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The effects of intravenous lipid emulsion and ethanol on the optic nerve and retina in methanol-intoxicated rats: a histopathological study

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Abstract

The effects of intravenous lipid emulsion and ethanol on the optic nerve and retina in methanol-intoxicated rats: a histopathological study

Objective: This study aimed to evaluate the therapeutic efficacy of intravenous lipid emulsion (ILE) compared with ethanol in the treatment of optic neuropathy induced by acute methanol intoxication in rats.
Methods: Sixty-four male rats were divided into seven groups: Group 1 (control), Group 2 (methanol), Group 3 (methanol + ethanol), Group 4 (methanol + ILE), Group 5 (methanol + ethanol + ILE), Group 6 (ethanol), and Group 7 (ILE). Blood samples were collected to assess liver and kidney functions. After sacrifice, the optic nerve and retina were examined histologically.
Results: The combination of methanol, ethanol, and ILE improved LDH and CK-MB levels. Histopathological analysis revealed marked vascularization and vacuolization in the optic nerve of the methanol group, whereas these changes were minimal in the methanol + ethanol + ILE group. Neither ILE nor ethanol caused significant apoptotic alterations in the retina ($p = 0.357$). Pronounced edema, vascularization, apoptotic changes, and vacuolization were observed in the methanol group, but these effects were largely absent in the methanol + ILE and methanol + ethanol + ILE groups compared with controls.
Conclusion: The combined administration of ethanol and ILE exerts a protective effect against methanol-induced cardiac and optic nerve damage. Histopathologically, this combination mitigates the degenerative effects of methanol on both the optic nerve and retinal tissues.
Keywords: Methanol intoxication, Ethanol, Intravenous lipid emulsion, Optic nerve, Retina.

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Öz

Metanol ile intoksike edilmiş sıçanlarda intravenöz lipid emülsiyonu ve etanolün optik sinir ve retina üzerindeki etkilerinin değerlendirilmesi: histopatolojik bir çalışma

Amaç: Bu çalışmanın amacı, sıçanlarda akut metanol zehirlenmesinden kaynaklanan optik nöropatinin tedavisinde intravenöz lipid emülsiyonu (ILE) uygulamasının etanole kıyasla etkinliğini değerlendirmektir.
Yöntem: 64 erkek sıçan 7 gruba ayrıldı: Grup 1 (kontrol grubu), Grup 2 (metanol grubu), Grup 3 (metanol + etanol grubu), Grup 4 (metanol + intravenöz lipid emülsiyonu grubu), Grup 5 (metanol + etanol + intravenöz lipid emülsiyonu grubu), Grup 6 (etanol grubu) ve Grup 7 (intravenöz lipid emülsiyonu grubu). Karaciğer ve böbrek fonksiyonlarını analiz etmek için kan örnekleri alındı. Sıçanlar öldürüldü. Optik sinir ve retina histolojik olarak incelendi.
Bulgular: Metanol, etanol ve intravenöz lipid emülsiyonunun kombinasyonu LDH ve CK-MB seviyeleri üzerinde olumlu bir etkiye sahiptir. Histopatolojik olarak, metanol grubunda optik sinirde belirgin vaskülarizasyon ve vakuolizasyon gözlendi. Bu değişiklikler metanol, intravenöz lipid emülsiyonu ve etanolü birlikte alan grupta anlamlı değildi. İntravenöz lipid emülsiyonu ve etanol retinada anlamlı apoptotik değişikliklere neden olmadı (p=0,357). Metanol grubunda retina dokusunda anlamlı ödem, vaskülarizasyon, apoptotik değişiklikler ve vakuolizasyon gözlendi. Bu etkiler metanol + intravenöz lipid emülsiyonu ve metanol + intravenöz lipid emülsiyonu + etanol gruplarında kontrol grubuyla karşılaştırıldığında gözlenmedi.
Sonuç: Metanol, etanol ve intravenöz lipid emülsiyonunun kombinasyonu, özellikle kardiyak hasar üzerinde faydalı bir etkiye sahiptir. Histopatolojik olarak metanol + etanol + intravenöz lipid emülsiyonu kombinasyonunun, metanolün optik sinir ve retina üzerindeki olumsuz etkilerini azaltabildiği görüldü.

Anahtar Kelimeler: Metanol Zehirlenmesi, Etanol, İntravenöz Lipid Emülsiyonu, Optik Sinir.

INTRODUCTION

Methanol is a type of alcohol derived from the distillation of wood. It is a colorless and highly toxic organic solvent widely used as a cleaner and solvent in the industry. It is used in the production of illicit spirits to cut costs. Methanol poisoning is a potentially fatal condition and continues to pose a major public health challenge in developing countries. Methanol poisoning is usually caused by the oral ingestion of methanol. However, it can also occur after accidental or suicidal ingestion of methanol (1). Methanol poisoning can also occur through the skin or inhalation. Metabolites, rather than methanol itself, are responsible for the toxic effect. Methanol is rapidly absorbed from the gastrointestinal tract, respiratory tract, and skin.

After oral ingestion, serum methanol levels typically peak within 30 to 90 minutes. In the liver, methanol is metabolized into more toxic compounds. It is first converted into formaldehyde by the enzyme alcohol dehydrogenase. Formaldehyde, a transient intermediate with a very short half-life, is then rapidly oxidized to formic acid by formaldehyde dehydrogenase. Formic acid, a more stable and harmful metabolite, is primarily eliminated through the urine. However, a portion is further broken down into carbon dioxide and water via a folate-dependent pathway catalyzed by the enzyme 10-formyltetrahydrofolate synthetase (3). Methanol is eliminated from the body at a slower rate than ethanol, leading to its accumulation and the development of toxic effects.

Methanol poisoning can cause various symptoms and signs, such as central nervous system depression, visual disturbances, and systemic metabolic acidosis. The affected person may experience initial unconsciousness, followed by a latent period lasting 12-24 hours. During this period, symptoms such as headache, blurred vision, decreased visual acuity, photophobia, vertigo, confusion, nausea, abdominal pain, and vomiting may develop (4).

Methanol-induced optic neuropathy (Me-ION) is a very serious condition that causes significant and irreversible damage to the optic nerve as well as other structures of the visual system (retina, chiasma, and optic tract) (5,6). The priorities in treating Me-ION are correcting metabolic acidosis, preventing the decomposition of methanol into its more toxic metabolites with appropriate antidotes, and facilitating the excretion of the formed metabolites from the body (4).

Intravenous lipid emulsion (ILE) therapy was originally developed to treat local anesthetic toxicity. In recent years, it has increasingly been used in emergency departments and intensive care units for the management of lipophilic drug toxicities (7).

This study aimed to evaluate the effectiveness of intravenous lipid emulsion (ILE) therapy in comparison to ethanol (E) in the treatment of optic neuropathy resulting from acute methanol intoxication in rats.

METHODS

A total of 64 male Wistar albino rats, aged 7–8 weeks and weighing between 250 and 300 grams, were included in the study. The animals were randomly assigned to seven groups, ensuring comparable body weights across groups. Throughout the experiment, each rat was housed individually in a temperature-controlled environment (21 ± 2°C) with 50 ± 10% relative humidity and a 12-hour light/dark cycle.

Tablo 1. Comparison of optic nerve vacuolization (ONVC) levels between groups

		Control [a] (n=8) (%)		M [a,b] (n=10) (%)		M+ILE [a, c] (n=10) (%)		E [d] (n=8) (%)		M+E [a,e] (n=10) (%)		M+E+ILE [b,c,d,e] (n=10) (%)		p
ONVC	Negative	4	%50.0	0	(%0.0)	0	(%0.0)	0	(%0.0)	0	(%0.0)	0	(%0.0)	<0.001*
	Positive/ Weak	4	%50.0	0	(%0.0)	0	(%0.0)	3	(%37.5)	1	(%10.0)	10	(%100.0)	
	Positive/ Moderate	0	%0.0	5	(%50.0)	9	(%90.0)	5	(%62.5)	9	(%90.0)	0	(%0.0)	
	Positive/ Strong	0	%0.0	5	(%50.0)	1	(%10.0)	0	(%0.0)	0	(%0.0)	0	(%0.0)	

* Chi-square test (Post hoc: Bonferroni, p<0.008)
a p≤0.001 vs Control (C vs M for p<0.001, C vs M+ILE for p<0.001, C vs E for p=0.010, C vs M+E for p=0.001, C vs M+E+ILE for p=0.011)
b p<0.001 vs M (M vs M+ILE for p=0.051, M vs E for p=0.019, M vs M+E for p=0.028, M vs M+E+ILE for p<0.001)
c p<0.001 vs M+ILE (M+ILE vs E for p=0.083, M+ILE vs M+E for p=0.368, M+ILE vs M+E+ILE for p<0.001)
d p<0.003 vs E (E vs M+E for p=0.163, M+ILE vs M+E+ILE for p=0.003)
e p≤0.003 vs M+E (M+E vs M+E+ILE for p=0.003)
Abbreviations: C: control, M: methanol, E: Ethanol, ILE: Intravenous lipid emulsion

Tablo 2. Comparison of optic nerve vascularization (ONVS) levels between groups

		Control a (n=8) (%)		M a,b (n=10) (%)		M+ILE b (n=10) (%)		E b (n=8) (%)		M+E b (n=10) (%)		M+E+ILE b (n=10) (%)		p
ONVS	Negative	8	%100.0	0	(%0.0)	7	(%70.0)	7	(%8.,5)	5	(%50.0)	10	(%100.0)	<0.001*
	Positive/ Weak	0	%0.0	7	(%70.0)	3	(%30.0)	1	(%12.5)	5	(%50.0)	0	(%0.0)	
	Positive/ Moderate	0	%0.0	3	(%30.0)	0	(%0.0)	0	(%0.0)	0	(%0.0)	0	(%0.0)	
	Positive/ Strong	0	%0.0	0	(%0.0)	0	(%0.0)	0	(%0.0)	0	(%0.0)	0	(%0.0)	

* Chi-square test (Post hoc: Bonferroni, p<0.008)
a p<0.001 vs Control (C vs M for p<0.001, C vs M+ILE for p=0.090, C vs E for p=0.302, C vs M+E for p=0.019)
b p<0.005 vs M (M vs M+ILE for p=0.003, M vs E for p=0.001, M vs M+E for p=0.005, M vs M+E+ILE for p<0.001)
(M+ILE vs E for p=0.375, M+ILE vs M+E for p=0.361, M+ILE vs M+E+ILE for p=0.060)
(E vs M+E for p=0.094, M+ILE vs M+E+ILE for p=0.250)
(M+E vs M+E+ILE for p=0.010)
Abbreviations: C: control, M: methanol, E: Ethanol, ILE: Intravenous lipid emulsion

All animals had ad libitum access to filtered tap water and a standard commercial diet (Korkutelim Food Company, Antalya, Turkey), which contained 88.0% dry matter, 23.5% crude protein, 3.3% ether extract, 6.1% crude fiber, 5.3% ash, and provided 2800 kcal/kg of metabolizable energy.

The surgical procedures for the animal experiments followed the Balıkesir University Animal Experiments Local Ethics Committee guidelines. No anesthetic agents were administered to avoid influencing the biochemical results, and all necessary precautions were taken to minimize animal discomfort throughout the experiment.

Since our study did not investigate the effects of sex differences on the outcomes of ethanol, methanol, and ILE, we chose to only include male rats to prevent sex differences from impacting the results.

Selected Reagents and Chemicals

Saline Solution (Isotonic sodium chloride at 0.9%): Sourced from Polifleks in Turkey.

Methanol (M) (methyl alcohol, CH3OH, purity: 99.9%): was obtained from LiChrosolv, a product of Merck.

Ethanol (E) (ethyl alcohol, CH3CH2OH, purity: 99.8%): was obtained from Chromosolv, a division of Sigma Aldrich.

Intravenous Lipid Emulsion (ILE): The ILE used in this study is marketed as Clinoleic by Baxter (Belgium). It is a 20% linoleic emulsion available in 500 mL containers, composed of 80% olive oil and 20% soybean oil. The formulation also contains egg phospholipids, glycerol, sodium oleate, and sodium hydroxide.

Tablo 3. Comparison of retinal edema (RE) levels between groups

		Control a (n=8) (%)		M a,b (n=10) (%)		M+ILE b,c (n=10) (%)		E b (n=8) (%)		M+E a (n=10) (%)		M+E+ILE b (n=10) (%)		p
RE	Negative	7	%87.5	0	(%0.0)	7	(%70.0)	5	(%62.5)	1	(%10.0)	7	(%70.0)	<0.010*
	Positive/ Weak	1	%12.5	7	(%70.0)	3	(%30.0)	3	(%37.5)	7	(%70.0)	3	(%30.0)	
	Positive/ Moderate	0	%0.0	2	(%20.0)	0	(%0.0)	0	(%0.0)	2	(%20.0)	0	(%0.0)	
	Positive/ Strong	0	%0.0	1	(%10.0)	0	(%0.0)	0	(%0.0)	0	(%0.0)	7	(%70.0)	

* Chi-square test (Post hoc: Bonferroni, p<0.008)
a p≤0.002 vs Control (C vs M for p=0.002, C vs M+ILE for p=0.375, C vs E for p=0.248, C vs M+E for p=0.004, C vs M+E+ILE for p=0.375)
b p<0.007 vs M (M vs M+ILE for p=0.002, M vs E for p=0.007, M vs M+E for p=0.572, M vs M+E+ILE for p=0.002)
(M+ILE vs E for p=0.737, M+ILE vs M+E for p=0.017, M+ILE vs M+E+ILE for p=1.000)
(E vs M+E for p=0.047, M+ILE vs M+E+ILE for p=0.737)
(M+E vs M+E+ILE for p=0.017)
Abbreviations: C: control, M: methanol, E: Ethanol, ILE: Intravenous lipid emulsion

Experimental Design and Group Allocation

A total of 64 male rats were randomly divided into seven groups. The first group consisted of 8 rats, groups two through five included 10 rats each, and the sixth and seventh groups contained 8 rats each.

Group 1: Control (Saline): Rats received an oral dose of saline solution (1.5 mL/kg) once daily.

Group 2: Methanol (M): Rats were administered methanol orally at a dose of 4 g/kg, diluted to 50%, once daily.

Group 3: M + E: Rats received oral methanol at a dose of 4 g/kg which was diluted to 50%, and 1 hour later, oral ethanol was administered via gavage at a dose of 1 g/kg which was diluted to 50%.

Group 4: M + ILE: Rats were given oral methanol at a 4 g/kg dose, diluted to 50%, and then received ILE at a 1.5 ml/kg dose via the IV route. The procedure was repeated three times in total, with the initial dose given within the first 30 minutes following methanol administration, and the subsequent doses administered at 6-hour intervals.

Group 5: M + E + ILE: Rats were administered oral methanol at a dose of 4 g/kg, diluted to 50%, followed by oral ethanol via gavage at a dose of 1 g/kg, diluted to 50%, 1 hour later. Additionally, the rats received ILE at a dose of 1.5 ml/kg via the IV route, which was repeated 3 times in total. The first dose was given within the first half hour after methanol ingestion, followed by doses every 6 hours.

Group 6: E: Rats were given oral ethanol via gavage once daily at a dose of 1 g/kg, which was diluted to 50%.

Group 7: ILE: Rats received ILE at a dose of 1.5 ml/kg via the IV route, which was administered within the first half hour and repeated every 6 hours, 3 times in total, within a single day.

In this study, Group 1 (control), Group 6 (ethanol), and Group 7 (ILE) initially comprised 10 male rats each. However, in line with the principle endorsed by the Local Ethics Committee for Experimental Animals, advocating for the minimal use of animals to avoid influencing study outcomes, the number of rats in these three groups had to decrease.

Collection of samples

The control group was exclusively administered saline, while ethanol, methanol, and ILE were administered to the remaining groups.

During the 5-day observation period following chemical administration, several notable events occurred. In the control group, the eighth rat exhibited a cystic structure in the right kidney, which necessitated the removal of the left kidney from the sample container and its inclusion in the study. In Group 3 (methanol + ethanol), blood sampling was not possible for the 4th and 10th rats on day 4 due to their deaths; however, organ samples were collected within 1–2 hours postmortem. In Group 5 (methanol + ethanol + ILE), the health of the 3rd rat deteriorated, leading to euthanasia on day 4, allowing for the collection of blood and organ samples.

After completion of chemical treatments, the rats were monitored for 5 days. Surviving animals were euthanized on day 5 by cervical dislocation (decapitation) without anesthesia, to prevent interference with biochemical measurements. The brain, eyes, optic nerves, liver, lungs, kidneys, heart, and testes were harvested for pathological examination. Among

Tablo 4. Comparison of retinal vacuolization (RVC) levels between groups

		Control a (n=8) (%)		M a,b (n=10) (%)		M+ILE b,c (n=10) (%)		E b,c (n=8) (%)		M+E a,b,c (n=10) (%)		M+E+ILE b,c (n=10) (%)		p
RVC	Negative	5	%62.5	0	(%0.0)	7	(%70.0)	1	(%12.5)	0	(%0.0)	2	(%20.0)	<0.001*
	Positive/ Weak	3	%37.5	0	(%0.0)	1	(%10.0)	7	(%87.5)	6	(%60.0)	8	(%80.0)	
	Positive/ Moderate	0	%0.0	4	(%40.0)	2	(%20.0)	0	(%0.0)	4	(%40.0)	0	(%0.0)	
	Positive/ Strong	0	%0.0	6	(%60.0)	0	(%0.0)	0	(%0.0)	0	(%0.0)	0	(%0.0)	

* Chi-square test (Post hoc: Bonferroni, p<0.008)
a p≤0.007 vs Control (C vs M for p<0.001, C vs M+ILE for p=0.207, C vs E for p=0.039, C vs M+E for p=0.007, C vs M+E+ILE for p=0.066)
b p<0.002 vs M (M vs M+ILE for p=0.002, M vs E for p<0.001 M vs M+E for p=0.002, M vs M+E+ILE for p<0.001)
c p≤0.006 vs M+ILE (M+ILE vs E for p=0.004, M+ILE vs M+E for p=0.004, M+ILE vs M+E+ILE for p=0.006)
(E vs M+E for p=0.086, M+ILE vs M+E+ILE for p=0.671)
(M+E vs M+E+ILE for p=0.043)
Abbreviations: C: control, M: methanol, E: Ethanol, ILE: Intravenous lipid emulsion

Table 5. Effect of interventions on serum levels of biochemical parameters

	AST (IU/L)	ALT (IU/L)	LDH (IU/L)	ALP (IU/L)	Urea (mg/dL)	Creatinine (mg/dL)	CK-MB (IU/L)
Control	223.75±39.253	66.38±7.708	1613.88±287.957	141.88±14.197	59.63±6.675	0.414±0.040	873.750±132.112
Group 2	215.70±20.881	79.30±10.625	1601.30±255.033	134.00±12.561	56.40±5.621	0.354±0.042	779.060±127.785
Group 3	176.13±24.920	53.00±33.522	1268.00±337.793	115.38±33.594	48.25±9.130*	0.336±0.054*	579.075±106.476*
Group 4	204.00±40.044	74.10±25.567	1341.70±296.622	170.40±61.134	52.70±5.376	0.357±0.031	618.370±167.858*
Group 5	191.30±39.920	75.10±20.415	1123.70±156.621*	145.20±28.794	58.20±6.697	0.340±0.049*	525.120±99.488*
Group 6	230.63±44.635	60.50±8.418	1633.00±304.212	120.00±25.879	51.63±6.209	0.347±0.035	778.263±149.275
Group 7	225.63±53.636	73.13±9.311	1509.63±378.065	157.13±35.942	47.88±5.055*	0.380±0.045	784.813±220.341
F/p values	2.26/0.051	2.06/0.073	4.30/0.001	2.82/0.018	4.44/0.001	3.35/0.007	6.95/0.000

*p<0.05; compared to control group, p<0.05; compared to group 2, p<0.05; compared to group 6, p<0.05; compared to group 3, p<0.05; compared to

these tissues, the liver, lungs, kidneys, heart, and testes were preserved for future studies, while analyses were performed on the brain, eyes, optic nerves, and biopsy samples.

Following decapitation, serum samples were promptly collected using a glass funnel for subsequent biochemical analysis. The glass funnels were washed and rinsed with saline, and each animal was subsequently allowed to dry. Blood collected in gel-separated tubes was kept at room temperature for approximately 30 minutes for clotting.

For biochemical analysis, each collected blood sample from each of the 7 groups was individually numbered and placed into blood centrifuge tubes. These were numbered from one to eight for Groups 1, 6, and 7; and from one to ten for the other groups. After the study concluded, the collected blood tubes were transported to the laboratory of the Department of Biochemistry for analysis. The samples were centrifuged at 2000 rpm and 4°C for 15 minutes and then stored at -40°C until analysis. Following centrifugation, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) levels were measured using a Beckman Coulter AU680 autoanalyzer.

Biochemical analyses

Liver function was assessed by measuring lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total protein levels. Kidney function was evaluated through urea and creatinine measurements. All analyses were conducted using a Beckman Coulter AU680 autoanalyzer.

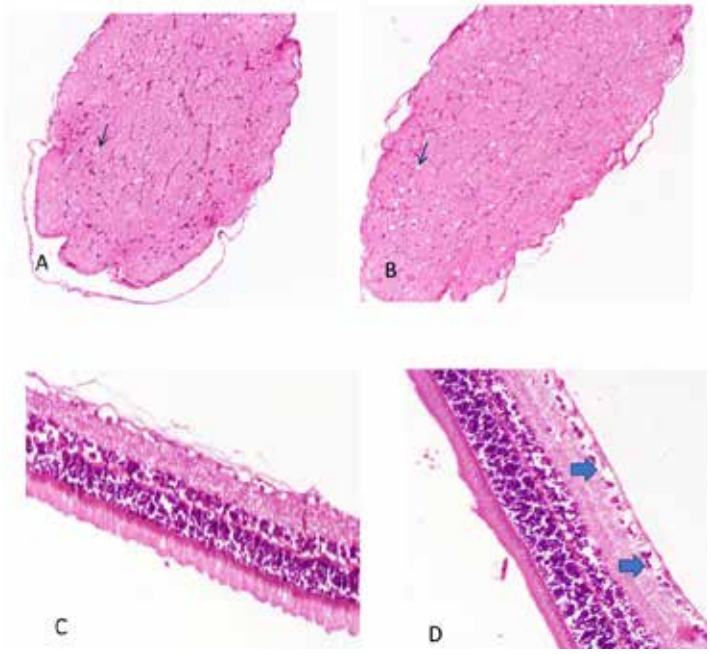

Figure 1A: Optic nerve vacuolization Hematoxylin & Eosin stain, x100 (indicated by the black arrow)

Figure 1B: Optic nerve vacuolization Hematoxylin & Eosin stain, x200 (indicated by the black arrow)

Figure 1C: Control group normal retinal tissue Hematoxylin & Eosin stain, x100.

Figure1D: Apopitotic changes in retinal tissue Hematoxylin&Eosin stain, x200 (indicated by the blue arrow)

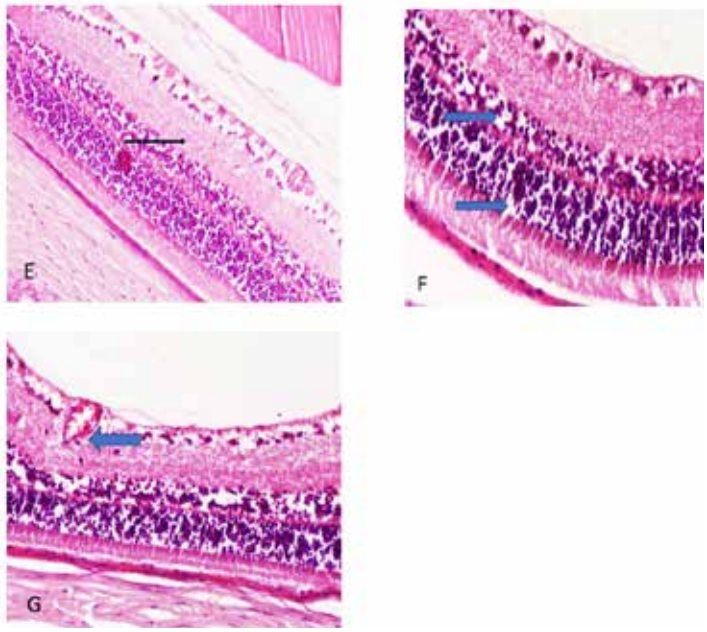

Figure 2E: Edema in retinal tissue Hematoxylin&Eosin stain, x100 (indicated by the black arrow)

Figure 2F: Vacoulization in retinal tissue Hematoxylin & Eosin stain, x200 (indicated by the blue arrow)

Figure 2G: Vascularization in retinal tissue Hematoxylin & Eosin stain, x200 (indicated by the blue arrow)

Histopathological Examinations

Tissue samples were immediately fixed in 10% buffered formalin after collection. Following fixation, the tissues were processed, embedded in paraffin, and sectioned into 4-micron-thick slices. The sections were then stained with hematoxylin and eosin (H&E) and examined under a light microscope to assess and document histopathological alterations.

Statistical Analysis

Biochemical data were analyzed using IBM SPSS Statistics Version 22.0 (SPSS Inc., Chicago, USA) and are presented as mean ± standard deviation. One-way ANOVA was applied to compare numerical variables across groups. Post hoc comparisons were performed using Bonferroni tests, except for ALP, where the Games-Howell test was used. A p-value of less than 0.05 was considered statistically significant.

Histopathological data analysis was conducted with IBM SPSS Version 27.0 (IBM Corporation, Armonk, NY, USA). Continuous variables are reported as medians with minimum and maximum values. Normality was assessed using the Kolmogorov-Smirnov test. Due to non-normal distribution, group comparisons were performed using the chi-square test. Pairwise post hoc analyses with Bonferroni correction identified specific group differences. A type I error rate of less than 5% indicated significance, with a Bonferroni-adjusted significance threshold of $p < 0.008$.

RESULTS

The results of histopathological examinations

A significant difference was observed in optic nerve vacuolization (ONVK) between the control and experimental groups ($p < 0.001$) (Figure 1A, B, C). Further analysis showed that ONVK levels in the methanol (M), methanol plus ILE (M+ILE), and methanol plus ethanol (M+E) groups were significantly higher compared to the control group ($p < 0.001$, $p < 0.001$, and $p = 0.001$, respectively). However, the differences between the methanol plus ethanol plus ILE group (M+E+ILE) and the control group ($p = 0.011$), as well as between the ethanol-only (E) group and control group ($p = 0.010$), did not reach statistical significance. Additionally, significant differences in ONVK were found when comparing the M group to the M+E+ILE group ($p < 0.001$), the M+ILE group to the M+E+ILE group ($p < 0.001$), the E group to the M+E+ILE group ($p = 0.003$), and the M+E group to the M+E+ILE group ($p = 0.003$) (Table 1).

Optic nerve vascularization (ONVS) levels were significantly

different between the control and experimental groups (p<0.001). When the group from which the difference originated was analyzed, it was determined that only the M group showed a statistically significant difference in terms of ONVS levels compared to the control group (p<0.001). In contrast, the other experimental groups did not show significant differences in terms of ONVS levels compared to the control group (p=0.090, p=0.302, p=0.019, respectively). There were significant differences between the M group and the M+ILE, E, M+E and M+E+ILE groups in terms of ONVS (p=0.003, p=0.001, p=0.005 and p<0.001, respectively). There were no statistically significant differences between the other experimental groups (p>0.05) (Table 2).

No optic nerve inflammation was observed in any group. Retinal edema levels were significantly different between the control and experimental groups (p=0.010), figure 2(E). When the group from which the difference originated was analyzed, it was determined that only the M and M+E groups showed a statistically significant difference in terms of retinal edema levels compared to the control group (p=0.002 and p=0.004, respectively), while the other experimental groups did not show significant differences in terms of retinal edema levels compared to the control group (p=0.375, p=0.248, p=0.375, respectively). There were significant differences between the M group and the M+ILE, E, and M+E+ILE groups in terms of retinal edema (p=0.002, p=0.007, and p=0.002, respectively). There were no statistically significant differences between the other experimental groups (p>0.05) (Table 3).

Retinal vacuolization (RVC) levels were significantly different between the control and experimental groups (p<0.001), figure 2(f). When the group from which the difference originated was analyzed, it was determined that only the M and M+E groups showed a statistically significant difference in terms of RVC levels compared to the control group (p<0.001 and p=0.007, respectively), while the other experimental groups did not show significant differences in terms of RVC levels compared to the control group (p=0.207, p=0.039, p=0.066, respectively). There were significant differences in the RVC between the M group and the M+ILE, E, M+E and M+E+ILE groups (p=0.002, p<0.001, p=0.002 and p<0.001, respectively), and there were significant differences between the M+ILE group and the E, M+E and M+E+ILE groups (p=0.004, p=0.004 and p=0.006, respectively). There were no statistically significant differences between the other experimental groups (p>0.05) (Table 4).

Retinal vascularization (RVS) levels were significantly different between the control and experimental groups (p<0.001), figure 2(G) . When the group from which the difference originated was analysed, it was determined that only the M and M+E groups showed a statistically significant difference in terms of RVS levels compared to the control group (p=0.001 and p=0.002, respectively), while the other experimental groups did not show significant differences in terms of RVS levels compared to the control group (p=0.180 and p=0.131, respectively). In terms of the RVS, there were significant differences between the M group and the M+ILE, E and M+E+ILE groups (p=0.005, p=0.007 and p<0.001, respectively), and there was a significant difference between the M+E group and the M+E+ILE group (p=0.001). There were no statistically significant differences between the other experimental groups (p>0.05).

It was found that retinal apoptotic change (RAC) levels showed a significant difference between the control and experimental groups (p<0.001), figure 1(D). When the group from which the difference originated was analysed, it was determined that only group M showed a statistically significant difference in terms of RAD levels compared to the control group (p=0.007), while the other experimental groups did not show significant differences in terms of RAD levels compared to the control group (p=0.357). There were significant differences between the M group and E, M+E and M+E+ILE groups in terms of RAD levels (p=0.007, p=0.003 and p=0.003). There were no statistically significant differences between the other experimental groups (p>0.05).

Results of Biochemical Analyses

Serum biochemical parameters in the rats are summarized in Table 5. No significant differences were observed among the groups for liver enzymes AST and ALT. However, LDH levels were significantly lower in the methanol plus ethanol plus ILE group (M+E+ILE) compared to the methanol-only (M) and ethanol-only (E) groups (p = 0.011 and p = 0.010, respectively). ALP levels were significantly higher in the methanol plus ILE group (M+ILE) than in the methanol plus ethanol group (M+E) (p = 0.030).

Regarding kidney function, urea levels were significantly decreased in the ILE-only and M+E groups compared to the control group (p = 0.013 and p = 0.018, respectively). Conversely, the M+E+ILE group showed a significant increase in urea levels relative to both the ILE-only (p = 0.029) and M+E groups (p = 0.041). Creatinine levels were significantly lower in the M+E and M+E+ILE groups compared to controls (p = 0.013 and p = 0.012, respectively).

The cardiac marker CK-MB was significantly reduced in the M+E, M+ILE, and M+E+ILE groups compared to the control group (p = 0.004, p = 0.012, and p < 0.001, respectively). Additionally, the M+E+ILE group had significantly lower CK-MB levels than the M-only (p = 0.006), E-only (p = 0.013), and ILE-only (p = 0.010) groups.

DISCUSSION

In this study, the effect of ILE on methanol-induced optic neuropathy and retinal damage was histopathologically examined in rats. ILE administration to methanol-poisoned rats resulted in a significant reduction of methanol-induced retinal damage and preservation of retinal structure. Additionally, we observed that ILE may positively affect reducing cardiac damage.

The number of histological studies on methanol toxicity in humans is limited to postmortem studies (8). Animal models were used to gather some important information on the subject (9).

Accumulation of formate leads to the inhibition of cytochrome oxidase activity the inhibition of oxygen utilization by mitochondria and decreased aerobic ATP production. Formate can impair mitochondrial energy production, causing toxicity in the retina and optic nerve (10). According to a study by Chen et al., histopathologic changes caused by methanol toxicity start in the outer layers of the retina, especially the photoreceptor layer, as mitochondria are damaged and spread to the inner layers. The photoreceptor layer is highly sensitive to formate-induced toxic damage and is the main target of methanol toxicity. There is marked axonal vacuolization in the prelaminar region of the optic nerve, edema of the oligodendroglia, and damage to the myelin sheath. Axonal vacuolization also occurs in the laminar and postlaminar layers (11).

Vacuolization in the retinal pigment epithelial layer is a prominent finding. In photoreceptors, there is edema in the inner parts, fragmentation in the outer parts, and vacuolization in the inner and outer segment junction (10,12).

According to a study conducted by Rashed et al. on rats that were exposed solely to methanol, it was observed that there was significant edema in the outer nuclear layer, fragmentation in the outer segment of the photoreceptor layer, and vacuolization in the inner segment. However, the histological structure remained intact among the group receiving methanol and ethanol. Moreover, less edema and vacuolization were observed in the retinal layers of this group (13). In our study, significant edema, vascularization, apoptotic changes, and vacuolization were detected in the retinal tissue in the methanol group, while these effects were not different in the methanol + ILE and methanol + ILE +E groups compared to the control group. Again in this study, marked vascularization and vacuolization were observed in the optic nerve in the methanol group, while these changes were not significant in the group in which methanol, ILE and ethanol were given together.

Intravenous lipid emulsion (ILE) is also utilized to treat toxicities from lipophilic drugs, herbicides, pesticides, and local anesthetics. The most widely accepted mechanism of ILE's action is the "lipid sink" phenomenon, as proposed by Weinberg. Additionally, ILE may theoretically enhance ATP production by replenishing the reduced intracellular fatty acid content in cardiomyocytes affected by local anesthetic toxicity (7,14,15).

Assuming that ILE may theoretically contribute to ATP production in cardiomyocytes by increasing the intracellular fatty acid content and may have a positive inotropic effect by increasing the intracellular calcium level, we can attribute the lower CK-MB level to the M+E+ILE combination (16). Fomepizole and ethanol, which are competitive inhibitors of alcohol dehydrogenase, are used in the first-line treatment of methanol toxicity. Fomepizole has a greater affinity for alcohol dehydrogenase than ethanol, but ethanol is preferred as an antidote in developing countries due to its high cost and poor availability (17,18).

Due to the similarity between methanol-induced optic nerve damage and optic neuritis, glucocorticosteroids are used to treat Me-ION (19,20). Erythropoietin, a glycoprotein that stimulates red blood cell differentiation, is also used in Me-ION due to its antioxidant and antiapoptotic effects (21). Pakravan et al. reported that intravenous erythropoietin administered in combination with high-dose steroids provided structural and functional improvement in vision in patients with methanol-induced optic neuropathy (22). In recent years, taxophylline, rutin, and TEMPOL, which have antioxidant properties, have been used in animal studies to treat Me-ION (23-25).

CONCLUSION

In conclusion, ethanol and ILE did not significantly affect liver or kidney function in methanol-intoxication rats. Although histopathologically, the combination of M+E+ILE reduced the adverse effects of methanol on the optic nerve and retina, it is not possible to conclude that ILE may have a role in the treatment of methanol intoxication based on a single study. Further studies are needed to evaluate the efficacy and possible side effects of ILE in the treatment of methanol toxicity.

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Peer-Review

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Conflict of Interest

The authors declare that they have no conflict of interests regarding content of this article.

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Ethical Declaration

Ethical permission was obtained from the Balıkesir University, Medical Faculty Research Ethics Committee for this study with decision no. 2022/1-15, and Helsinki Declaration rules were followed to conduct this study.

Authorship Contributions

Concept: HY, MC, ÖB Design: HY, MC, ÖB Supervising: Ö: GK AAP Financing and equipment: HY, MC Data collection and entry: HY, MC, GT, AAH, MHY Analysis and interpretation: HY, MC, GT, AAH, MHY, Literature search: HY, MC, GT, AAH, MHY Writing: HY, MC, Critical review: HY, MC, ÖB, GT, CG, AAH, MHY

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