



Original Research

Protective Effects of Vinpocetine on Methotrexate-Induced Hepatic Oxidative Stress in Rats

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Abstract

Objectives: Methotrexate (MTX) is a potent antineoplastic and immunosuppressive drug; nevertheless, its therapeutic use is limited by hepatotoxicity, mostly driven by oxidative stress (OS). This research sought to examine the possible preventive impact of vinpocetine (VPC) against MTX-induced liver injury in rats.

Methods: Thirty-two female Wistar albino rats were randomly allocated into four groups: Control, MTX (20 mg/kg, single dose, intraperitoneally), VPC (10 mg/kg/day, intraperitoneally for 7 days), and MTX+VPC. Upon conclusion of the experiment, liver tissues and serum samples were obtained. Hepatic OS indicators, including malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI), were assessed. Serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed. Histopathological evaluation focused on sinusoidal dilation and congestion.

Results: The treatment of MTX markedly elevated liver levels of MDA, TOS, and OSI, while concurrently diminishing SOD, CAT, GPx, and TAS values, signifying substantial OS. VPC co-administration markedly reduced MTX-induced oxidative imbalance, as shown by decreased MDA and OSI levels and a partial recovery of antioxidant enzyme activity. Serum AST and ALT levels exhibited no significant differences among the groups. Histopathological examination revealed that MTX caused considerable sinusoidal dilatation, which was somewhat reduced by VPC, but this reduction did not achieve statistical significance.

Conclusion: VPC offers partial protection against MTX-induced hepatic OS and early histopathological changes without influencing serum transaminase levels. These data indicate that VPC may function as a possible supplementary treatment to alleviate MTX-induced hepatotoxicity.

Keywords: Methotrexate, vinpocetine, hepatotoxicity, oxidative stress, rat model

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Methotrexate (MTX) is an antifolate drug widely used in the treatment of several malignancies, including acute lymphoblastic leukemia and non-Hodgkin lymphoma, and is also commonly prescribed for chronic inflammatory conditions such as rheumatoid arthritis and psoriasis.^[1,2] Although clinically successful, prolonged use of MTX is often linked to significant side effects, including hepatotoxicity. MTX-induced hepatic injury presents via several pathogenic mechanisms, including oxidative stress (OS), inflammation, mitochondrial dysfunction, apoptosis, and, in some instances, fibrosis and cirrhosis.^[3,4]

The pathophysiology of MTX-induced hepatotoxicity is marked by elevated production of reactive oxygen species (ROS), diminished endogenous antioxidant defenses like glutathione (GSH), and increased expression of pro-inflammatory cytokines, including tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β). These mechanisms together induce hepatocyte injury and necrosis, resulting in compromised liver function, as shown by increased serum transaminase levels and significant histological damage.^[3,5] Consequently, the identification of safe and efficacious medicines that may safeguard the liver against MTX-induced toxicity has emerged as a significant objective in both clinical and preclinical research.

Vinpocetine (VPC; ethyl apovincamate), a semi-synthetic derivative of the vinca alkaloid vincamine, is best known for its cerebral vasodilatory, neuroprotective, and nootropic properties.^[6] The pharmacological actions include the inhibition of phosphodiesterase-1 (PDE-1), blocking of voltage-gated sodium channels, and anti-inflammatory activity predominantly via the suppression of the nuclear factor kappa B (NF- κ B) signaling pathway.^[7-9] VPC has significant antioxidant capabilities by neutralizing ROS and enhancing the activity of essential antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT).^[9] Experimental investigations have shown VPC's protective benefits in models of ischemia-reperfusion damage, diabetic nephropathy, and hepatic ischemia.^[7,10] VPC augments antioxidant enzyme activity, diminishes lipid peroxidation, and inhibits inflammatory pathways. VPC enhances protective factors such as nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase 1 (HO-1), while inhibiting apoptosis-related proteins, leading to better liver histology and function in rats subjected to MTX exposure^[11].

We hypothesize that VPC may mitigate MTX-induced hepatic injury by reducing OS and preserving liver architecture. The findings of this study may support the potential repositioning of VPC as an adjunct hepatoprotective agent in patients receiving MTX-based therapy.

Methods

A total of 32 female Wistar albino rats (300–350 g), sourced from the Inonu University Laboratory Animal Production and Research Center, were randomly assigned to four experimental groups. The animals were kept under regulated laboratory settings, with a controlled ambient temperature of 21 ± 2 °C, relative humidity of $60\pm 5\%$, and a 12-hour light/12-hour dark photoperiod. All rats were provided with a regular pellet diet and had unrestricted access to tap water throughout the research period.

Randomization methods were used for both group allocation and for data collection and analysis, conducted by investigators unaware of the treatment conditions. The experimental methodology adhered to the National Institutes of Health criteria for animal research and conformed to the ARRIVE reporting requirements.^[12] Ethical permission was obtained from the Inonu University Faculty of Medicine Animal Experiments Local Ethics Committee (approved date: October 11, 2018; meeting number: 2015/A-37). A simple randomization approach guaranteed an impartial allocation of animals among the experimental groups. The required minimum sample size was determined a priori using G*Power software (version 3.1.9.7, Heinrich Heine University, Düsseldorf, Germany). Based on effect sizes reported in previous experimental studies investigating MTX-induced hepatic OS and antioxidant interventions, a large effect size was assumed ($f=0.40$). The calculation was performed for a one-way ANOVA with four independent groups, with a significance level (α) of 0.05 and a statistical power ($1-\beta$) of 0.80. Under these assumptions, the minimum total sample size required was 28 animals (7 per group). To compensate for potential experimental losses and to increase the robustness of the statistical analysis, 32 rats were included in the study ($n=8$ per group).

32 female Wistar albino rats were randomly divided into four groups:

- Control Group ($n=8$): The vehicle solution (0.5 mL) was administered intraperitoneally (i.p.) once daily for seven days.
- MTX Group ($n=8$): On day 1, a single dose of MTX (20 mg/kg) was administered i.p., followed by daily i.p. administration of 0.5 mL of the vehicle solution for seven days.
- VPC Group ($n=8$): A single daily dose of 10 mg/kg VPC was administered i.p. for 7 days.
- MTX+VPC Group ($n=8$): On the first day, a single dose of 20 mg/kg MTX was administered i.p., followed by a single daily dose of 10 mg/kg VPC administered i.p. for 7 days.

The dosages, administration routes, and dosing regimens for MTX (Methotrexate DBL, 500 mg/20 mL; Koçak Farma, Istanbul, Türkiye) and VPC (CAS No. 42971-09-5; Sigma-Aldrich, St. Louis, MO, USA) were determined according to prior research conducted by Samdanci et al. and Ristić et al., respectively.^[13,14] On the eighth day of the experimental phase, the body weights of the rats were documented. Animals were anesthetized with urethane (1.2 g/kg, intraperitoneal; CAS No. 51-79-6; Sigma-Aldrich St. Louis, MO, USA), followed by the collection of blood samples from the inferior vena cava. Euthanasia was conducted surgically to guarantee exsanguination.

Subsequent to sacrifice, liver tissues were removed, irrigated with 0.9% NaCl isotonic saline to eliminate leftover blood, and weighed with a precision scale. Blood samples were centrifuged at 2000 rpm for 7 minutes to isolate serum. The harvested liver tissues were divided symmetrically for biochemical and histopathological assessments; one portion was fixed in 10% formaldehyde for histological evaluation, while the remaining liver tissue and serum samples were appropriately packaged and stored at -80°C until biochemical analyses were conducted.

Tissue Biochemical Analysis

Upon the commencement of the analyses, the tissues were washed by submerging them in a beaker filled with Tris-HCl. Their weights were subsequently measured and documented. Tissues were homogenized for one minute in glass tubes using an IKA-WERKE T 25 B device with the addition of pH 7.4 Tris-HCl buffer. Homogenization was completed by including an additional quantity of buffer solution and homogenizing for an additional minute. A segment of the resultant homogenate was reserved for examination. The residual homogenate was subjected to centrifugation at 4000 rpm for 45 minutes at $+4^{\circ}\text{C}$ using a Hettich D 78532 chilled centrifuge. The transparent supernatant was isolated for examination. Spectrophotometric measurements were conducted with SHIMADZU UV-160A and BIOTEK SYNERGY LX multi-mode reader instruments.

Malondialdehyde (MDA)

MDA was measured according to the method of Uchiyama and Mihara^[15]. The results obtained were expressed in nmol/g tissue.

Superoxide Dismutase (SOD)

SOD activity was determined using the method of Sun et al.^[16] Results were calculated in U/mg protein.

Protein Quantification

Protein quantity analysis was performed using the modified Lowry method to calculate the data for the other mark-

ers studied.^[17] The results were calculated according to the standard graph obtained and expressed in $\mu\text{g/mL}$.

Catalase (CAT)

CAT activity was determined according to Aebi's method.^[18] Results were reported as K/g protein.

Glutathione Peroxidase (GPx)

GPx activity was measured using the Paglia and Valentine method.^[19] The activity calculated from the observed absorbance change during this process was given as U/mg protein.

Total Antioxidant Status (TAS), Total Oxidant Status (TOS), and Oxidative Stress Index (OSI)

The TAS measurement was determined in the supernatant using the Erel method.^[20] The unit is mmol Trolox equivalent per liter. TOS was quantified colorimetrically using the Erel technique, utilizing the supernatant produced.^[21] The unit is $\mu\text{mol H}_2\text{O}_2$ equivalent/L. The OSI was calculated by dividing the TOS by the TAS. The unit is arbitrary unit (AU).^[22]

Serum Biochemical Analysis

Serum samples were extracted from the deep freezer one day before biochemical analysis and allowed to defrost prior to processing at the Inonu University Turgut Özal Medical Center Central Laboratory.

Histopathological Analysis

Upon completion of the experiment, liver tissues were preserved in 10% formaldehyde. After standard tissue preparation, paraffin-embedded blocks were created, and sections measuring 4–5 μm in thickness were acquired. The sections were stained with hematoxylin and eosin (H&E) for comprehensive histological evaluation.

Histopathological evaluation focused on sinusoidal dilatation and congestion. Tissue injury was assessed using a semi-quantitative scoring system as follows: 0, no pathological change; 1, mild injury; 2, moderate injury; and 3, severe injury. For each animal, ten randomly selected, non-overlapping microscopic fields were examined at $\times 20$ magnification. Scoring was performed by an experienced histopathologist who was blinded to the experimental groups in order to minimize observer bias. The mean score for each animal was used for statistical analysis.

All histological analyses were performed using a Leica DFC-280 research microscope alongside the Leica Q Win Image Analysis System (Leica Micros Imaging Solutions Ltd., Cambridge, UK).

Statistical Analysis

Statistical analyses were performed using statistical software developed by the Department of Biostatistics and Medical

Informatics, Faculty of Medicine, Inonu University [23]. Data distribution was assessed using normality tests, and since the variables did not follow a normal distribution, non-parametric tests were applied. Overall group comparisons were performed using the Kruskal–Wallis test. When a significant difference was detected, pairwise comparisons were conducted using the Mann–Whitney U test. To control for inflation of Type I error due to multiple comparisons, a Bonferroni correction was applied, and the adjusted significance threshold was set at $p < 0.0083$ ($0.05/6$). Data were expressed as median (minimum–maximum), and p values below the adjusted threshold were considered statistically significant.

Results

Body and Liver Weights of Rats

Table 1 demonstrates that liver weights exhibited no significant differences across the experimental groups ($p > 0.05$), suggesting that neither MTX nor VPC treatment resulted in a notable alteration in liver mass. The ultimate body weight of rats in the MTX group was considerably lower than that of the Control group ($p < 0.05$). The VPC therapy alone did not significantly affect the final body weight in comparison to the Control group. The co-administration of VPC with MTX considerably mitigated the body weight loss generated by MTX, resulting in final body weights that were markedly greater than those in the MTX group ($p < 0.05$).

Tissue Biochemical Findings

As shown in Figure 1 and Table 2, MTX administration caused a marked disruption of oxidative balance in liver tissue, as indicated by significant increases in MDA, TOS, and OSI levels, along with significant decreases in antioxidant defense parameters, including SOD, CAT, GPx, and TAS, compared with the Control group ($p < 0.05$).

Treatment with VPC alone induced considerable alterations in OS indicators, shown by increased MDA, TOS, and OSI levels, and diminished CAT and GPx activity compared to the Control group ($p < 0.05$), although SOD and TAS levels were sim-

ilar. Co-administration of VPC with MTX markedly reduced MTX-induced oxidative damage, as shown by decreased MDA and OSI values and a partial recovery of antioxidant enzyme activity relative to the MTX group ($p < 0.05$), while some parameters continued to deviate from Control values.

Serum Biochemical Findings

As shown in Table 3, serum liver enzyme levels did not differ significantly among the experimental groups. Neither MTX administration nor VPC treatment, whether individually or in conjunction, resulted in a statistically significant alteration in serum aspartate aminotransferase (AST) or alanine aminotransferase (ALT) levels compared to the Control group ($p > 0.05$), signifying the lack of pronounced hepatocellular damage under the experimental conditions employed.

Histopathological Findings

Liver injury was assessed for sinusoidal dilatation and congestion. In the Control and VPC groups, the liver had a normal histological appearance, with the exception of modest alterations. In these groups, hepatocyte cords exhibiting anastomoses surrounding the central veins and the sinusoids situated between these cords were clearly visible (Fig. 2a and b). In the MTX group, sinusoidal congestion paralleled that of the Control group; however, a substantial increase in sinusoidal dilatation was seen ($p < 0.001$) (Fig. 2c). The MTX+VPC group exhibited a little reduction in sinusoidal dilatation; yet, this reduction was not statistically significant in comparison to the MTX group (Fig. 2d). The scores for histopathological assessment are shown in Table 4.

Discussion

The present study investigated the protective effects of VPC against MTX-induced liver injury in rats, focusing on OS parameters, serum liver enzymes, and histopathological alterations. The main findings show that MTX administration induced a marked oxidative imbalance in liver tissue, characterized by increased lipid peroxidation and oxidant status along with a significant reduction in endogenous antioxidant defenses. Co-administration of VPC partially attenuated these changes, supporting its antioxidant and hepatoprotective potential; however, this effect was not fully reflected in serum transaminase levels or in complete normalization of histological alterations.

OS is widely recognized as a central mechanism underlying MTX-induced hepatotoxicity.^[5,24] Excessive production of ROS and depletion of antioxidant enzymes such as SOD, CAT, and GPx contribute to lipid peroxidation, mitochondrial dysfunction, and hepatocellular injury^[25,26]. Consistent with previous studies, MTX-treated rats in the present study exhibited sig-

Table 1. Body and liver weights of rats

Groups	Liver weight (g)	End of experiment rat weight (g)
Control	9.46 (5.73-11.16)	279 (242-340) ^a
MTX	9.05 (6.25-10.95)	229.5 (217-300)
VPC	8.17 (6.56-9.04)	265.5 (215-310)
MTX+VPC	8.17 (6.03-10.12)	252.5 (229-303) ^a

a: There is a statistically significant difference according to the MTX group ($p < 0.05$). MTX: Methotrexate-treated group; VPC: Vinpocetine-treated group; MTX+VPC: Methotrexate- and Vinpocetine-treated group.



Figure 1. Liver tissue oxidative stress parameters.

nificantly elevated hepatic MDA, TOS, and OSI levels, along with decreased SOD, CAT, GPx, and TAS values, confirming the establishment of an oxidative injury model.^[25,27,28] These findings align with earlier reports demonstrating MTX-induced redox imbalance as a key driver of liver damage.

VPC administration alone produced moderate changes in certain OS parameters without causing marked histopathological damage or alterations in serum AST and ALT levels. This finding suggests that VPC does not exert overt hepatotoxic effects under the applied experimental conditions. Importantly, co-administration of VPC with MTX significantly reduced hepatic MDA and OSI levels and partially restored antioxidant enzyme activities compared with the MTX group. These results indicate that VPC mitigates MTX-induced oxidative injury, likely through its free radical scavenging capacity and enhancement of endogenous an-

tiioxidant systems. The observed effects are consistent with previous experimental studies reporting VPC-mediated up-regulation of antioxidant enzymes and suppression of oxidative damage in various organ injury models, including hepatic ischemia-reperfusion and drug-induced toxicity. VPC, a synthetic vincamine derivative, shows consistent antioxidant and hepatoprotective effects across in vitro and animal liver models of toxic and metabolic injury. In human liver L02 cells, VPC (1–30 µM) showed strong, concentration-dependent radical scavenging in the ABTS assay (≈87% at 30 µM) and reduced H₂O₂- and paracetamol-induced ROS, while increasing intracellular GSH. VPC upregulated Nrf2 and HO-1, key regulators of endogenous antioxidant defenses, partly by competing with Nrf2 for kelch-like ECH-associated protein 1 (Keap1) binding and stabilizing Nrf2 protein.^[30] In multiple rat models, VPC increased hepatic GSH and SOD and

Table 2. Liver tissue biochemical analysis results

Groups	MDA (nmol/g tissue)	SOD (U/mg protein)	CAT (K/g protein)	GPx (U/mg protein)	TAS (mmol Trolox Eqv./L)	TOS ($\mu\text{mol H}_2\text{O}_2$ Eqv./L)	OSI (AU)
Control	4.75 (2.99-6.18)	1.68 (1.43-1.97)	36.80 (23.50-51.90)	161.59 (96.97-187.39)	1.96 (1.44-2.83)	27.18 (13.25-32.72)	11.83 (7.11-19.29)
MTX	8.76 (7.99-13.10) ^{a,b}	1.24 (0.97-1.49) ^{a,b}	26.76 (17.03-33.10) ^a	87.02 (63.83-126.72) ^{a,b}	1.38 (0.80-2.19) ^{a,b}	32.98 (26.51-50.33) ^a	24.62 (13.68-35.72) ^a
VPC	6.38 (4.16-9.14) ^a	1.50 (1.16-1.98)	21.86 (15.95-27.96) ^a	126.04 (97.82-163.63) ^a	1.92 (1.56-2.38)	35.26 (30.03-43.91) ^a	18.62 (13.95-26.89) ^a
MTX+VPC	6.35 (4.98-8.90) ^a	1.28 (0.91-1.56) ^a	21.39 (18.41-30.52) ^a	116.53 (91.14-165.97) ^a	2.07 (1.17-3.18)	32.83 (24.65-48.05) ^a	19.62 (7.74-25.84) ^a

a: There is a statistically significant difference compared to the Control group ($p < 0.05$). b: There is a statistically significant difference compared to the VPC group ($p < 0.05$). MDA: Malondialdehyde; SOD: Superoxide Dismutase; CAT: Catalase; GPx: Glutathione Peroxidase; TAS: Total Antioxidant Status; TOS: Total Oxidant Status; OSI: Oxidative Stress Index; AU: Arbitrary Unit; MTX: Methotrexate-treated group; VPC: Vinpocetine-treated group; MTX+VPC: Methotrexate- and Vinpocetine-treated group.

reduced MDA and nitric oxide (NO/NOx), indicating reduced lipid peroxidation and nitrosative stress.^[11,30-33]

Despite the clear biochemical evidence of oxidative injury and partial protection by VPC, serum AST and ALT levels did not differ significantly among groups. This finding suggests that the degree of hepatocellular damage induced by a single MTX dose in the present model may be

subclinical or insufficient to elicit measurable elevations in circulating transaminases. Serum liver enzymes are known to be relatively insensitive in early or mild hepatic injury, particularly when oxidative damage precedes overt cell necrosis. OS can clearly precede overt hepatocyte necrosis and histologic liver injury, so standard serum liver enzymes [ALT, AST, alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT)] may stay normal in early or mild oxida-

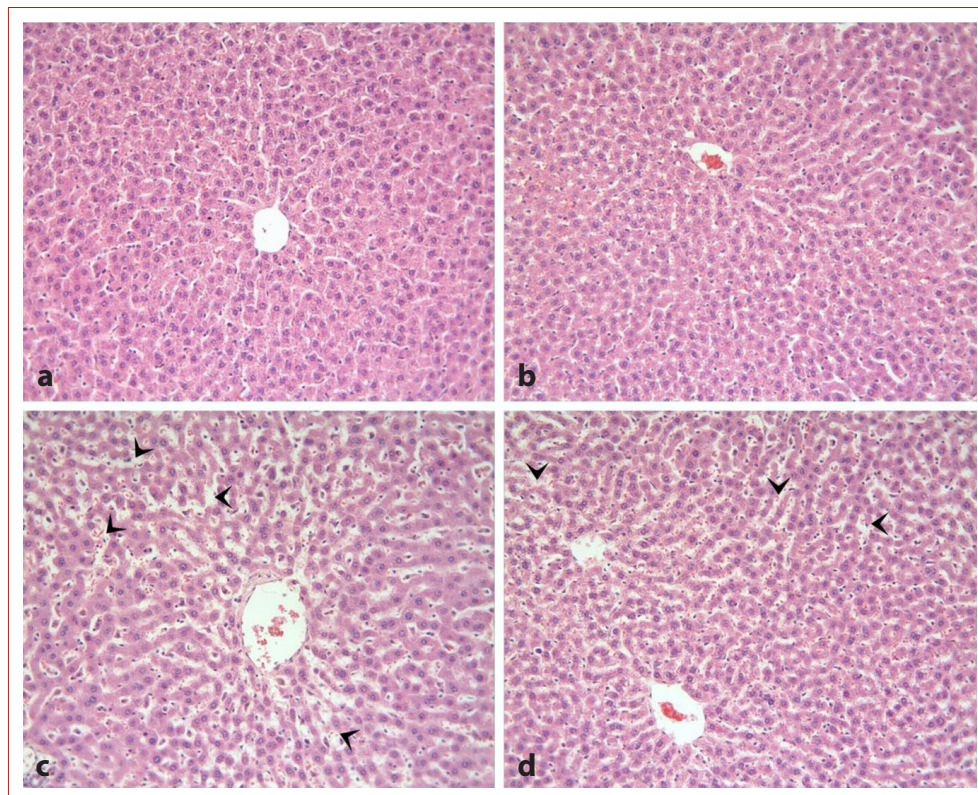


Figure 2. The liver shows a normal histological appearance in the control (a) and VPC (b) groups. In the MTX group (c), sinusoidal dilatation (arrowheads) is noted. In the MTX+VPC group (d), sinusoidal dilatation (arrowheads) persists, albeit to a lesser extent. H&E; 20x.

Table 3. Serum biochemical analysis results

Groups	AST (IU/L)	ALT (IU/L)
Control	177 (118-222)	35 (29-51)
MTX	164.5 (117-194)	35.5 (24-67)
VPC	182.5 (89-260)	30.5 (17-43)
MTX+VPC	122 (78-294)	26 (18-78)

There is no statistically significant difference between the groups ($p > 0.05$). AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; MTX: Methotrexate-treated group; VPC: Vinpocetine-treated group; MTX+VPC: Methotrexate- and Vinpocetine-treated group.

Table 4. Histopathological Evaluation Results

Groups	Sinusoidal dilatation	Sinusoidal congestion
Control	0.0 (0.0-1.0)	0.0 (0.0-0.1)
MTX	1.0 (0.0-3.0) ^a	0.0 (0.0-0.2)
VPC	0.0 (0.0-1.0)	0.0 (0.0-0.1)
MTX+VPC	1.0 (0.0-2.0)	0.0 (0.0-0.2)

a: Significant increase compared to the Control group ($p < 0.001$). MTX: Methotrexate-treated group; VPC: Vinpocetine-treated group; MTX+VPC: Methotrexate- and Vinpocetine-treated group.

tive damage and do not reliably reflect its onset or severity. ALT/AST rises often correlate with necrosis or substantial membrane damage, not with the earliest oxidative events. OS and lipid peroxidation can occur days before necrosis in liver and other organs in dietary choline deficiency models.^[34] Reviews of OS in acute liver injury and drug-induced liver injury emphasize that mitochondrial OS, GSH depletion, and ROS formation occur upstream of cell death and clinical enzyme release.^[34-38] There is poor correlation at times between serum enzyme levels and true liver integrity; selective enzyme release can occur without necrosis, and necrosis can progress despite relatively modest enzyme changes.^[37,39] Subclinical or mild chronic hepatitis in humans and dogs can have normal ALT despite histologic inflammation or copper-associated injury, highlighting insensitivity in early disease.^[40] Therefore, the discrepancy between tissue OS markers and serum biochemistry underscores the importance of evaluating both biochemical and histopathological parameters when assessing hepatotoxicity. In this context, the lack of significant changes in serum AST and ALT, despite clear OS and histopathological alterations in liver tissue, suggests that the present MTX model represents an early or subclinical stage of hepatotoxicity. At this stage, intracellular oxidative injury and microstructural disturbances may occur before sufficient hepatocyte membrane damage develops to cause enzyme leakage into the circulation. Therefore, tissue OS markers and histo-

logical evaluation appear to be more sensitive indicators of early MTX-induced liver injury in this experimental setting. Histopathological examination further supported the biochemical findings. MTX administration resulted in a significant increase in sinusoidal dilatation, indicating microcirculatory disturbance and early structural liver injury. VPC co-treatment produced a modest reduction in sinusoidal dilatation; however, this improvement did not reach statistical significance. VPC generally shows protective effects on hepatic structure, and in liver models, it can reduce histologic damage, but specific data on sinusoidal dilatation are limited, and any reduction is likely modest. In diethylnitrosamine-induced early hepatocellular carcinoma in rats, VPC improved liver ultrastructure, reduced OS and inflammatory signaling, and favorably modulated remodeling markers, indicating attenuation of structural liver damage and fibrosis.^[33] In human hepatic L02 cells, VPC protected against H₂O₂ and acetaminophen-induced hepatotoxicity, reducing ROS and restoring antioxidant defenses through Nrf2/HO1 activation.^[29] These effects would be expected to blunt microvascular/sinusoidal injury, though sinusoidal dilatation per se was not quantified. This partial histological protection may reflect the short duration of VPC treatment or the severity of MTX-induced injury, suggesting that longer treatment periods or different dosing regimens may be required to achieve more pronounced structural recovery.

Body weight loss observed in the MTX group further supports the systemic toxic effects of MTX, whereas partial restoration of body weight in the MTX+VPC group indicates an overall protective influence of VPC. Importantly, liver weights were not significantly altered, suggesting that functional and oxidative changes preceded gross morphological alterations in liver mass. In cyclosporine A-induced hepatotoxicity in rats, liver function markers and OS indices were markedly altered, with clear microscopic damage, despite preserved lobular architecture and no mention of early gross organ enlargement.^[41] Fluoride exposure in mice produced significant OS, enzyme leakage, and ultrastructural damage (vacuolar degeneration, dilated endoplasmic reticulum, mitochondrial membrane damage) before advanced structural collapse, again emphasizing that functional and oxidative disruption precede massive morphologic change.^[42] High-sugar/high-energy diets in young rats increased serum AST/ALT and oxidative/inflammatory markers along with early histopathological alterations, linking proinflammatory and OS mechanisms to early hepatic dysfunction before end-stage remodeling.^[43] Reviews of liver OS emphasize that disturbed redox balance and mitochondrial dysfunction drive progression from simple steatosis to steatohepatitis, fibrosis, and hepatocellular carcinoma, acting upstream of gross fibrotic and mass changes.^[44,45]

Several limitations of the present study should be acknowledged. The evaluation was limited to a short-term MTX exposure model, and inflammatory cytokines, apoptotic markers, or molecular signaling pathways. A limitation of the present study is that mechanistic signaling pathways involved in OS, inflammation, and cell death, such as the Nrf2/HO-1 and NF- κ B pathways, inflammatory cytokines, and apoptotic markers, were not directly investigated. Although the observed biochemical and histopathological findings strongly support an antioxidant and hepatoprotective effect of VPC, the proposed molecular mechanisms are based on previous experimental evidence reported in the literature. Future studies incorporating molecular and protein-level analyses are warranted to clarify the precise signaling pathways responsible for the protective effects of VPC in MTX-induced hepatotoxicity. It should be noted that this study employed a short-term, single-dose MTX model that primarily reflects early or subclinical hepatotoxicity; therefore, future studies using longer-term or repeated-dose MTX protocols are warranted to better simulate chronic clinical exposure and to further clarify the long-term hepatoprotective potential of VPC.

Conclusion

The findings of this study demonstrate that MTX induces significant OS and early histopathological alterations in rat liver tissue without causing overt changes in serum liver enzymes. VPC co-administration partially attenuates MTX-induced oxidative damage and improves antioxidant capacity, supporting its potential role as an adjunctive protective agent against MTX-related hepatotoxicity. Further experimental and clinical studies are warranted to clarify its therapeutic relevance and optimal treatment strategies.

Disclosures

Ethics Committee Approval: This study was approved by The Inonu University Faculty of Medicine Animal Experiments Local Ethics Committee on October 11, 2018, at the meeting numbered 2015/A-37.

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