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Preventive and therapeutic effects of the peel powder of *P. granatum* in a rat sepsis model

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ABSTRACT

The aim of the present study was to evaluate the treatment potential of *Punica granatum* L. peel powder in an experimentally induced sepsis model in rats. Sepsis was induced in 10-week-old, male, Wistar Albino (n=24) rats. The animals were divided into four groups: Sham-operated (S) Group, Control (C) Group, Treatment-1 (T1) Group, and Treatment-2 (T2) Group. To induce the sepsis model, the cecal ligation and puncture procedure was performed. The *P. granatum* peel powder (200 mg/kg; *per os*) was applied one hour before (T1) and 10 hours after (T2) surgery in a volume of 2 mL. At the end of the experimental procedure, microbial and histopathological analyses were performed. The histopathological scores on the liver, lungs, heart, kidney, spleen, and pancreas were evaluated. *Escherichia coli, Staphylococcus aureus* and *E. coli* + *S. aureus* were isolated from blood cultures. Severe bacteria were detected in the blood of the group C animals. It was found that there were fewer bacteria in groups T1 (n=2) and T2 (n=4) compared with group C. There were no lesions in the pancreas tissues of any groups. Vascular changes, degeneration, and necrosis were common in the organs in all cases of group C compared to group S. The findings in group T1 were similar to those in group C, however, it was seen in fewer animals. It was determined that there was a general improvement in group T2, and in addition the existing lesions were moderate in severity. In conclusion, *P. granatum* L. peel powder prevented CLP-induced lung injury in experimental rats. Thus, *P. granatum* L. peel powder may be an alternative therapeutic agent against lung tissue injury induced by sepsis. The recovery from inflammation was better in group T2 than in the other groups. According to the results of the current study, *P. granatum* peel powder was found to be effective in the treatment of sepsis with antimicrobial and anti-inflammatory functions.

Key words: cecal ligation and puncture; inflammation; *Punica granatum*; sepsis

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Introduction

Sepsis is a disease with a high incidence and mortality, resulting in death as a result of immune response disorder and circulatory and/or organ dysfunction due to infection. The original source of sepsis is an infection, and the path from infection to sepsis is a complicated pathophysiological process that includes invasion by pathogens, cytokine release, capillary leakage, and microcirculation disorder (KAUKONEN et al., 2014). Sepsis is the primary cause of death from infection, especially if not recognized and treated promptly. Diagnosis is important and urgent. Sepsis is a syndrome that is shaped by pathogen factors and host factors (sex, race and other genetic determinants, age, and environment) and its features develop over time. What distinguishes sepsis from infection is the presence of an abnormal or irregular host response and organ dysfunction. Sepsis-induced organ dysfunction may be occult, therefore, its presence should be considered in every patient presenting with an infection (SINGER et al., 2016). The underlying mechanism of tissue and organ dysfunction in sepsis is decreased oxygen delivery and oxygen use in cells as a result of hypoperfusion. Hypoperfusion occurs due to the cardiovascular dysfunction seen in sepsis. Impairment of the barrier function in the endothelium, vasodilation and increased leukocyte adhesion occur. This causes edema fluid to accumulate in the interstitial spaces, body cavities, and subcutaneous tissue (POELAERT et al., 1997; JONES and PUSKARICH, 2011; VIEILLARD-BARON, 2011). There is a disruption of the alveolar-endothelial barrier, with an accumulation of protein-rich fluid in the lungs, interstitial lung spaces and alveoli. In extreme cases, this can cause a ventilation-perfusion mismatch, hypoxia and decreased lung compliance, resulting in acute respiratory distress syndrome (ARDS). Decreased renal perfusion, acute tubular necrosis and varying degrees of acute kidney injury occur in the kidneys. In the liver, the clearance of bilirubin is suppressed, which produces cholestasis. Endothelial changes weaken the blood-brain barrier, causing the entry of toxins, inflammatory cells, and cytokines. Cerebral edema, neurotransmitter disruption, oxidative stress and subsequent changes in white matter damage lead to a clinical spectrum of septic encephalopathy, ranging from mild confusion to delirium and coma. Sepsis is known to produce a catabolic state. Rapid and significant muscle breakdown occurs to produce amino acids for gluconeogenesis to nourish immune cells. In addition, increasing insulin resistance causes a state of hyperglycemia (SINGER et al., 2016). It is reported that more than 30 million people worldwide are affected by sepsis every year, and 6 million people die. As a result, Chinese emergency medicine specialists introduced the concept of "prevention and prevention" of sepsis, and conducted the "Campaign for Sepsis Prevention in China" (PSCC) throughout China. In addition, they put forward the principles of performing targeted diagnosis, examination and treatment in the "early stage of sepsis" and "peri-sepsis period" in order to realize early prevention, early discovery and early intervention, and reduce morbidity. Research on the prevention of sepsis-related deaths, and thus the diagnosis and treatment of patients with acute severe infection is important (LEVY et al., 2012).

Recently, natural resources have been investigated for the treatment of many diseases. *Punica granatum* L., a member of the Punicaceae family, is one of the oldest edible fruits. It is known that *Punica granatum* is known to possess pharmacological properties, such as antioxidant, radical scavenging and anticancer properties (LERMAN et al. 2005). However, previous studies demonstrated that various parts of *P. granatum* also showed anti-oxidant, anti-bacterial, anti-diarrheal, anti-viral, anti-diabetic, antihelmintic, hypolipidemic, hepatoprotective, anti-neoplastic and protective activity for vessel and digestive systems (MIGUEL et al. 2010). Although *P. granatum* has been used against various diseases, there are no studies on the anti-microbial and histopathological effects of the peel powder of *P. granatum.* Therefore, the aim of this study was to investigate the potential anti-microbial and histopathological effects of *P. granatum* peel powder on cecal ligation and puncture-induced (CLP) sepsis in rats.

Materials and methods

Preparation of P. granatum peel powder. The extraction procedure was conducted according to our previously published data (STOJANOVIĆ et al., 2017). *P. granatum* peel powder (100 g) was extracted with 50% ethanol in an ultrasonic bath for 40 min at 60ºC. The obtained extract was filtered and evaporated to dryness using a rotary evaporator.

Animals. Twenty-four, healthy, 10-week-old male Wistar Albino rats were used in this experiment. The rats were housed in polysulfone cages at 21–24 °C and 40–45% humidity, and with light-controlled (12 h light/12 h dark) conditions at the Laboratory of the Animals Breeding and Experimental Research Center of Etlik Central Veterinary Control and Research Institute (Ankara, Turkey). The animals were fed with a standard pellet diet and water *ad libitum* throughout the experimental procedure. The rats were maintained in accordance with the directions of the Guide for the Care and Use of Laboratory Animals. All experimental protocols were approved by the Experimental Animal Ethics Committee of Etlik Central Veterinary Control and Research Institute (EDAM/2020-4). After acclimation for one week, all the rats were randomly divided into four groups consisting of six rats in each group, as follows: Sham-operated (S) Group, Control (C) Group, Treatment-1 (T1) Group, and Treatment-2 (T2) Group.

Induction of rat sepsis model. Sepsis was induced by cecal ligation and puncture (CLP) as previously described (HU et al., 2019). The rats were intraperitoneally anesthetized with xylazine hydrochloride (10 mg/kg) and ketamine hydrochloride (50 mg/kg). Abdominal shaving was performed after the anesthesia procedure. The rats were placed in the supine position. After routine disinfection of the abdomen, a 3-cm midline vertical incision was performed. The subcutaneous and muscle layers were separated, and the abdominal cavity was opened. The cecum was exposed, and the ileocecal region was ligated with USP 4/0 polyglactin (Lactasorb PGLA, Orhan Boz, Turkey). The cecum was perforated with an 18-gauge needle and gently squeezed to remove a small amount of feces. The cecum was then placed back into the abdominal cavity. The muscle layers of the abdomen and skin were closed with USP 4/0 polyglactin (Lactasorb PGLA, Orhan Boz, Turkey). In the sham-operated group, the same procedures were performed, however, cecal ligation and puncture were not applied.

The treatment procedure. The 50% ethanol extract of *Punica granatum* peel powder (200 mg/kg; FADDLADDEEN and OJAIMI, 2019) prepared in distilled water was administered one hour before (group T1) and 10 hours after (group T2) the operation in 2 mL volume *per os*. The sham-operated and control groups were given distilled water at 2 mL dose by oral gavage.

Termination of the experimental procedure. 72 hours after the treatment procedure, all the rats were sacrificed by taking blood from the heart under general anesthesia (10 mg/kg xylazine hydrochloride and 50 mg/kg ketamine hydrochloride). Blood samples were collected by cardiac puncture for bacterial culture analysis. The liver, lung, heart, kidney, spleen, and pancreas were dissected. The blood samples were collected in heparinized tubes for bacterial culture analysis, and added to nutrient agar. In addition, blood agar, MSA agar, and EMB agar were used to identify the isolated bacteria.

Histopathological evaluations. The liver, lung, heart, kidney, spleen, and pancreas were sampled from rats in all groups (n=6 for each group). These organs were examined according to macroscopic evaluation criteria, and all tissue samples were fixed in 10% buffered formalin for 48 hours. After the fixation, the tissues were treated with graded alcohol and xylene series (Leica, TP1020, Germany) and blocked in paraffin (Thermo Shandon, Germany). Five µm thickness sections were cut by rotary microtome (Shandon). From paraffin blocks, sections were stained according to the hematoxylin-eosin (H&E) staining procedure (LUNA, 1968) and evaluated under a digital optical light microscope, and images were taken with a camera attachment (Olympus BX51 digital microscope, DP25 Japan). For scoring histopathological findings, a number was obtained by counting 10 fields at 400x magnification (10 HPF). The counted fields were calculated as proportions and expressed as (%) percentages. According to the density of findings

(inflammation, vascular changes, degeneration and necrosis), the mean results per animal in each group were calculated. Then, the total mean results were analyzed statistically.

Statistical analysis. Statistical analyses were performed using Graphpad Prism 8.4.2 The results are expressed as the mean ± standard error of the mean (SEM). The two-way analysis of variance test and post-hoc Bonferroni multiple comparison test were used to determine the significance of differences between groups. Statistical significance was assumed at the level of $P<0.05$.

Results

Survival rate. The survival rate was 83.33% (5/6 rats) in group T2, whereas the survival rate was 100% for the other groups. There was no statistically significant difference in survival between the *P. granatum* -treated CLP groups and the distilled water-treated CLP group.

Blood bacterial culture. After the cecal ligation and puncture were performed, sepsis occurred due to fecal spillage in this model. The blood culture results are given in Table 1. At the end of the experimental procedure, a bacterium (*E. coli*) was isolated in only one case from group S. Bacteria colonies were detected in all cases in group C. While *E. coli* and *Staphylococcus aureus* were identified in four cases, the presence of *E. coli* was noticed in two cases in group C. Bacteria (*S. aureus* and *E. coli* + *S. aureus*) were determined in fewer cases (n=2) in group T1 when compared with groups C and T2. In group T2, *S. aureus* and *E. coli* + *S. aureus* were isolated in two case each, but there were no bacteria in two cases.

Table 1. Blood culture results according to groups

Groups	*E. coli*	*S. aureus*	*E.* coli + *S. aureus*
S group	1 case	-	-
C group	2 cases	-	4 cases
T1 group	-	1 case	1 case
T2 group	-	2 cases	2 cases

Histopathological findings. Inflammation, degeneration, necrosis, and vascular changes were the main lesions in the organs mentioned. However, the inflammatory cells were mainly composed of neutrophils and macrophage-inflammatory proteins. In degenerative changes, cells lost their nuclei and their cytoplasms shrank into a dark pink color. Cells that underwent degenerative changes had vacuoles with clear pronounced edges. In some areas, degeneration of parenchymal cells increased in many areas, and necrotic areas were observed. In the necrotic areas, cellular borders were lost. Vascular changes were also prominent in the histopathological findings. Some vessels, including veins and arterioles, were enlarged with many erythrocytes. In some areas, hemorrhage also occurred. Some areas, especially the lungs and liver, showed microhemorrhages and blood extravasations. Edema was also present in these dense vascular disturbances. Edema was seen densely in the alveolar lumina of lungs, sinusoids and portal region of the liver, and the interstitium of the kidney.

In group C, only vasculature changes, which constituted predominantly mild hyperemia, were observed in each case. Acute cell swelling and vacuolar degeneration and necrosis in the hepatocytes of the liver, the alveolar epitheliums of the lungs, and cortical tubule epitheliums of the kidneys were encountered in many areas in all cases. Degeneration and necrosis were found less often in the islet cells of the pancreatic glands. Parenchyma degeneration was seen densely in myocardiocytes. In the spleen, hyperplastic lymph follicles were

present. Some of them included free erythrocytes along with hyperemia. There were no inflammatory cells in any of the organs mentioned in this group. In group T1, the findings were localized in the same organs in many cases. Lesion distribution, in terms of degeneration and necrosis, was less than the control group findings in these cases.

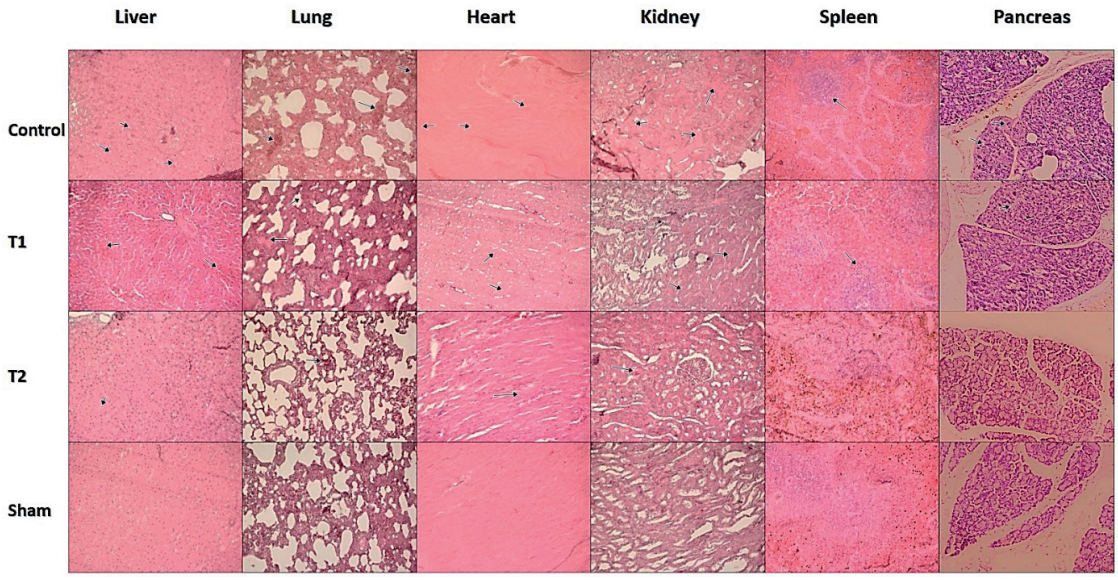

Fig. 1. Vacuolar degeneration (arrows) in liver, hyperemia and vascular changes (arrows) in lungs, parenchymal degeneration in myocardiocytes (arrows) in heart, vacuolar degeneration in cortical tubule epitheliums (arrows) in kidney, hyperplastic follicle in spleen, x100, degeneration and necrosis (arrows), x100, H&E staining

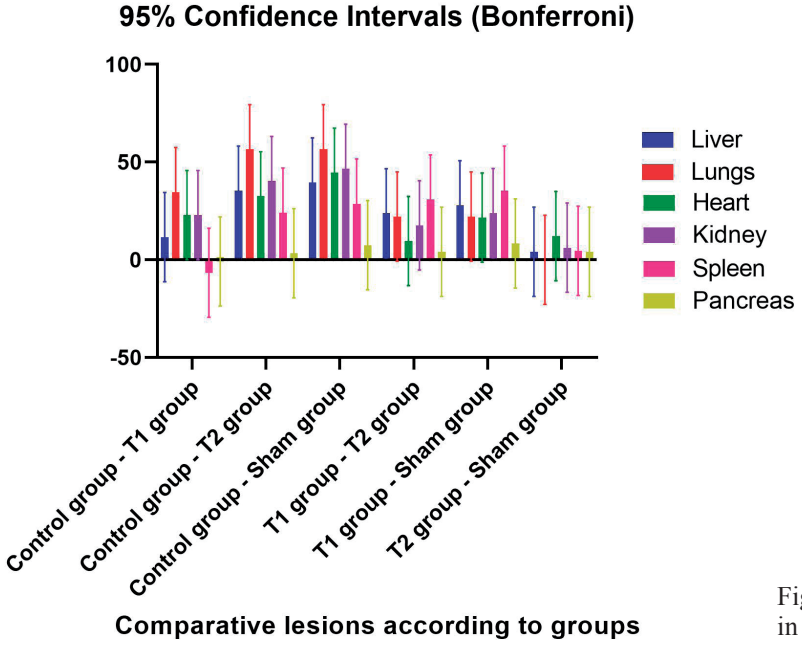

Fig. 2. Comparative evaluation of lesions in organs between experimental groups

Table 2. Histopathological score of organs in experimental groups

Groups	Liver				Lungs				Heart				Kidney				Spleen				Pancreas			
	I	V	D	N	I	V	D	N	I	V	D	N	I	V	D	N	I	V	D	N	I	V	D	N
Control group																								
1	-	+++	+++	+++	+	+++	+++	+++	-	++	+++	+++	-	++	+++	+++	-	+++	+	-	-	-	-	-
2	-	++	+++	+++	-	++	+++	+++	-	++	++	++	-	++	+++	+++	-	+	++	-	-	-	-	-
3	-	++	+++	+++	-	++	+++	+++	-	++	++	++	-	+	+++	++	-	+++	++	-	-	-	+	+
4	-	++	++	++	-	+++	+++	+++	-	++	+++	+++	-	++	+++	++	-	+++	+	-	-	+	++	+
5	-	++	++	++	-	+++	+++	+++	-	++	+++	+++	-	++	+++	++	-	+++	+	-	-	+	+	-
6	-	+	++	+++	-	++	++	++	-	++	-	-	-	-	++	++	++	-	-	-	-	-	-	-
T1 group																								
1	-	+	+++	+++	-	-	+++	+++	-	-	+++	+++	-	-	+++	+++	+++	+++	++	++	-	-	-	-
2	-	+	++	++	-	-	++	+++	-	-	++	++	-	+	++	++	++	++	++	++	-	-	-	-
3	-	++	++	++	-	-	++	++	-	-	++	++	-	+	++	++	++	++	++	++	-	+	+	-
4	-	++	++	++	+	++	++	++	-	-	+	++	-	+	++	++	-	++	++	++	-	-	-	-
5	-	++	++	++	+	++	++	++	-	++	++	-	-	+	++	+	++	-	-	-	-	+	+	-
6	-	+	++	++	-	++	++	++	-	-	++	-	-	-	-	-	-	-	-	-	-	-	-	-
T2 group																								
1	-	+	+	-	-	+	-	-	-	+	-	-	-	+	-	-	-	-	+	-	+	-	-	-
2	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
3	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	+	-	-	-	-	-	-	-	+++	+	-	-	+	+	-	-	-	-	-	-	-	-
5	-	+	-	-	-	-	-	-	-	-	++	-	-	+	+	-	-	+	+	-	-	-	-	-
6	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Sham group																								
1	-	-	+	-	-	++	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	+	-	-	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

I: inflammation, V: vascular changes (Hyperemia, hemorrhage and edema), D: degeneration, N: necrosis

Lymphoid follicles were hyperplastic in some areas. Islet cells were not affected by degeneration in any case in this group. Lesions were generally restricted to certain areas. They were not widespread in every field. When the histopathological findings of group T2 are examined, they show similarities with the findings of group T1. Likewise, the density of inflammatory cells differed less when comparing groups T1 and T2. In group T2, these findings were observed in the same organs, while the number of affected cases was found to be low. In the liver, fewer degeneration and vasculature changes were found in four cases. In the kidney and spleen, milder lesions were found with the same appearance. In the lungs, mild vasculature changes, including hyperemia and edema, were found in two cases. In the heart, there were moderate changes in three cases. There was no inflammatory cell infiltration in this group as in previous groups. In group S, the findings were localized in the liver, lungs, heart, spleen, and kidneys in general. There was no inflammatory infiltration. Vascular changes and cellular alterations, including degeneration and necrosis, were common in every field of the organs in almost all cases. No lesions were found in the pancreas in group S, even though there were severe degenerative and necrotic changes in group C, and milder or less degeneration in groups T1 and T2 (Fig. 1.). The histopathological scores of the liver, lungs, heart, kidneys, spleen, and pancreas are given in Tables 2, 3 and 4 and Fig. 2.

Table 3. Statistical correlation between groups by Two-way ANOVA

Two-way RM ANOVA	Matching: both factors				
Assume sphericity	Yes				
Alpha	0.05				
Source of variation	% of total variation	P value	P value correlation	Significant	F (DFn, DFd)
Organs	7.452	0.0190	*	Yes	F (5.15) = 3.857
Histopathological findings	40.44	0.0061	**	Yes	F (3.9) = 8.176
Organs x Histopathological findings	10.07	0.0077	**	Yes	F (15.45) = 2.558

Table 4. Comparative group results according to histopathological findings in organs by post- hoc Bonfererroni's multiple comparison test

Bonferroni's multiple comparisons test	Mean difference	Significant	Summary	Adjusted P value
Liver				
Control group vs. T1 group	11.65	No	ns	0.9930
Control group vs. T2 group	35.40	Yes	***	0.0006
Control group vs. Sham group	39.57	Yes	***	0.0001
T1 group vs. T2 group	23.75	Yes	*	0.0370
T1 group vs. Sham group	27.92	Yes	**	0.0091
T2 group vs. Sham group	4.165	No	ns	>0.9999
Lungs				
Control group vs. T1 group	34.58	Yes	***	0.0008
Control group vs. T2 group	56.66	Yes	****	<0.0001
Control group vs. Sham group	56.66	Yes	****	<0.0001
T1 group vs. T2 group	22.08	No	ns	0.0628
T1 group vs. Sham group	22.08	No	ns	0.0628
T2 group vs. Sham group	-3.553e-015	No	ns	>0.9999
Heart				
Control group vs. T1 group	22.92	Yes	*	0.0483
Control group vs. T2 group	32.50	Yes	**	0.0017
Control group vs. Sham group	44.58	Yes	****	<0.0001
T1 group vs. T2 group	9.583	No	ns	>0.9999
T1 group vs. Sham group	21.66	No	ns	0.0714
T2 group vs. Sham group	12.08	No	ns	0.9045
Kidney				
Control group vs. T1 group	22.84	Yes	*	0.0495
Control group vs. T2 group	40.42	Yes	****	<0.0001
Control group vs. Sham group	46.67	Yes	****	<0.0001
T1 group vs. T2 group	17.58	No	ns	0.2335
T1 group vs. Sham group	23.83	Yes	*	0.0361
T2 group vs. Sham group	6.248	No	ns	>0.9999
Spleen				
Control group vs. T1 group	-6.665	No	ns	>0.9999
Control group vs. T2 group	24.17	Yes	*	0.0323
Control group vs. Sham group	28.75	Yes	**	0.0068
T1 group vs. T2 group	30.83	Yes	**	0.0032
T1 group vs. Sham group	35.41	Yes	***	0.0006
T2 group vs. Sham group	4.580	No	ns	>0.9999

Table 4. Comparative group results according to histopathological findings in organs by post- hoc Bonfererroni's multiple comparison test (continued)

Bonferroni's multiple comparisons test	Mean difference	Significant	Summary	Adjusted P value
Pancreas				
Control group vs. T1 group	-0.8325	No	ns	>0.9999
Control group vs. T2 group	3.335	No	ns	>0.9999
Control group vs. Sham group	7.498	No	ns	>0.9999
T1 group vs. T2 group	4.168	No	ns	>0.9999
T1 group vs. Sham group	8.330	No	ns	>0.9999
T2 group vs. Sham group	4.163	No	ns	>0.9999

Statistical correlation degree: ns not significant * mild ** moderate ***high

Discussion

In the management of sepsis, providing tissue oxygenation and perfusion, and applying appropriate antimicrobial therapy against the causative organism are some of the therapeutic goals. For this purpose, the appropriate antibiotic use, at the right time, fluid therapy, vasopressors and inotropes, airway support and oxygen, and cortisone are used for sepsis treatment (KEELEY et al., 2017). Although there are many treatment options used in sepsis cases, specific therapy, targeting the sepsis mediators has not yet been proven to be effective (EVANS, 2018). Therefore, new drug candidate molecules, including promising natural products, have been investigated for the treatment of sepsis. The effectiveness of medicinal plants in the treatment of many diseases has been investigated for many years. The use of polyphenols in the treatment of inflammatory diseases has become increasingly important due to their anti-inflammatory effects. Phenolic compounds are generally found in the fruit, leaves, seeds, bark, and roots of plants (COLOMBO et al., 2013; MANSOURI et al., 2016) According to previous studies, many plants such as *Ferulago pauciradiata* (KUTLU et al., 2020), *Andrographis paniculata, Zingiber officinale, Curcuma longa, Piper nigrum, Syzygium aromaticum, Momordica charantia,* and *Centella asiatica* (LIEW et al., 2020) are used for the treatment of sepsis due to their anti-inflammatory and antioxidant properties. The antibacterial, antioxidant, and anti-inflammatory effects *of P. granatum* have been revealed in previous studies (AVIRAM et al., 2004; LANSKY and NEWMAN, 2007; DE NIGRIS et al., 2007). Therefore, in the present study, we investigated the preventive and therapeutic effects of the peel powder of *P. granatum* in a rat sepsis model. The results showed that the peel powder of *Punica granatum* used in group T2 displayed beneficial effects in the sepsis model in rats, considering the histopathological changes when compared to the control and T1 groups.

The endotoxic model induced by lipopolysaccharide (LPS) mimics poisoning rather than infection. The cytokines peak early in the LPS model, whereas in the CLP model, the pro-inflammatory response is delayed and continues over time. In the LPS model, mortality is thought to occur early, most likely due to the effects of the intense inflammatory response on the cardiovascular system (RUIZ et al., 2016). In the CLP model, mortality is delayed by multi-organ failure complicating induced peritonitis. The most widely used CLP model for experimental sepsis is currently considered as the gold standard in research because it mimics the nature and evolution of severe sepsis in humans (RUIZ et al., 2016). The timing of antibiotic administration is directly related to

overall survival. When antibiotic administration is 12 hours after CLP, mice with IL-6 concentrations higher than 14,000 µpg/mL have 0% survival, while administration of antibiotics at 6 hours to mice with similar IL-6 concentrations is reported to increase overall survival to 25% (LEWIS et al., 2016). When choosing an experimental animal species in experimental sepsis models, it is important for the purpose of the study that the animal species is easily accessible and cost-effective. For these reasons, small experimental animals, such as mice, rats, and guinea pigs are frequently used. These species are also used in survival studies, and histopathological examinations (İSKİT, 2005). In the present study, the survival rate of the rats in group T1 was higher than that of rats in group T2. It was considered that the administration of *P. granatum* L. peel powder 6 hours before CLP may increase the survival rate in the treatment of sepsis.

A literature search showed that antimicrobial therapy is an essential factor for sepsis management. Therefore, bacterial identification should be performed in the treatment process (MONTRAVERS et al., 2009; VAITTINADA et al., 2020). In a study of 16 rats, blood cultures after CLP found *E. coli* 88% (14/16), *Enterococcus faecalis* 81% (13/16), and *Enterobacter cloacae* 75% (12/16) (VAITTINADA et al., 2020). In this CLP-induced study, blood cultures (*E coli* and *S aureus*) were determined to be compatible with common polymicrobial infections in humans with stercoral peritonitis. It was observed that bacteria were isolated in fewer animals in group T1 (n=2) than in C (n=6) and T2 (n=4). It was thought that the administration of *P. granatum* L. peel powder 6 hours before CLP may have a beneficial effect on blood bacterial elimination in sepsis.

In conclusion, the present study using a rat sepsis model, induced by cecal ligation and puncture, showed that inflammation, vascular changes, degeneration, and necrosis of visceral organs, especially the lungs, were caused by *S. aureus* and/or *E. coli*. However, it was indicated that blood cultures could be used as a diagnostic marker in the pathogenesis of sepsis. In addition, pomegranate peel powder administration was determined to be effective in the treatment of sepsis, with its antimicrobial and anti-inflammatory functions. Thus, ellagic acid may be an alternative therapeutic agent against sepsis.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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ÜLKER, U., M. A. DEMİREL, B. BAYRAKTAR, M. E. ALÇIGIR, A. AKSOY: Preventivni i terapijski učinci praha kore pitomog šipka (*P. Granatum*) na modelu sepse u štakora. Vet. arhiv 93, 719-730, 2023.

SAŽETAK

Cılj je rada bıo procıjenıtı potencıjal lıječenja prahom kore pıtomog šıpka *Punica granatum* L. kod eksperimentalno izazvane sepse u štakora. Ukupno 24 mužjaka Wistar Albino štakora, u dobi od 10 tjedana, uključeno je u istraživanje. Životinje su podijeljene u četiri skupine: placebo-skupinu (S), kontrolnu skupinu (C), pokusnu skupinu 1 (T1) i pokusnu skupinu 2 (T2). Kako bi se potaknula sepsa, provedena je cekalna ligacija i punkcija. Prah kore pitomog šipka (200 mg/kg peroralno) dan je jedan sat prije (T1) i 10 sati poslije (T2) kirurškog zahvata u dozi od 2 mL. Na kraju pokusnog postupka provedena je mikrobna i histopatološka analiza jetre, pluća, srca, bubrega, slezene i gušterače. Iz krvi su izolirane bakterije *Escherichia coli, Staphylococcus aureus* i *E. coli + S. aureus*. Teška bakterijemija otkrivena je u krvi životinja u kontrolnoj skupini. Uočena je manja prisutnost bakterija u skupinama T1 (n=2) i T2 (n=4) u usporedbi s kontrolnom skupinom. U tkivu gušterače nije bilo lezija ni u jednoj skupini. U svih životinja u kontrolnoj skupini u usporedbi s placebo-skupinom uočene su krvožilne promjene i degenerativne promjene te nekroza. Vrijednosti nalaza u skupini T1 bili su slični onima u kontrolnoj skupini, no u manjeg broja životinja. Zapaženo je opće poboljšanje u skupini T2, osim toga su postojeće lezije bile umjerene težine. Zaključuje se da je prah kore pitomog šipka spriječio oštećenje pluća uzrokovano ligacijom i punkcijom cekuma u pokusnih štakora. Stoga bi prah kore *P. granatum* L. mogao biti alternativno terapijsko sredstvo kod oštećenja plućnog tkiva uzrokovanog sepsom. Oporavak od upale bio je brži u skupini T2 nego u drugim skupinama. Prema rezultatima ovog istraživanja prah kore *P. granatum* učinkovit je u liječenju sepse s obzirom na svoja antimikrobna i protuupalna svojstva.

Ključne riječi: cekalna ligacija i punkcija; upala; *Punica granatum;* sepsa