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The effects of the pulsed magnetic field on sepsis-induced liver tissue injury in rats

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Abstract. Sepsis is the host's response to infection and can lead to severe and life-threatening cases. We aimed to investigate the effects of pulsed magnetic field (PMF) on septic liver tissue injury. A total of 28 adult Wistar albino rats were divided equally into four study groups: Sham, PMF-1, PMF-2, and Sepsis, with seven rats in each. Sepsis was performed using the CLP method. PMF-1 and PMF-2 were exposed to 7.5 Hz and 15 Hz PMF, respectively, for 24 hours. After having their livers removed, liver tissues were analysed using histological techniques. We observed remarkable healing in PMF groups. Apoptotic cells decreased in the PMF-treated groups compared with the Sepsis group ($p < 0.05$). Immune expressions of Acas-3, Bax, and HIF-1 increased in the Sepsis group, while Bcl-2 expression decreased ($p < 0.05$). The results imply that PMF application has anti-apoptotic, anti-inflammatory, and therapeutic effects on septic liver tissue injury.

Key words: Apoptosis — Inflammation — Liver — Pulsed magnetic field (PMF) — Sepsis

Highlights

- PMF treatment inhibits apoptotic cell death.
- PMF application upregulates anti-apoptotic marker protein Bcl-2 expression and downregulates apoptotic marker proteins like Acas-3 and Bax expression.
- PMF exposure has anti-inflammatory effects by reducing HIF-1α expression.

Introduction

Sepsis is a condition created by entering toxin substances and microorganisms into the bloodstream and the response of host cells to the resulting inflammation (Bone et al. 1992). Sepsis is a difficult-to-treat clinical condition that still has a high mortality rate. Severe conditions such as septic shock, organ dysfunctions, and multiple organ failures that can ultimately lead to the death of an organism occur in sepsis (Greco et al. 2017; Purcarea and Sovaila 2020). Although many treatment protocols are applied in the fight against sepsis, deaths from sepsis are still high in intensive care units (Liu et al. 2017). Cell hypoxia and apoptosis are among the most prominent causes of sepsis-related deaths, as they can lead to organ dysfunction (Ferreira et al. 2021). A multi-organ deficiency is one of the most critical factors causing death in sepsis. The liver, kidneys, lungs, heart, central nervous system, and hematologic system are the most frequently affected in the septic picture (Caraballo and Jaimes 2019).

The liver is an organ that contains large circulating vessels and has heterogeneous cell diversity. The liver performs many essential functions with vital values for the organism, such as metabolic functions, cleaning of the blood, regeneration of some blood cells, storage of vitamins and iron, detoxification of drugs and toxins, mediation of toxic substances, production of bilirubin and bile, production

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of blood factors and complements (Trefts et al. 2017). As sepsis progresses, this organ is severely injured and cannot fulfill its vital functions, which ultimately brings the organism to the verge of death (Woznica et al. 2018). Histopathological findings such as portal mononuclear cell infiltrates, Kupffer cell hyperplasia, steatosis, focal hepatocyte changes, and intrahepatic cholestasis are common in the liver with sepsis (Woznica et al. 2018; Garofalo et al. 2019) . The deterioration of the histological architecture of the liver will not only affect itself but will adversely affect the whole organism. Therefore, implementing timed and effective treatment methods before the liver damage in sepsis becomes irreversible will protect the liver and ensure the survival of the whole organism.

Understanding the histopathology and pathophysiology of septic organs might help optimize patient management and provide valuable targets for expanding new therapies (Lelubre and Vincent 2018). The effects of impaired cellular functions, such as mitochondrial and altered cell-death mechanisms, on the progression of organ dysfunction, are starting to be understood (Lelubre and Vincent 2018; Cao et al. 2019). Various cell death routes, like necrosis, apoptosis, necroptosis, pyroptosis, and autophagy-induced cell death, can be triggered during sepsis (Galluzzi et al. 2012). Many cell death pathways are altered in sepsis, directly affecting the pathophysiology of sepsis and its accompanying inflammation or *via* direct interaction with pathogens (Pinheiro da Silva and Nizet 2009; Lelubre and Vincent 2018). The reorganization of these cellular functions is regulated by different transcriptional factors (Hirota and Fukamizu 2010).

Hypoxia-inducible factor (HIF)-1α is frequently encountered and promotes oxidative stress and inflammation (Guan et al. 2018). HIF-1α is a nuclear transcriptional factor associated with oxygen insufficiency, and hypoxia regulates several cellular mechanisms like apoptosis (Greijer and van der Wall 2004). The effect of HIF-1α on apoptosis arises through the mitochondrial pathway, which includes the down-regulation of the anti-apoptotic protein Bcl-2 and the up-regulation of apoptotic molecules like Bax, Caspase-3, and Caspase-9 (Greijer and van der Wall 2004; Karatas et al. 2020).

The therapeutic effects of electromagnetic fields began with treating bone fracture through current passing through a needle inserted into the tissues near the broken bone in 1812 (Steinberg 1993; Furse et al. 2009). The ions of each tissue cell can be affected by the electromagnetic field change around them during their biochemical and physiological activities. Pulsed magnetic field (PMF) can serve as electromagnetic stimulation without harmful side effects due to its low energy level. At the same time, it has been regarded that PMF helps the treatment by accelerating blood circulation with the ionic activities in the tissues (Adey 1993; Bassett 1993).

In the literature, we could not find any study regarding the liver tissue damage of an organism with sepsis treated with PMF. As can be understood from the information described above, electromagnetic fields have therapeutic effects on living systems. In addition, after being exposed to electromagnetic fields, cell division rate, mRNA and protein synthesis levels, cell membrane permeability and changes in Ca^{2+}, Na^{+}, and K^{+} ion transfers, changes in ion/ligand binding on the cell surface, wound healing, blood circulation rate was observed generally positively affected in the living organism (Bassett 1993; Shupak et al. 2003). In light of this literature information, we hypothesized that PMF might have protective and therapeutic properties in sepsis-induced liver tissue injuries.

Several methods are used against sepsis, including drugs, chemicals, and antioxidants. When treatment with such substances is not possible or sufficient, alternative treatment options may be required. There is no study in the literature in which PMF is applied to treating sepsis, especially sepsis-induced liver damage. Therefore, the aim of this study is to investigate the therapeutic effect of PMF on liver tissue damage caused by sepsis. We hope that the results obtained from our study will contribute to research on this subject, and PMF may be thought of as an alternative supplementary treatment in clinical practice.

Materials and Methods

Experimental animals

The study was conducted on 28 Wistar albino rats, aged 2.5 months and weighing 250 g, obtained from the Animal Experimentation Research Unit of Tokat Gaziosmanpaşa University (DETAB). After the Tokat Gaziosmanpaşa University Animal Experiments Local Ethics Committee approved Decision No. 51879863-220, we started the study. The rats were randomly divided into four groups of seven rats each, as follows: Sham, PMF-1 (sepsis + 7.5 Hz PMF), PMF-2 (sepsis + 15 Hz PMF), and Sepsis. Rats were kept in standard polycarbonate plastic cages on sawdust beds during the experiment on a 12-hour day-night cycle in a stable environment at 20–22°C and 50–60% relative humidity, with free access to food and water.

Experiment procedure

The rats' abdominal wall was incised in the Sham group to see the cecum and reinserted. Twenty-four hours later, after suturing, the rats were sacrificed. The lower part of the cecum was tied tightly with a thread, and some little feces was allowed to come out through a hole opened at the end of the cecum with a 2cc syringe needle to make the sepsis

model with the cecal ligation perforation method (CLP), as seen in Figure 1. Five hours after sepsis was induced, the cecum went entirely black. So the rats were successfully made septic. The rats in Sepsis group were kept in a Faraday cage for 24 h without PMF exposure. The rats of PMF-1 and PMF-2 groups were left in a Faraday cage under the exposure of 1 mT 7.5 Hz PMF and 1 mT 15 Hz PMF to treatment for 24 h, respectively. At the end of the experiment, the rats were sacrificed, and their livers were removed.

PMF exposure

Five hours after sepsis was induced the rats were placed into plastic boxes (26×17×13 cm) and located in the center of a pair of Helmholtz coils (60 cm in diameter, placed 30 cm apart). Coils were constructed by electrically and thermally insulated copper wire of 2.5 mm diameter with 50 turns (Resistance: 0.78 Ω, inductance: 8.8 mH). The time-varying magnetic field consisted of a quasi-triangular waveform with a rise time of 0.3 ms and a fall time of 9.7 ms, as previously described Gul et al. (2018). The corresponding induced peak electrical field value in the plastic box between the Helmholtz coils was 0.24–0.27 V/m, calculated based on Faraday's law (Mert et al. 2010; Gul et al. 2018). The Helmholtz coils' magnetic field values and the ambient were measured before application using a Tesla meter equipped with a Hall-effect probe (Sypris 6010; F.W. Bell, CA, USA). The Helmholtz coils were placed in a 90×90×55 cm grounded Faraday cage to minimize the effect of environmental fields and keep the researcher away from the magnetic field. The magnetic fields created in Helmholtz coils were homogeneously distributed. These coils create a magnetic field with peak amplitude of 1 mT once connected to a signal generator (ILFA Electronic, Mersin, Turkey). The rats were left with PMF exposure for the same duration to ensure the same physiological effects. PMF application was carried out with two different pulses, one with a 7.5 Hz frequency and magnetic flux density of 1 mT and the other with a 15 Hz frequency and magnetic flux density of 1 mT, for 24 h in a silent room with a temperature of 22–24°C and 50–60% relative humidity conditions monitored throughout the experiment. The experiments detected no considerable temperature changes between two activated Helmholtz coils. The International Commission on Non-Ionizing Radiation Protection (ICNIRP), the American Industrial Hygienists Association (ACGIH), and the European Union (EU) have all approved the dose. In some different studies, 7.5 and 15 Hz of 1 mT PMFs applications have promoted cell proliferation and differentiation, as well as tissue healing and bone mass enhancement (Chang et al. 2006; Zhai et al. 2016; Wang et al. 2017), so we administered these two different frequencies of PMF at 1 mT magnetic flux density.

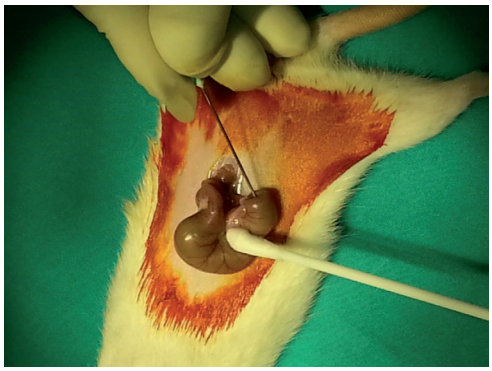

Figure 1. A representative picture of rats undergoing sepsis with Cecal Ligation Perforation (CLP) in the study groups.

Histological analyzes

Liver samples were fixed in 4% buffered neutral formalin for 72 h, washed under running tap water for 12 h, dehydrated through ascending alcohol series from 70% to absolute, cleared in xylene series, and embedded in paraffin blocks at 60°C. Serial sections were cut at 5 µm thickness from the liver samples embedded in the paraffin blocks with a rotary microtome (Leica RM2135, Wetzlar, Germany). Tissue sections were taken on lams covered Poly-L-Lysine and stained according to hematoxylin, eosin, triple, tunnel, and immunohistochemical staining protocols.

Immunohistochemistry

An indirect immunohistochemistry protocol for formalin fixed paraffin embedded rat tissues was conducted on the liver sample sections to detect immune expression of some apoptosis pathway molecules (Bax, Bcl-2, and Acas-3) and hypoxia-inducible factor alpha (HIF-1α). In brief, liver tissue sections were mounted on slides, deparaffinized in etüve at 60°C and xylene series, and rehydrated through descending alcohols (from absolute to 70% and distilled H_2O). After antigen retrieval with citric acid (10 mM), the sections were incubated in 3% hydrogen peroxide (H_2O_2) to quench endogenous peroxidase activity washed in phosphate buffer solution (PBS) (3×5 dak) and encircled by a pap pen (hydrophobic pen). The slides were incubated in 1% bovine serum albumin (BSA) for 15 min to block them. Removed the BSA without washing, and the slides were incubated with primary antibodies to Acas-3, Bax, and Bcl-2 (1:50, Santa Cruz) and HIF-1α (1:100) at 4°C in a humidified and dark environment overnight. Having been washed with PBS (3×5 min), the samples were incubated with biotinylated secondary antibody (Vector Laboratories, USA) at room temperature in a humidified dark environment for 45 min. They were washed with PBS (3×5 min) and incubated with

horseradish peroxidase (HRP)-labeled streptavidin secondary antibody (GE Healthcare, UK) at room temperature for 30 min. Samples were incubated with amino ethyl carbazole (AEC, ScyTek Laboratories Inc.) chromogen after washing with PBS (3×5 min) to visualize immunoreactivity. Sections were counterstained with hematoxylin and were mounted under a coverslip in an aqueous mounting reagent (Invitrogen, Carlsbad, CA) after washing in distilled water. In some slides, PBS was dropped onto the sections instead of a primary antibody for the negative control, and no immunostaining was observed.

Randomly selected five areas in each section were analyzed under the light microscope at 400× magnification (Nikon Eclipse E200) using the NIS-Elements software program (Hasp ID: 6648AA61; Nikon) to define the immune-stained cells. The immune staining intensity of these molecules was graded at four categorical levels to determine the immune expression levels of the molecules. The criteria of the immunostaining scoring scale are in Table 1. The cells were counted categorically according to these criteria, and immunostaining intensities were defined. The results were converted into H-score values, a semiquantitative evaluation system of immunohistochemistry, using the formula [∑Pi(i + 1)]. In this formula, i is the staining intensity score, and Pi is the percentage of stained cells. The mean immunostaining H-scores of the groups were accounted for and compared statistically.

TUNEL staining

The terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) technique was used to identify apoptosis resulting from DNA fragmentation in the liver tissue of the study groups. The formalin-fixed paraffin-embedded liver tissue sections were stained according to the standard Tunel kit (In Situ Cell Death Detection Kit, AP; Roche) protocol as follows: After deparaffinization and rehydration, the sections were subjected to microwave irritation in citrate solution (pH 6.0) for 5 min and incubated in Tris-HCL (0.1 M, pH 7.5) at room temperature for 30 min. After washing with PBS (3×5 min) and drying with a paper towel were encircled with a pap pen (Liquid Blocker, Japan), then the tunnel reaction mixture (vial1+vial2) was dropped on the sections. They were incubated at 37°C in a moist and dark environment for 30 min. Except for vial1, only vial2 was dropped on a few sections for the negative control. After washing with PBS (3×5 min), slides were incubated in a dark, humidified chamber for 30 min before being washed with PBS (3×5 min). Substrate solution (Fast Red) was dropped onto the samples, incubated for 10 min at room temperature in the dark, and then washed with PBS. After counterstaining with hematoxylin, we mounted the slides under a coverslip in an aqueous mounting reagent (Invitrogen, Carlsbad, CA). Cells with bright red nuclei were considered apoptotic. To calculate the apoptotic cell index, we counted TUNEL-positive cells at least *per* 500 cells in 5 microscopic fields under a 40× objective.

Hematoxylin-eosin staining

Formalin-fixed paraffin-embedded liver tissue sections were deparaffinized in xylenes (3×5 min) and rehydrated in descending alcohol series (from 100 to 70%) and distilled water, then incubated in hematoxylin for 10 min. After being washed with running tap water for 5 min, dipped in acid alcohol, and put in distilled water, the slides were incubated in eosin solution. The sections were immersed in distilled water to remove excess dye, dipped in 80% alcohol, and left in 90%, 96%, and 100% alcohol series for 2, 3, and 5 min, respectively. They were cleared in xylene (3×10 min) and mounted under a coverslip. After drying, the sections were analyzed histologically through a light microscope.

Triple (Modified Masson Trichrome) staining

The sections were deparaffinized in xylene series (3×5 min) after being melted in etüve at 60°C and rehydrated in descending alcohols (100 to 70%) and distilled water (2×5 min). The slides were incubated in Weigert's

Table 1. Immunohistochemical staining intensity H-scores values of Acas-3, Bax, and Bcl-2 proteins in rat liver tissues of the study groups.

	Sham	PMF-1	PMF-2	Sepsis	*p*	*F*	*df*	*n*
Acas-3	7.33 ± 2^{a}	$32.68 \pm 12^{b,**}$	$39.40 \pm 12^{b,**}$	87.70 ± 22^{c}	0.001	53.2	3	7
Bax	25.29 ± 14^{a}	$68.52 \pm 30^{b,**}$	$60.76 \pm 20^{b,**}$	168.88 ± 14^{c}	0.001	86.6	3	7
Bcl-2	$170.41 \pm 51^{a,**}$	96.78 ± 30^{b}	$143.55 \pm 38^{a,**}$	72.49 ± 25^{b}	0.001	13.8	3	7

Data are means ± SD. Letters on the values express statistical similarities and differences for each molecule in the same line (different letters: differences, same letters: similarities). ** statistical differences for each molecule from other groups without asterisks in the same row; *n,* the number of rats. Acas-3, $p = 0.732$ for PMF-1 *vs.* PMF-2; Bax, $p = 0.841$ for PMF-1 *vs.* PMF-2; Bcl-2, $p = 0.395$ for Sham *vs.* PMF-2, $p = 0.483$ for PMF-1 *vs.* Sepsis. PMF, pulsed magnetic field; PMF-1, sepsis + 7.5 Hz PMF group; PMF-2, sepsis + 15 Hz PMF group.

iron hematoxylin solution for 10 min, then dipped in distilled water after washing with tap water for 5 min. The slides were incubated in acid fuchsin solution for 1 min, immersed in distilled water, then left in the phosphotungstic acid solution until the connective tissue was whitened. Sections were immersed in distilled water and left in an aniline blue staining solution for 30 s. Slides were mounted after treatment with gradually ascending ethanol series (80, 90, 96, 100%) and xylenes (3×5 min) to be ready for microscopy.

Statistical analysis

The program IBM SPSS 20 Windows Statistics package (IBM Co., Somers, NY, US) was used for the statistical analysis. After testing with Kolmogorov-Smirnov for homogeneity of variances, we analyzed the data with One-way ANOVA. Then, comparisons were made between groups with Tukey's honestly significant difference (Tukey HSD) and Tamhane tests from the *post hoc* multiple comparison tests. The standard deviation was used to express variability in the

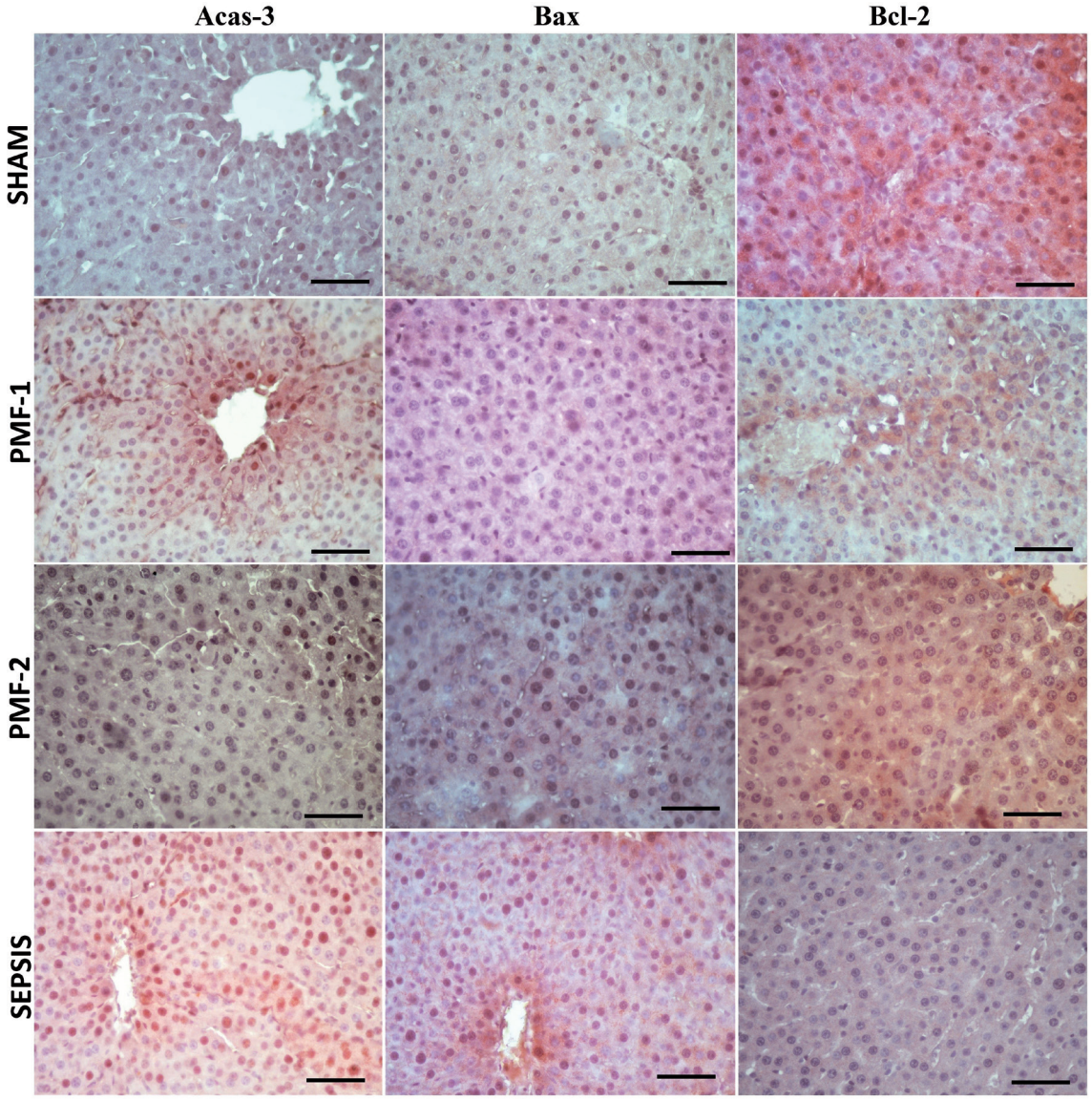

Figure 2. Representative photomicrographs of immune staining of the apoptotic (Acas-3, Bax) and antiapoptotic (Bcl-2) proteins are shown in the liver tissues of the study groups. Acas-3 and Bax's immunohistochemical expressions are stronger in Sepsis than in the other groups. In groups PMF-1 and PMF-2, a considerable decrease has been seen *versus* Sepsis group. The immunohistochemical expression of Bcl-2 in the groups is *versa verse* with that of Acas-3 and Bax expression (scale bars: 50 μm). PMF, pulsed magnetic field; PMF-1, sepsis + 7.5 Hz PMF group; PMF-2, sepsis + 15 Hz PMF group.

data. The outcome was considered significant if a p value less than 0.05.

Results

Immunohistochemistry results

Bax and Acas-3 ($p < 0.01$, $n = 7$, F = 86.6 (Bax) and 53.2 (Acas-3), df = 3) immune expression, which are apoptotic pathway proteins, increased in the Sepsis group compared to the control (Sham group), and decreased in both PMF groups *versus* the Sepsis group ($p < 0.01$, $n = 7$, F = 86.6 (Bax) and F = 53.2 (Acas-3), df = 3). The decrease of Acas-3 immune expression was more significant in PMF-1 group (7.5 Hz PMF) and was statistically similar to the Sham and PMF-2 group (15 Hz PMF) ($p = 0.732$, $n = 7$, F = 53.2, df = 3). Both PMF administrations increased immune expression of the anti-apoptotic protein Bcl-2; only the 15 Hz PMF application was statistically significant. Immunohistochemistry H-score values and representative images of apoptosis markers Acas-3, Bax, and Bcl-2 have been shown in Figure 2 and Table 1. HIF-1α immune expression increased in the Sepsis group significantly compared to the control ($p < 0.001$, $n = 7$, F = 114.16, df = 3) and both PMF groups ($p < 0.01$, $n = 7$, F = 114.16, df = 3). Immune staining intensity of HIF-1α in groups PMF-1 and PMF-2 reduced compared with the Sepsis group ($p < 0.001$, $n = 7$, F = 114.16, df = 3). However, there were no statistical differences between groups PMF-1 and PMF-2 ($p = 0.283$, $n = 7$, F = 114.16, df = 3). Immunohistochemistry staining images of HIF-1α were illustrated in Figure 3A, and immune staining H-score mean values were shown in Figure 3B.

TUNEL results

In the microscopic analyzes of the TUNEL-stained preparations, we observed that the apoptotic cell index increased significantly in the Sepsis group compared to the other groups ($p < 0.01$, $n = 7$, F = 33.9, df = 3). In the PMF-1 and PMF-2 groups, the apoptotic cell index decreased in favor of the control, and both were statistically similar ($p = 0.630$, $n = 7$, F = 33.9, df = 3). Representative TUNEL staining

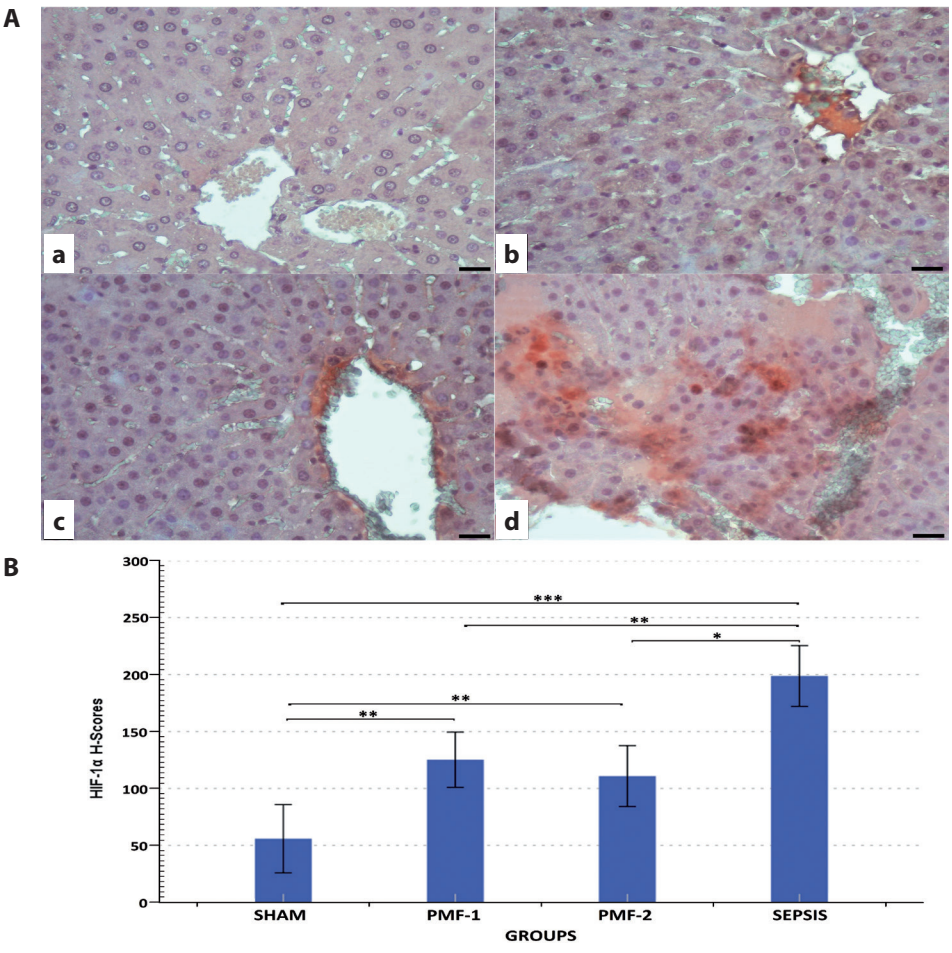

Figure 3. A. Representative immunohistochemistry staining images of the HIF-1α molecule in the study groups: Sham (**a**), PMF-1 (**b**), PMF-2 (**c**) and Sepsis (**d**). Immunohistochemistry staining density is stronger in Sepsis than in the other groups. In groups PMF-1 and PMF-2, a considerable decrease has been seen *versus* Sepsis group. Scale bars: 20 μm. **B.** H-score values of the HIF-1α immunohistochemistry in the study groups. One-way ANOVA and Tukey's tests were used. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, nonsignificant $p = 0.283$ for PMF-1 *vs.* PMF-2, $n = 7$, F = 114.16, df = 3. For abbreviations, see Figure 2.

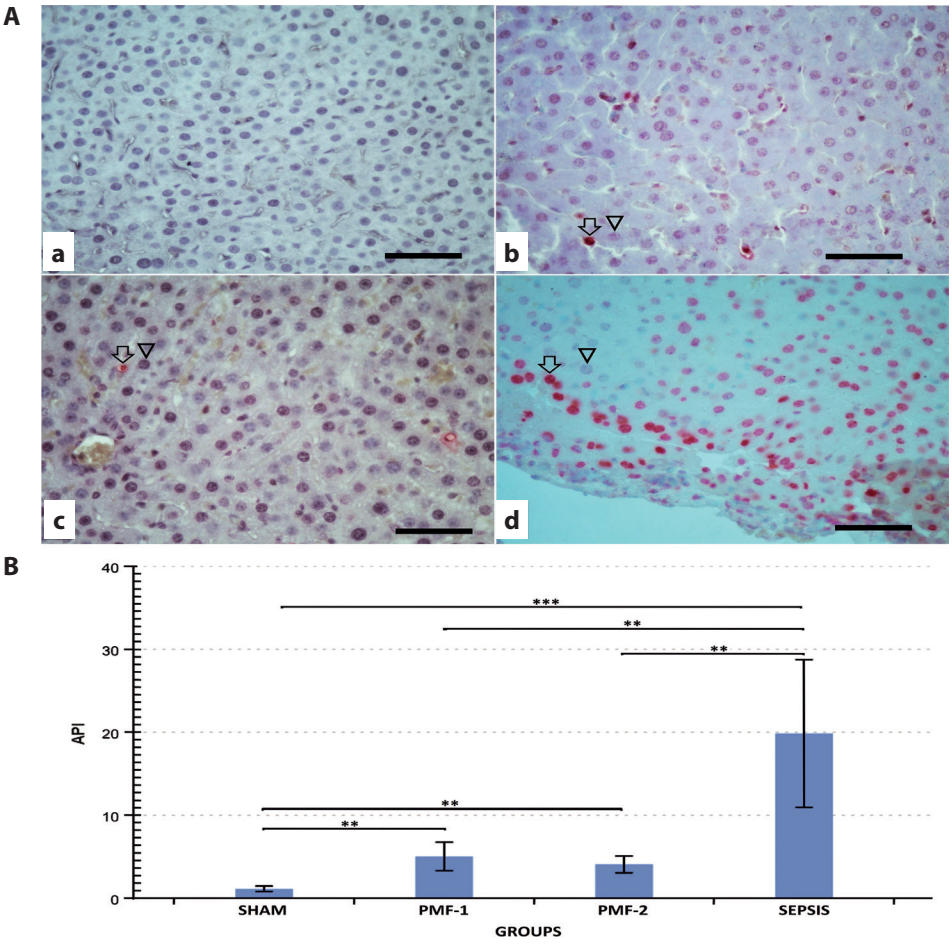

Figure 4. A. Micrographs show TUNEL labeling representative samples in the liver tissue of the study groups: Sham (**a**), PMF-1 (**b**), PMF-2 (**c**) and Sepsis (**d**). TUNEL-stained apoptotic cells appear pink-red. Arrow icons show a TUNEL-reactive apoptotic cell, and triangle indicators indicate one sample of non-stained normal cells (TUNEL-AP, scale bars: 50 μm). **B.** Apoptotic index (API) values of the groups. One-way ANOVA and Tamhane tests were used. ** $p < 0.01$, *** $p = 0.001$, non-significant $p = 0.630$ for PMF-1 *vs*. PMF-2, $n = 7$, F = 33.9, df = 3. For abbreviations, see Figure 2.

images are shown in Figure 4A, and mean apoptotic index values are given in Figure 4B.

Histopathologic results

In microscopic analysis, we observed that the Sham group of rats' liver tissue had a general histological structure. However, there were severe histological damages in the Sepsis group, such as sinusoidal and vascular congestion, hemorrhagic and necrotic areas, diffuse inflammatory cell infiltration, apoptotic cell characterized pyknotic nuclei, Remark cordon disarray, and some pale stained regions.

We determined the reduction of histopathological injuries in both PMF groups. Both PMF groups were almost similar appearances to each other (Fig. 5).

Discussion

Bacterial toxins and cytokines have a disproportionate activation in sepsis cases, a clinical condition characterized by a systemic inflammatory response against a focus of infection in the organism (Bone et al. 1992). The cytokines, especially tumor necrosis factor-alpha (TNF-α), interleukins (IL-1, IL-6, IL-10), and the complement system can cause damage of varying severity, ranging from systemic inflammation to septic shock. In this process, cardiovascular functions may be interrupted due to the deterioration of homeostatic balance and endothelial dysfunction (Shapiro et al. 2011). Depending on the impairment of physiological and biochemical mechanisms, intracellular hemostasis may also be disturbed (Morelli and Passariello 2016). It has been reported that apoptotic cell death associated with the mitochondrial pathway increases due to the increase of free radicals, reactive oxygen, and nitrogen derivatives in the parenchymal tissues of the liver, lung, kidney, and digestive system organs in sepsis cases. Cell death regulation is essential to the host's reaction to infectious stress (Bone et al. 1992; Pinheiro da Silva and Nizet 2009).

Sepsis can lead to severe consequences, up to the death of an organism, when it occurs in one of the primary vital organs, like the liver (Caraballo and Jaimes 2019; Zou et al.

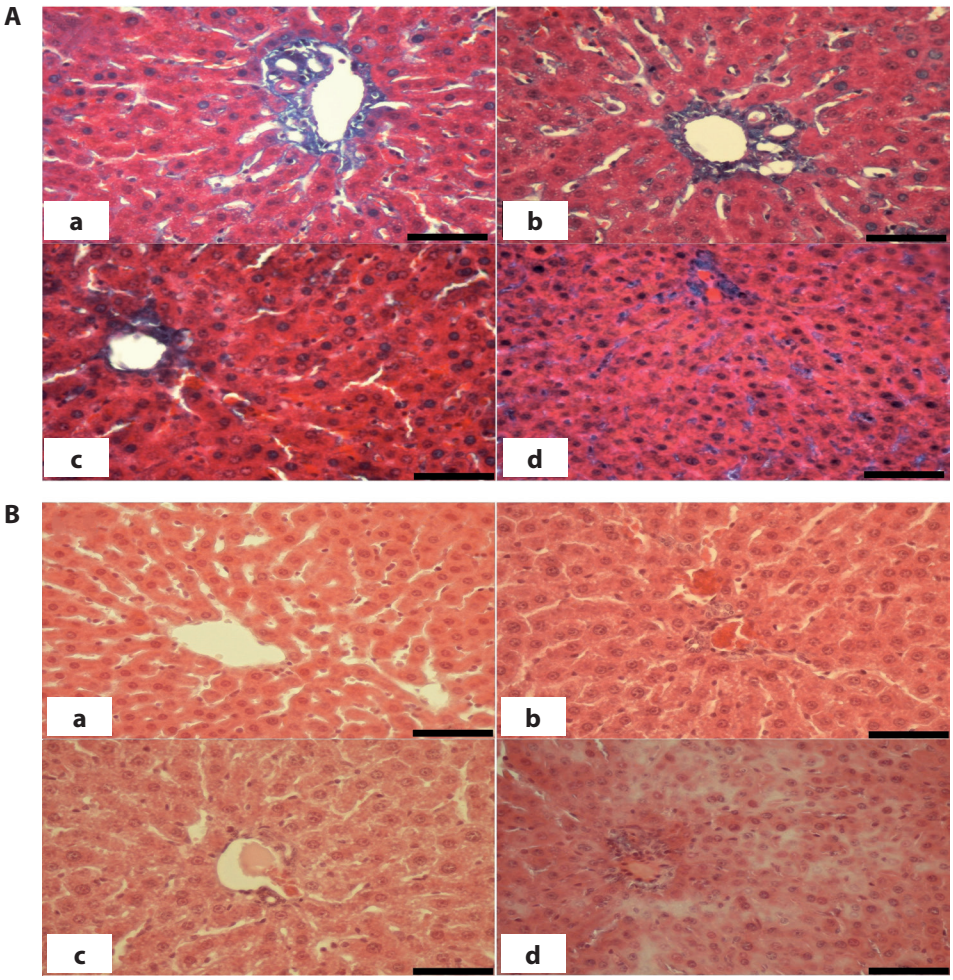

Figure 5. Representative microscopic images of modified Masons' trichrome (**A**) and hematoxylin-eosin (**B**) stained cross-sections of the rat liver tissues in the study groups: Sham (**a**), PMF-1 (**b**), PMF-2 (**c**) and Sepsis (**d**). Sham appears as a typical histological liver tissue structure. PMF-1 and PMF-2 have moderate tissue damage. Sepsis has severe tissue damage (scale bars: 50 μm). For abbreviations, see Figure 2.

2022). The liver plays the most substantial role at the organ level in sepsis. The liver provides clearance and detoxification of bacteria, endotoxins, and vasoactive substances formed during sepsis, and it also regulates the activities of cells involved in host defense. The liver is a source of inflammatory mediators and a target organ affected by these mediators (Yan et al. 2014). Histopathological changes in the liver tissues with sepsis are mostly intrahepatic cholestasis, Kupffer cells hyperplasia, portal mononuclear cell infiltrates, focal hepatocyte deformities, and steatosis (Garofalo et al. 2019; Zou et al. 2022). In this study, we observed similar histological injuries like sinusoidal and vascular congestion, hemorrhagic areas, inflammatory cell infiltrates, abundant necrotic and apoptotic cells characterized by necrotic and pyknotic nuclei, disruption of hepatocytes radial arrays in the Sepsis group. The livers of the Sham group rats had a typical general histological structure. Both groups receiving PMF experienced healing of the sepsis-related tissue damage.

Considering that sepsis is one of the most important causes of death in intensive care units, it is crucial to develop and apply effective treatment methods against it. Throughout the biochemical and physiological processes of the cells, electromagnetic changes in their surroundings impact the ions in each of the organism's cells. PMF is an electromagnetic stimulation without side effects due to low energy levels. It is assumed to accelerate treatment by accelerating blood circulation with ionic activity in the tissues (Vadalà et al. 2016).

Since sepsis is a condition that can lead to severe health consequences, there are many studies about treating sepsis. In those studies, drugs and chemical agents were generally used to treat tissue damage caused by sepsis (Thompson et al. 2019). In a study, one example, antibiotics prevented hepatic tissue damage in rats with CLP-induced sepsis was reported (Mert et al. 2014). In some cases where drug use is not possible or sufficient, alternative treatment methods may also be required.

In some different several studies, PMF has been utilized therapeutically to cure different pathological conditions such as diabetes, edema, inflammation, hyperalgesia, pain, oxidative stress and neuropathic symptoms (Bassett 1993; Mert

et al. 2010, 2014; Vadalà et al. 2016; Multanen et al. 2018). Coskun et al. showed that 50 Hz–1 mT PMF decreased the rat sciatic nerve's amplitude value and hyperpolarization time of action potentials (Coskun et al. 2011). Comlekci and Coskun stated that 50 Hz, 1 mT magnetic field exposure was helpful in more effective and safe physical therapy of the human median nerve (Comlekci and Coskun 2012). Bellossi et al. (1996) determined that a total of 96 hours (1 h application/1 h rest) of 6 mT 12 Hz PMF administration to rats reduced cholesterol and triglyceride levels in rats. Coskun and Comlekci (2013) found rising leukocyte cell numbers in rats after being subjected to PMF. It can imply that PMF activates the immune system. Xie et al. (2014) exposed the rabbits to PMF to repair anterior knee cruciate ligament injuries, and they detected a significant decrease in apoptotic cell index and Acas-3 expression levels. These results are similar to ours and indicate that PMF application increases cell survival by inhibiting apoptosis. Greenough suggested that PMFs may have achieved tissue healing through vascularization (Greenough 1992). In another study, PMF was used to treat shoulder cuff muscle inflammation in rats, and a significant improvement was observed (Binder et al. 1984). In a study, after crushing damage to the sciatic nerve, the rats were subjected to PEMF. At the end of the study, it was reported that PEMF could be helpful during the initial recovery phase (Bademoğlu et al. 2021). Another study evaluating the therapeutic effects of low-frequency pulsed electromagnetic fields on the recovery of neurological function following spinal cord injury in rats discovered that PEMF application facilitates the restoration of neurological functions by enhancing the differentiation of oligodendrocyte precursor cells into oligodendrocytes, promoting remyelination, inhibiting inflammation, and enhancing neurotrophic effects (Li et al. 2019).

Several studies observed increased apoptotic cell death, Bcl-2 gene suppression, and Bax and Acas-3 gene upregulation occurred in septic liver tissue cells, as we found in our current study result (Buchman et al. 1993; Hamada et al. 1999; Hotchkiss et al. 1999; Jao et al. 2001; Kobayashi et al. 2002; Maitra et al. 2005; Koskinas et al. 2008). Our present study also revealed a significant increase in HIF-1α immune expression, which induces cell death through the mitochondrial apoptotic pathway in the Sepsis group. These findings indicate that the rats developed sepsis after exposure to CLP in an experimental setting. After the rats were exposed to PMFs at 7.5 and 15 Hz mT frequencies, we found that the hepatocyte apoptotic index decreased along with Bax and Acas-3 immune expressions, but Bcl-2 and HIF-1α increased. These results imply the curative effect of PMF against sepsis-induced liver tissue damage.

The research mentioned above, and our study results have shown that PMF could have therapeutic effects by inducing blood circulation (Coskun and Comlekci 2013), slowing down macrophage-mediated intracellular signals, reducing Bax and Acas-3 expression, enhancing Bcl-2 expression (Chao and Korsmeyer 1998), downregulating HIF-1α expression, preventing the movement of the cytochrome-c from the mitochondria to the cytoplasm causing apoptosomes formations and inactivating the caspases (Renehan et al. 2001).

In conclusion, PMF application at different frequencies (7.5 or 15 Hz mT) to rats with sepsis-induced liver tissue injury inhibits apoptotic cell death, downregulates the apoptotic pathway molecules Acas-3 and Bax expression, and upregulates the anti-apoptotic pathway molecule Bcl-2. The PMF exposures also reduce HIF-1 expression, an inflammation marker molecule. Within the limits of the present study, it can be postulated that PMFs have anti-apoptotic, anti-inflammatory, and therapeutic effects on septic liver tissue. Further repetitive studies have to be conducted before PMF treatment of septic liver tissue injury could be considered as a supplementary approach in clinical practice.

Conflict of interest. The authors have no conflicts of interest to disclose.

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