liver tissues were measured using appropriate commercial ELISA kits (Bioassay Technology Laboratory, BT-E1648Ra, and BT-E0071Ra, respectively). Measurements were performed in supernatants obtained from tissue homogenates according to the kit protocol. The cardiac and hepatic levels of caspase-3 and p53 were presented relative to the total protein levels in each tissue.

Histological evaluation

Specimens of heart and liver were harvested and then fixed in 10 % neutral formalin. After routine histological processing steps, the tissues were embedded in paraffin according to the region to be examined (lobus sinister in the liver, ventricles in the heart), and then 5 µm thick sections were obtained using a microtome. To evaluate the general histological structure of the tissues, the sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope. Additionally, they were scored for tissue damage and cellular degeneration on a scale from 0 to 3 (0: none, 1: mild, 2: moderate, 3: severe) and photographed.

Statistical analysis

Results were presented as mean ± standard deviation. The normal distribution of the data was tested using the Kolmogorov-Smirnov test. One-way analysis of variance (ANOVA) followed by Tukey post hoc test was used to evaluate statistical differences between groups. Values with $p<0.05$ were considered statistically significant. GraphPad 4.0 software package was used for statistical evaluations.

Results

Effects of MTX and AP treatments on serum biomarkers

The impact of AP pretreatment on MTX-induced cardiac and hepatic damage was assessed with conventional serum biomarkers. As depicted in Table 1, serum CK-MB, troponin T, ALT, and AST values were comparable in the Control, Vehicle, and AP groups. A single dose of MTX injection resulted in a significant elevation in the serum levels of CK-MB, troponin T, ALT, and AST. However, in the animals treated with AP, MTX injection did not alter these serum biomarkers. In comparison to the MTX group, the values were found to be significantly lower in the AP+MTX group (Table 1).

Cardiac effects of MTX and AP treatments

In the Control group, the MDA level in cardiac tissue was found to be 92.73±22.84 µmol/mg protein. Neither vehicle nor AP treatment caused a significant change in this value. However, in the MTX group, a significant increase in cardiac MDA level to 154.64±25.18 µmol/mg protein was observed (difference from the Control group $p<0.01$; difference from the Vehicle and AP groups $p<0.05$). In the AP+MTX group, lipid peroxidation was at a similar level to the Control group (104.64±25.98 µmol/mg protein) and significantly lower than that in the MTX group ($p<0.05$, Fig. 1A). Cardiac GSH concentration was recorded at similar levels in the Control, Vehicle, and AP groups (64.27±10.69, 62.19±8.36, and 69.65±11.49 mg/mg protein, respectively). MTX injection caused a significant decrease in GSH levels in the MTX group (43.47± 12.68 mg/mg protein, difference from the Control group $p<0.01$; difference from the Vehicle group $p<0.05$; difference from the AP group $p<0.001$). However, the values increased to the control level in the AP pretreated AP+MTX group (68.76±11.07 mg/mg protein, difference from the MTX group, $p<0.001$) (Fig. 1B). Caspase-3 levels in control cardiac tissue homogenates were determined as 77.02±7.91 µg/mg protein. Vehicle or AP treatments did not cause a significant change, however, MTX injection induced a significant increase in caspase-3 levels to 94.57±12.01 µg/mg protein (difference from the Control group, Vehicle and AP groups $p<0.05$). In the AP+MTX group, the values were significantly lower than those of the MTX group ($p<0.05$) and were found to be close to the control level (79.49±8.45 µg/mg protein) (Fig. 1C). In the Control group, the concentration of p53 in cardiac tissue was found to be 8.84±1.82 pg/mg protein, and this value remained unaffected by vehicle

Table 1. The Effects of MTX and AP treatments on serum biomarkers

	CK-MB (ng/ml)	Troponin T (pg/ml)	ALT (U/L)	AST (U/L)
Control	6.94±0.78	372.14±60.61	30.71±2.88	71.79±2.85
Vehicle	7.18±0.21	365.71±41.48	29.73±3.46	71.61±5.96
AP	8.15±2.05	368.57±35.08	32.61±2.73	72.19±4.99
MTX	11.30±1.05 **##⊥⊥⊥ϕϕ	431.25±39.26 **##⊥⊥⊥ϕϕ	39.76±4.79 **##⊥⊥ϕ	80.63±4.12 *#⊥ϕϕ
AP+MTX	7.79±1.36	368.13±69.84	33.34±3.49	67.34±7.18

The values were presented as mean ± standard deviation. Difference from the Control group *p<0.05, **p<0.001; difference from the Vehicle group #p< 0.05, ##p< 0.001; difference from the AP group ⊥p< 0.05, ⊥⊥p< 0.01, ⊥⊥⊥p< 0.001; difference from the AP+MTX group ϕp<0.05, ϕϕp<0.001.

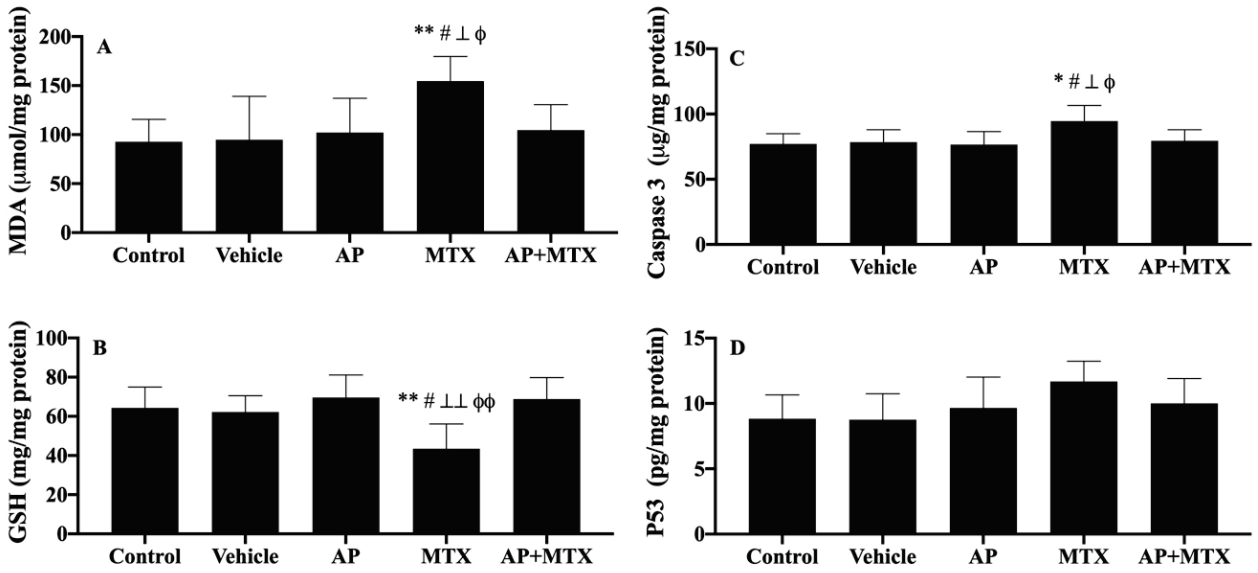

Fig. 1. The Effects of MTX and AP treatments on cardiac oxidative stress and apoptosis. Levels of MDA (**A**), GSH (**B**), Caspase-3 (**C**), and p53 (**D**) in cardiac tissue. Results are presented as mean ± standard deviation. Difference from the Control group *p<0.05, **p<0.01; difference from the Vehicle group #p<0.05; difference from the AP group ⊥p<0.05, ⊥⊥p<0.001; difference from the AP+MTX group ϕp<0.05, ϕϕp<0.001.

or AP treatments alone. The MTX group exhibited a slight increase in cardiac p53 levels (11.68±1.56 pg/mg protein), however, this increase was not statistically significant. Lastly, in the AP+MTX group, the tissue level of p53 was comparable to that of the other groups (10.01±1.89 pg/mg protein, Fig. 1D).

In the cardiac ventricles of the Control, Vehicle, and AP groups, normal histological myocardial structure was observed with a regular distribution of cells with centrally located nuclei and well-organized muscle bundles (Fig. 2). In the MTX group, histological alterations such as the absence of nuclei in some myocytes, myocyte vacuolization, the presence of hemorrhagic areas, and occasional lymphocytic infiltration were noted. In the AP+MTX group, a reduction in myocytic damage, absence of hemorrhagic areas and lymphocytic infiltration, and an overall return to normal histological appearance of cardiac tissue were observed (Fig. 2).

Upon reviewing the mean scores derived from the histological examination, similar results were observed in the Control (0.086±0.11), Vehicle (0.114±0.11), and AP (0.116±0.12) groups, while a significant increase was found in the MTX group (2.171±0.08, difference from the Control, Vehicle, and AP groups, p<0.001). The histological score recorded in the AP+MTX group was comparable to the Control group (0.229±0.18) and significantly lower than that of the MTX group (p<0.001).

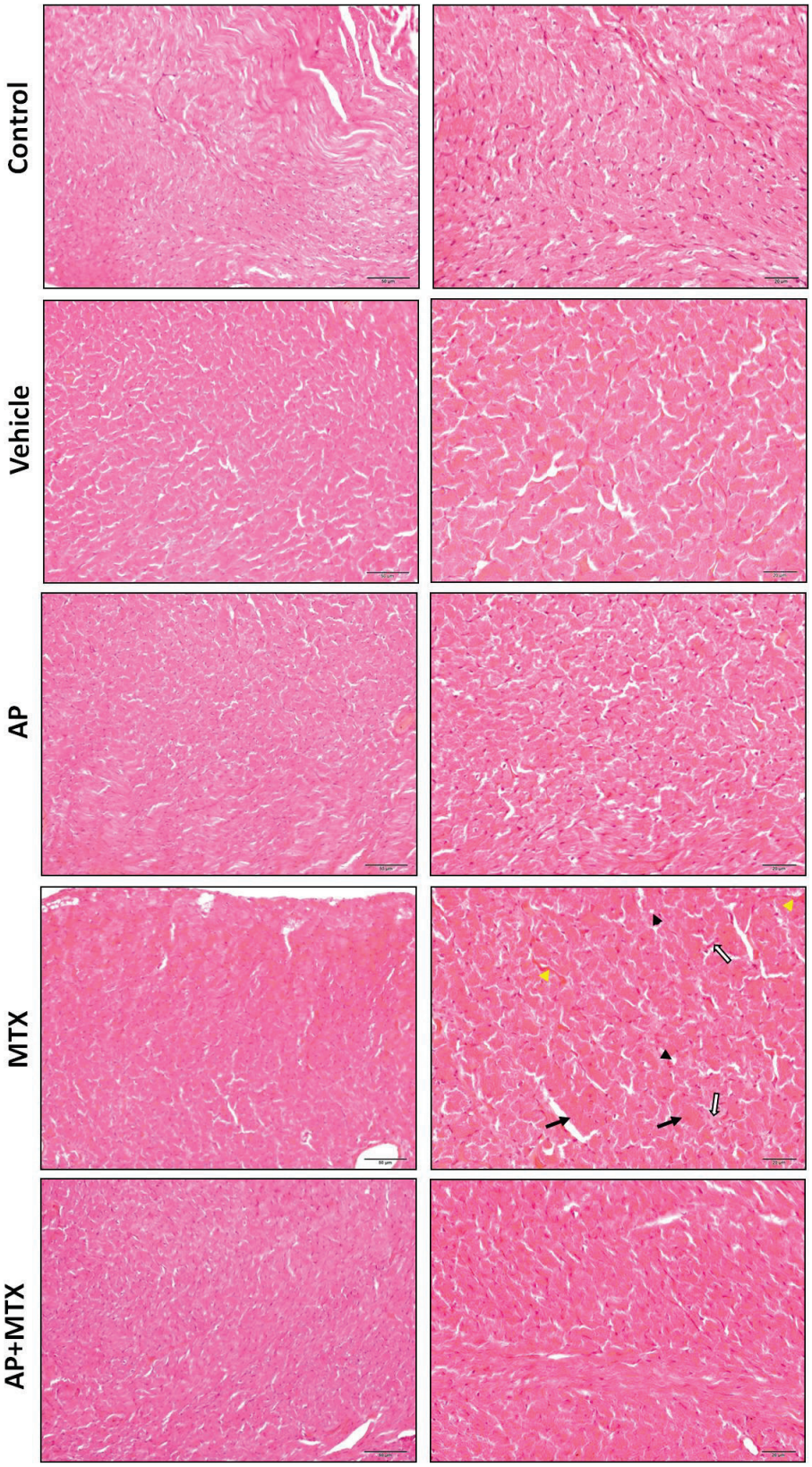

Fig. 2. H&E-stained cardiac tissues from the Control, Vehicle, AP, MTX, and AP+MTX groups. 1st column: 20x magnification, 2nd column: 40x magnification. Black arrows indicate myocytes lacking nuclei, black arrowheads indicate myocyte vacuolization, yellow arrowheads indicate hemorrhagic areas and white arrows indicate lymphocytic infiltration.

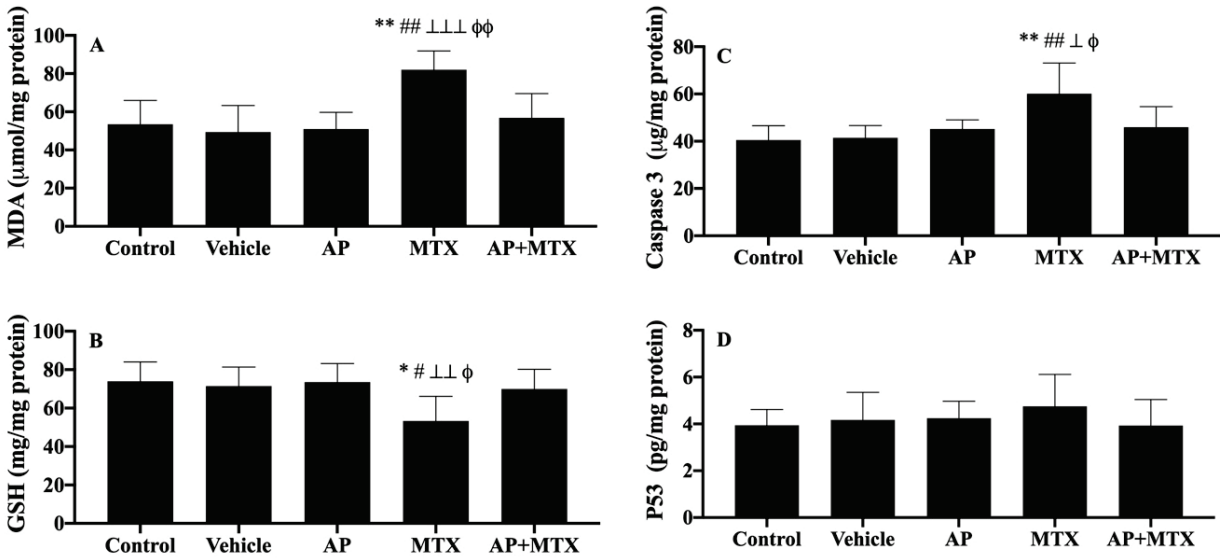

Fig. 3. The Effects of MTX and AP treatments on hepatic oxidative stress and apoptosis. Levels of MDA (**A**), GSH (**B**), Caspase-3 (**C**), and p53 (**D**) in liver tissue. Results are presented as mean ± standard deviation. Difference from the Control group *p<0.01, **p<0.001; Difference from the Vehicle group #p<0.05, ##p<0.01; Difference from the AP group ⊥p<0.05, ⊥⊥p<0.01, ⊥⊥⊥p<0.001; Difference from the AP+MTX group ϕp<0.05, ϕϕp<0.01.

Hepatic effects of MTX and AP treatments

Hepatic lipid peroxidation was determined as 53.49±12.50 μmol/mg protein in the Control group, and it did not significantly change in the Vehicle and AP groups. MTX injection resulted in a significant elevation of hepatic MDA levels to 82.06±9.80 μmol/mg protein (difference from the Control and AP groups p<0.001, difference from the Vehicle group, p<0.01). In the AP+MTX group, this value was significantly lower than that of the MTX group (p<0.01) but did not significantly differ from the Control group (Fig. 3A).

Hepatic GSH level was determined to be 73.94±10.08 mg/mg protein in the Control group. Treatment with vehicle or AP did not cause a significant change in tissue GSH levels. However, MTX injection led to a significant decrease in hepatic GSH levels (53.29±12.81 mg/mg protein, the difference from the Control and AP groups, p<0.01; difference from the Vehicle group, p<0.05). In the AP+MTX group, the GSH level was similar to the control values and significantly higher than that of the MTX group (69.93±10.26 mg/mg protein, p<0.05) (Fig. 3B).

The level of caspase-3 in the liver tissue was determined as 40.51±6.05 μg/mg protein in the Control group. No significant difference was observed compared to the Control group in the Vehicle and AP groups. In the MTX group, caspase-3 level increased to 60.11± 12.98 μg/mg protein (difference from the Control group, p<0.001; difference from the Vehicle group, p<0.01; difference from the AP group, p<0.05). In the AP+MTX group, the values returned to the control level (45.93±8.74 μg/mg protein) and were found to be significantly lower than those of the MTX group (p<0.05, Fig. 3C).

The level of p53 in liver tissue in the Control group was 3.95±0.67 pg/mg protein, unaffected by vehicle or AP treatments. The mild increase in hepatic p53 level in the MTX group was not statistically significant compared to the other groups (4.75±1.36 pg/mg protein). Similarly, the value did not significantly change in the AP+MTX group (Fig. 3D).

The liver lobus sinister regions of the Control, Vehicle, and AP groups exhibited histologically normal hepatic structure, featuring hepatocytes arranged in radially organized cords separated by sinusoids, with well-defined cytoplasm, one or more distinct nuclei, and nucleoli. In the MTX group, there were notable disruptions in the histological structure, with areas of hydropic degeneration and fatty change. In the AP+MTX group, a significant improvement in the overall histological structure of the liver was noted, with no signs of hydropic degeneration or fatty changes (Fig. 4).

The average histological scores obtained in the liver were similar in the Control (0.114±0.11), Vehicle (0.143±0.15), and AP (0.171±0.14) groups. In the MTX group, the histological score was significantly elevated to 2.029±0.34 (difference from the Control, Vehicle, and AP groups, p<0.001). However, in the AP+MTX group, it was close to the values of the Control group (0.200±0.16) and significantly lower than the MTX group (p<0.001).

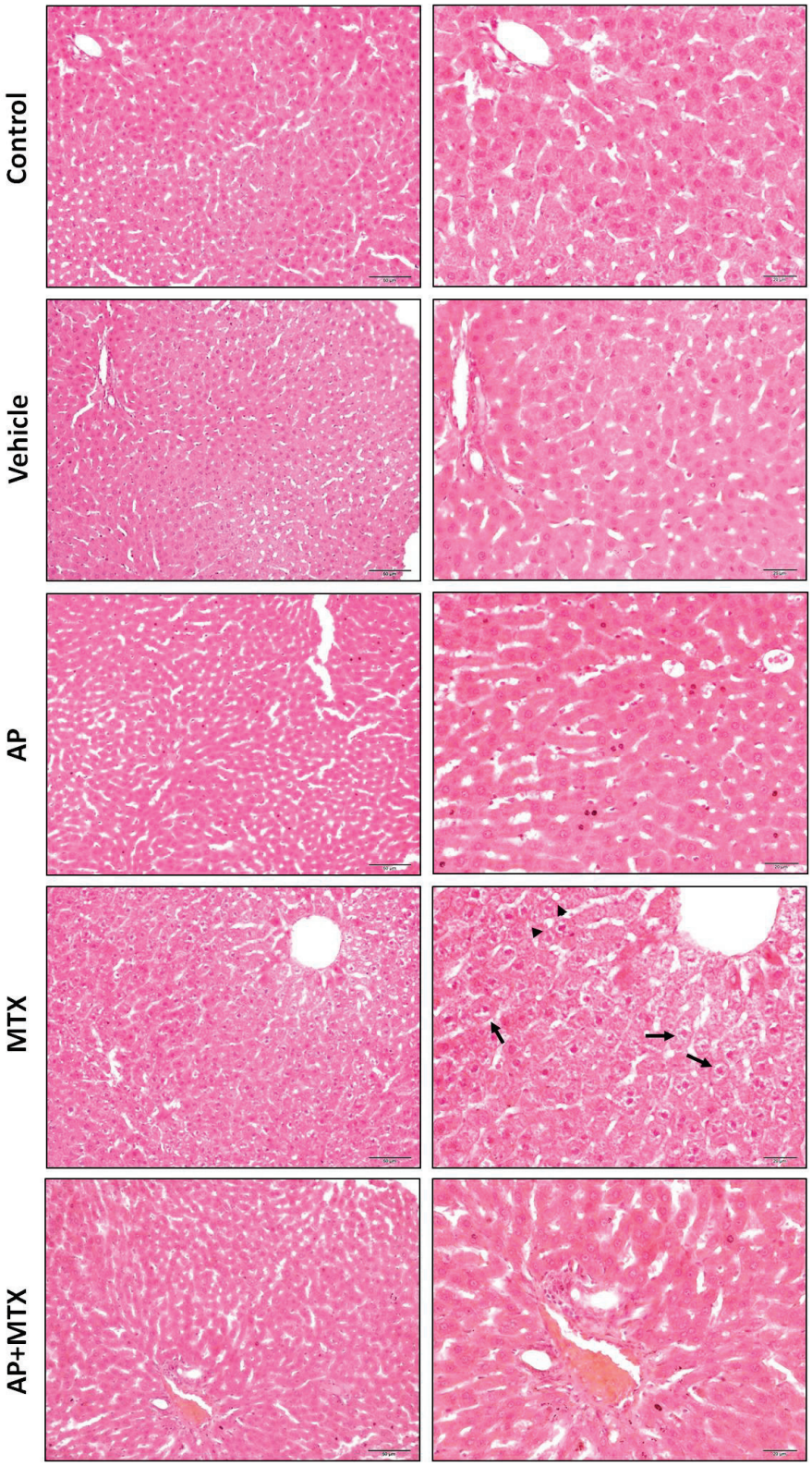

Fig. 4. H&E-stained liver tissues of Control and experimental groups. 1st column: 20x magnification, 2nd column: 40x magnification. Arrows indicate hepatocytes undergoing hydropic degeneration, and arrowheads indicate fatty changes.

Discussion

In this study, the effects of AP on MTX-induced cardiac and hepatic damage were investigated and our results notably revealed that the pretreatment with AP successfully prevented tissue damage through antioxidant and anti-apoptotic effects.

The cytotoxic impact of MTX extends beyond

cancer cells, affecting healthy cells and causing damage to various organs, notably to the heart and liver [3-7]. Numerous experimental studies have demonstrated that the toxic effects of MTX involve different mechanisms such as increased oxidative stress, inflammatory response, and apoptotic cell death. For instance, El-Sheikh and colleagues showed that 3 days after a single dose of MTX administration (20 mg/kg) in rats, serum levels of ALT, AST, creatinine, and blood urea nitrogen were found to be increased [26]. The researchers have also reported that hepatic and renal levels of GSH and activity of catalase (CAT) were lower, while MDA, nitric oxide, TNF-alpha, NF-κB, iNOS, and caspase-3 levels were higher than those of the control group. In a different experimental study, Al-Abkal and colleagues demonstrated an elevation in serum ALT, AST, urea, creatinine, CK, CK-MB, and troponin levels 3 days after a single dose of MTX injection (10 mg/kg) [3]. The researchers observed an increase in inflammatory response in liver, kidney, and heart tissues through elevated NF-κB and TNF-alpha levels. Additionally, there was an increase in oxidative stress, as indicated by reduced levels of superoxide dismutase (SOD), CAT, GSH, and ATP, along with elevated levels of MDA. Moreover, apoptotic cell death was noted through increased caspase-3 and p53 levels.

In line with previous reports, we observed an increase in serum CK-MB, troponin T, ALT, and AST levels 3 days after MTX administration (20 mg/kg). Histological evaluation revealed structural deterioration in both liver and heart tissues in the MTX group. These findings suggest that MTX causes hepatic and cardiac damage, consistent with previous studies. Further examinations of liver and heart tissues showed a decrease in GSH and an increase in lipid peroxidation in both tissues of rats injected with MTX. These results, in accordance with the literature findings, indicate the role of oxidative stress in the hepatic and cardiac damage caused by MTX [3, 4]. Since apoptosis is a significant mechanism in MTX-mediated tissue damage, caspase-3 and p53 levels were measured in the cardiac and hepatic tissues. Our findings demonstrate a significant increase in caspase-3 levels in both tissues. However, mild increase in the cardiac and hepatic levels of p53 in the MTX group did not reach statistical significance. Contrary to our results, previous studies have highlighted the role of p53 in MTX-mediated apoptosis [3]. It is known that p53-induced mitochondrial cytochrome-c release is crucial in the conversion of pro-caspase-3 to active caspase-3, however, it is not the sole mechanism, and the activation of the extrinsic pathway can also result in caspase-3 activation [27]. Therefore, under the current experimental conditions, in addition to the contribution of slightly increased p53, other mechanisms may also be involved in the elevation of cardiac and hepatic caspase-3 levels.

Since MTX-induced side effects limit its clinical usage, ongoing research aims to develop approaches that reduce or prevent them. Considerable amounts of previous studies presented that various natural compounds with antioxidant and anti-inflammatory properties could be used against MTX-induced drug toxicity [28]. AP, one of the important components of essential oils, possesses antioxidant, anti-inflammatory, anti-apoptotic, and anti-tumor properties [12, 18]. Among the limited number of *in-vivo* studies demonstrating the effects of AP, its cardioprotective properties were established in a damage model induced by isoproterenol [25]. In this model, researchers showed that the increments in CK, CK-MB, troponin T, and troponin I levels induced by isoproterenol were significantly improved in rats receiving AP pretreatment (50 mg/kg, 21 days). Furthermore, the isoproterenol-induced decrease in SOD, CAT, and GPx activities, as well as GSH depletion, along with the elevation in inflammatory markers such as TNF-alpha, IL-6, and NF-κB levels in cardiac tissue, were significantly prevented in the rats treated with AP. The researchers have concluded that the cardioprotective effects of AP may be attributed to its antioxidant and anti-inflammatory properties.

In this study, it is addressed whether the AP had similar protective effects in MTX-induced cardiac damage. Our results showed that the MTX-induced increments in the serum levels of cardiac damage markers, CK-MB and Troponin T, were significantly prevented in the group pretreated with AP. The histological evaluation also revealed that structural deterioration of ventricular myocytes caused by MTX, such as the presence of hemorrhagic areas and lymphocytic infiltration, was significantly reduced in the group pretreated with AP, and normal ventricular histological structure was preserved. The role of oxidative stress in the effects of MTX and AP treatment was assessed by determining the lipid peroxidation and GSH levels. According to the current results, MDA elevation and GSH depletion in cardiac tissue associated with MTX were prevented in the group treated with AP. These results suggest that the antioxidant property of AP

is an important factor for its protective effect in MTX-induced cardiac damage. Although the activity of antioxidant enzymes such as SOD, CAT and glutathione peroxidase (GPx) has not been investigated in cardiac tissue, which is a limitation of our study, changes in GSH levels can be interpreted that the AP increases the total antioxidant capacity and thus reduces oxidative stress. Another significant finding observed in the AP+MTX group was the prevention of MTX-induced increase in caspase-3 level in cardiac tissue. Caspase-3 is one of the key executor enzymes in apoptosis, that can be activated by both intrinsic and extrinsic pathways. Therefore, the prevention of caspase-3 increments indicates the anti-apoptotic effect of AP. In line with previous literature, this outcome suggests that AP not only possesses antioxidant properties, but also exhibits anti-apoptotic effects; therefore, it can be proposed that at least these two mechanisms might play a role in mitigating the MTX-induced cardiac damage.

While there is no literature finding demonstrating the isolated effects of AP on the liver, certain essential oils known to contain AP have been reported to exhibit protective role in various liver damage models. For instance, Rosemary (*Rosmarinus officinalis L.*) essential oil, which comprises approximately 11 % AP, has exhibited protective effects against hepatic damage induced by CCl_4, when orally administered at a dose of 10 mg/kg for one week [29]. The elevations in total bilirubin, direct bilirubin, AST, urea, and creatinine caused by CCl_4 application were significantly prevented with a 7-day rosemary oil treatment. Changes in MDA, GSH, CAT, GPx, and glutathione reductase activities induced by CCl_4 in the liver were significantly prevented with rosemary oil treatment, emphasizing the importance of the reduction of oxidative stress in this protective effect [29]. Nevertheless, it may not be accurate to directly attribute the results of the mentioned study to AP. In this regard, our study is the first of its kind to investigate the effects of AP in any damage model. The current results indicate that AP pretreatment prevented the MTX-induced increase in serum ALT and AST levels and significantly protected the general histological structure of the liver by preventing hydropic degeneration and fatty changes. These findings suggest that AP is protective for the liver, similar to its effects on the heart. The changes induced by MTX in hepatic MDA and GSH levels used to evaluate oxidative stress, were completely prevented with AP pretreatment. Additionally, the MTX-induced increase in hepatic caspase-3 level was not observed in the group pre-treated with AP. All these data suggest that AP prevents MTX-induced hepatic damage through antioxidant and anti-apoptotic mechanisms, in line with its effects on the heart.

Conclusion

In this study, the effectiveness of AP, which has antioxidant, anti-inflammatory and anti-apoptotic properties, in MTX-induced cardiac and hepatic damage was tested. According to the present results, AP successfully reduces MTX-induced cardiac and hepatic damage by preventing oxidative stress and apoptosis. Especially considering that MTX is a drug used in cancer treatment, AP appears to have very promising potential as a cardioprotective and hepatoprotective agent in antitumoral therapy.

Conflict of Interest

There is no conflict of interest.

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