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Research Article

The Protective Effects of Peganum harmala Extract on Lung and Kidney in Sepsis Induced by Cecal Ligation and Perforation in Rats

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Abstract

Sepsis is characterized by multiple organ dysfunction, tissue damage and hyper-inflammation. Peganum harmala (PH) is a plant considered for its antibacterial, antioxidant, anticarcinogen and antiinflammatory properties. This study was aimed to evaluate the protective effects of PH extract on tissues and cytokines in sepsis induced by cecal ligation and perforation (CLP) in rats. Forty rats were divided into five groups. Groups were sham-operated (control), CLP, 90 mg/kg PH-treated CLP, 180 mg/kg PH-treated CLP and 180 mg/kg PH-treated control healthy. Animals were sacrificed at the 16th h of the study. Biochemical and histopathological analyses were performed in lung, kidney and blood samples. Both 90 mg/kg and 180 mg/kg doses of PH decreased the level of interleukin-1 beta (IL-1β) and interleukin-10 (IL-10), and high dose of PH reduced the tumor necrosis factor-alpha (TNF-α) in the serum compared to CLP group. The PH also increased the activity of superoxide dismutase (SOD) and the total levels of glutathione (GSH) in the lung and kidney tissues of septic rats. The level of malondialdehyde (MDA) in the lung and kidney tissues was reduced in both PH treated CLP groups. The histopathological results were in accordance with the biochemical results. The CLP + 180 mg/kg PH group had the lowest inflammation score in the lung. In conclusion, the administration of PH has prevented the oxidative stress, the cytokine response and the inflammation in CLP-induced septic rats.

Keywords: *Inflammation, polymicrobial sepsis, oxidative stress, cytokine, tissue damage*

Peganum harmala Ekstraktının Ratlarda Çekal Bağlama ve Delme ile İndüklenen Sepsiste Akciğer ve Böbrek Üzerine Koruyucu Etkileri

Özet

Sepsis organlarda fonksiyon bozukluğu, doku hasarı ve hiper-inflamasyon ile karakterizedir. Peganum harmala (PH) antibakteriyel, antioksidant, antikarsinojenik ve antiinflamatuar özellikleri olduğu kabul edilen bir bitkidir. Bu çalışma çekal bağlama ve delme (CLP) ile sepsis oluşturulan ratlarda PH ekstraktının dokular ve sitokinler üzerine koruyucu etkilerinin değerlendirilmesi amacıyla yapılmıştır. Kırk rat 5 gruba ayrıldı. Gruplar sham-operasyon (kontrol) CLP, 90 mg/kg PH-uygulanan CLP, 180 mg/kg PH-uygulanan CLP ve 180 mg/kg PH-uygulanan sağlıklı kontroldür. Hayvanlar çalışmanın 16. saatinde sakrifiye edildi. Akciğer, böbrek ve kan örneklerinde biyokimyasal ve histopatolojik analizler yapıldı. CLP grubuna kıyasla PH'nın 90 mg/kg ve 180 mg/kg her iki dozu da serum interlökin-1 beta (IL1β) ve interlökin-10 (IL-10) düzeylerini azalttı ve yüksek doz PH tümör nekrozis faktör-alfa (TNF-α) düzeyini düşürdü. PH uygulaması septik ratların akciğer ve böbrek dokularındaki süperoksit dismutaz (SOD) aktivitesini ve total glutatyon (GSH) düzeyini de arttırdı. Akciğer ve böbrek dokularındaki malondialdehit (MDA) düzeyi PH uygulanan CLP gruplarında azaldı. Histopatolojik bulgular biyokimyasal bulgular ile uyumluydu. CLP grupları içerisinde, CLP + 180 mg/kg PH grubu akciğerde en düşük inflamasyon skoruna sahipti. Sonuç olarak, PH uygulaması ratlarda CLP ile indüklenen sepsiste oksidatif stres, sitokin yanıtı ve inflamasyonu önledi.

Anahtar sözcükler: *Yangı, polimikrobiyal sepsis, oksidatif stres, sitokin, doku hasarı*

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INTRODUCTION

Sepsis, a complex syndrome, is a systemic response of an organism against microorganisms and/or their toxins in the bloodstream [1]. It is one of the major causes of mortality in worldwide [2]. Sepsis leads to multiple organ dysfunction, coagulopathy, hypoglycemia, systemic inflammation, metabolic acidosis, hypotension, and oxidative damage [3-6]. During sepsis, hyper-inflammation and oxidative damage contribute to the immune response with mainly effects on tissues of lungs, liver, kidney, and intestines [7-9]. Sepsis is currently treated with specific antibiotics and other pharmacological agents that antioxidants and anti-inflammatory reagents [10-12].

Peganum harmala (PH) is a traditional plant that has a long history of use as a folk medicine in Turkey, Iran and China to treat diseases. The PH contents chemical ingredients such as alkaloids, steroids, flavonoids, anthraquinones, amino acids, and polysaccharides from its seeds, leaves, flowers, stems and roots. Among these compounds, the alkaloids, mostly β-carbolines such as harmine, harmaline, harmalol, harmol and harman [13,14]. These alkaloids were found to be the main substances responsible for the analgesic, antiinflammatory, anti-bacterial, anti-parasitic, antioxidant, insecticidal, anti-tumoral and vaso-relaxant activities of PH [15-25].

However, to the best of our knowledge, the effects of PH on pro-inflammatory mediators and oxidative response have not been documented in rat experimental sepsis model, until now. Due to high alkaloids content and anti-oxidative and anti-inflammatory effects of PH, we hypothesized that PH may protect the organism against sepsis mortality. Thus, the purpose of this study was to evaluate biochemically and histopathologically the protective effects (antioxidative/anticytokine properties) of PH extract on lung and kidney tissues and blood samples in sepsis induced by cecal ligation and perforation (CLP) in rats.

MATERIAL and METHODS

Animals

In the present study, a total of 40 male Wistar rats were used for the experiments. The rats were housed in standard plastic cages on sawdust bedding in an air-conditioned room at 22±1°C under lighting controls (14 h light/10 h dark cycle). Standard rat ration and tap water were given ad libitum. Each rat weighed 230-250 g, and all were obtained from Experimental Animal Laboratory of Medicinal and Experimental Application and Research Center (ATADEM). Animal experiments and procedures were performed in accordance with the national guidelines for the use and care of laboratory animals and they were approved by the university's local animal care committee (Decision No: 75/2014).

Chemicals

All chemicals were purchased from Sigma Chemical Co (Germany). ELISA kits were supplied from Invitrogen. IL-1β, IL-6 and TNF-α from each sample were measured with highly sensitive ELISA kits; Invitrogen-KRC0011, Invitrogen -KRC0101 and Invitrogen-KRC3011 (Grand Island, USA), respectively.

Preparations of PH Extract

The plant material was collected in July 2012 from Kayseri, a city in Middle Anatolia region of Turkey. The PH seeds were dried and grounded. Then, it was extracted with methanol at 40ºC for 4 h. The mixture was filtered and allowed for phase separation. The resultant supernatant was concentrated by using a rotary evaporator.

Experimental Design

The rats were allocated into five groups, each composed of 8 individual rats as shown below.

Group I (Sham): sham operated control group,

Group II (CLP): CLP group,

Group III (CLP+PH1): CLP + 90 mg/kg PH (oral administration),

Group IV (CLP+PH2): CLP + 180 mg/kg PH (oral administration),

Group V (Sham+PH2): 180 mg/kg PH (oral administration).

Sepsis Model

A CLP polymicrobial sepsis model was applied to the rats. Polymicrobial sepsis was induced through cecal ligation and two-hole puncture [7]. Briefly, rats were not fasted prior to the procedure and anesthesia was induced through intraperitoneal administration of thiopental sodium 25 mg/kg. After the abdomen was shaved, the peritoneum was opened. Once the diaphragm exposed the abdominal organs, the cecum was isolated and ligated with a 3/0 silk ligature just distal to the ileo-cecal valve. Two punctures were made with a 16-gauge needle through the cecum distal to the point of ligation, and the cecum was placed to the peritoneal cavity. The muscle and skin of abdominal incision was then closed with a 4/0 sterile synthetic, absorbable suture. The wound was bathed in 1% lidocaine solution to ensure analgesia.

The sham-operated groups received laparotomies, and the cecum were manipulated, but not ligated or perforated. All of the animals were given 2 ml/100 g body weight of normal saline subcutaneously at the time of surgery and 6 h after the operations, for fluid resuscitation. Immediately after the surgical procedure, the rats in the PH2-sham and the PH-treated CLP groups received 90 and 180 mg/kg doses of PH extract, which were administered with an oral gavage. An equal volume of saline was administered to the sham group and the CLP group. The rats were deprived of food postoperatively but had free access to water for the next 16 h until they were sacrificed.

All groups were sacrificed 16 h later with an overdose of a general anesthetic (thiopental sodium, 50 mg/kg), and whole blood samples were withdrawn via the intra-cardiac method. The serum was immediately separated by centrifugation at 2.500 g for 10 min at +4°C, and stored at –80°C. The lungs and kidney were then quickly removed from all of the rats and washed in ice-cold saline. Half of the tissue was transferred to biochemistry laboratory and kept at –80°C for biochemical analyses, while the other half were fixed in a 10% formalin solution for histopathological analyses.

Biochemical Analyses of Cytokine Levels in the Serum

Cardiac blood samples were collected immediately and transferred to the laboratory to facilitate the estimation of the inflammatory cytokines, IL-1β, IL-10 and TNF-α levels in the serum. Sera from the five rat groups were separated and stored at –80°C until they were thawed for the assay. IL-1β, IL-10 and TNF-α from each sample were measured with highly sensitive ELISA kits; rat IL-1β immunoassay Kit (Invitrogen, Cat. No: KRC0011), IL-10 Elisa Kit (Invitrogen, Cat. No: KRC0101), TNF-α Elisa Kit (Invitrogen, Cat. No: KRC3011), respectively. Kits were specific for rat cytokines, and all measurements were performed according to the manufacturer's instructions by using Bio-Tek µQuant (USA) multi plate spectrophotometer. Cytokine assays for each animal and its correlated control were run in the same lot.

Biochemical Analyses of Lung and Kidney Tissues

After macroscopic analysis, the lung and kidney tissues of the rats were kept at –80°C. First, 100 mg of tissue from each rat was perfused with phosphate buffered saline (PBS)/heparin (137 mM NaCl, 2.7 mM KCL, 10 mM Na_2HPO_4, 2 mM KH_2PO_4 + heparin (1.000 units/L), pH 7.2). After grinding in liquid nitrogen, tissues were homogenized in buffers specific for each parameter on ice bath by a tissue homogenizer.

Malondialdehyde (MDA): MDA level was determined according to the methods of Ohkawa et al.[26]. Tissue samples were homogenized in 2.5 ml 10% KCl over 25 mg sample using an ultraturrax homogenizer (IKA-Germany). Then, homogenates were centrifuged at 4000g and 4°C for 30 min. The supernatant was used to determine the MDA level. Within the capped tubes, 250µl homogenate, 100 µl 8% sodium lauril sulphate, 750 µl 20% acetic acid, 750 µl 0.08% thiobarbituric acid and 150µl distilled water were vortexed for 1 min. This mixture was incubated in 100°C for 60 min, centrifuged at 4.000 rpm for 10 min after adding 2.5ml n-butanol/pyridine and 200 µl supernatant was pipetted into microplates. The occurrence of red color was measured at 532nm by an ELISA reader (µQuant, BioTek). Standard curve was generated using 1, 1, 3, 3–tetramedhoxypropane. All samples were measured in triplicate. The results were expressed as nanomol MDA per milligram protein (nmol/mg protein).

Total Gluthathione (GSH): GSH analysis was measured according to methods described by Sedlak and Lindsay [27]. Twenty five mg tissue sample was homogenized by the ultraturrax homogenizer with 2.5 ml buffer (50 mM Tris-HCl, pH 7.4). The homogenate was centrifuged at 3.000g and 4°C for 30 min to obtain supernatant for determination of GSH level. In capped tubes, 125µl supernatant, 375 µl buffer (200 mM Tris-HCl including 0.2 mM EDTA, pH 8.2) + 25 µl DTNB + 1975µl methanol mixture was incubated at 37°C for 30 min. The resultant yellow color was measured at 412nm by the ELISA reader. The results were expressed as GSH nmol/mg protein.

Superoxide Dismutase (SOD): SOD activity was analyzed according to the method described by Sun et al.[28]. Twenty five mg tissue sample was homogenized by the ultraturrax homogenizer with 2.5 ml buffer (0.2 mM Tris-HCl, pH 7.4). The samples were centrifuged at 5.000 g and 4°C for 60 min to obtain the supernatant. 200 µl supernatant, 980 µl measurement mixture (0.3 mM xanthine, 0.6 mM EDTA, 150 µM NBT, 0.4M Na_2CO_3, 1g/L BSA) and 20 µl xanthine oxidase incubated at 25°C for 20 min. The reaction was inhibited by $CuCl_2$ and measured at 560 nm. The results were expressed as SOD U/mg protein.

Histopathology Process

For histopathology, tissue samples from lung and kidney were obtained and fixed in 10% buffered formalin solution. After the routine histopathology process, paraffin sections in 5 µ stained with hematoxylin and eosin (HE). All slides were examined under the light microscopy (Olympus BX52 with DP72 camera system).

Inflammation Scoring in Tissues

For inflammation scoring, histopathological changes in lung and kidney tissues were semi quantitatively assessed. Ten different areas were examined under 40X magnification. Histopathological changes in lung (hyperemia, vasculitis, alveolar or bronchiolar exudate, desquamation of bronchiolar epithelium) and kidney (glomerulitis, degeneration of tubulary epithelium, interstitial cellular infiltration and hyaline casts within the tubulary lumina) were graded as follows: none: -, mild: +, moderate: ++, and severe: +++.

Statistical Analyses

The IBM SPSS Statistics 20 computer program package was used for statistical calculations. Data for the serum cytokine levels measured by the ELISA and the oxidant and antioxidant enzymes were subjected to one-way ANOVA followed by Tukey's post hoc test and were considered significant at $P<0.05$. All data were expressed as mean ± standard deviation (SD) in each group.

RESULTS

The Effects of PH on Cytokines in Serum

The levels of IL-1β, IL-10, and TNF-α of serum were shown in *Table 1*. The cytokine levels significantly increased in the CLP groups (IL-1β: 84.40 ± 10.40 pg/ml, IL-10: 494.33±79.28 pg/ml, and TNF-α: 18.46±5.33 pg/ml) 16 h after sepsis, when compared to the sham operated rats (control group) that the IL-1β, IL-10, and TNF-α were 34.53±3.06 pg/ml, 18.70±4.36 pg/ml, and 2.95±0.62 pg/ml, respectively (P<0.05). On the contrary, the serum levels of IL-1β, IL-10, and TNF-α decreased as a result of the administration of both PH1 and PH2 in CLP induced rats (P<0.05). The administrations of PH1 and PH2 decreased the serum levels of the IL-1β to 52.60±4.24 pg/ml and 49.84±17.95 pg/ml, respectively. The administrations of PH1 and PH2 decreased the serum levels of the IL-10 to 236.20±182.15 pg/ml and 141.70±70.18 pg/ml, respectively. The administration of PH1 and PH2 decreased the serum levels of the TNF-α to 24.96±1.88 pg/ml and 12.16±6.00 pg/ml, respectively. As shown in *Table 1* the administration of PH2 in the sham control rats did not affect the serum levels of cytokines (IL-1β; 40.79±2.15 pg/ml, IL-10: 20.22± 3.73 pg/ml, and TNF-α: 3.96±0.24pg/ml), when compared to the control group.

The Effects of PH on Oxidants and Antioxidants in Tissues

The levels of MDA, SOD, and GSH in lung tissues were shown in *Table 2*.

The MDA level significantly increased in the CLP groups (17.00±0.58 nmol/mg), when compared to the sham operated rats that the MDA level was 12.93±1.19 nmol/mg. The administrations of PH1 and PH2 decreased the MDA levels in the lung tissue to 15.85±0.75 nmol/mg and 13.98±0.69 nmol/mg respectively. On the contrary, the SOD and GSH levels in lung tissue decreased in the CLP group (110.05±7.56 U/mg protein and 2.30±0.37 nmol/mg protein, respectively), when compared to the sham operated rats that the SOD and GSH levels were 129.79±5.77 U/mg protein and 4.29±0.41 nmol/mg protein, respectively. However, the levels of SOD and GSH were significantly increased in lung tissues of PH1+CLP and PH2+CLP group, respectively (SOD: 150.79±5.58 U/mg protein, GSH: 4.52±0.56 nmol/mg protein and SOD: 166.14±5.50 U/mg protein, GSH: 4.79±0.53 nmol/mg protein). The administration of PH2 in the sham control rats did not significant affect the lung levels of oxidant and antioxidants, when compared to the control group.

The levels of MDA, SOD, and GSH in kidney tissues were shown in *Table 3*.

The MDA level significantly increased in the CLP groups (25.26±2.06 nmol/mg), when compared to the sham operated rats (control group) that the MDA level was 18.81±0.71 nmol/mg. The administrations of PH1 and PH2 decreased in the kidney tissue levels of the MDA to 20.33±1.84 nmol/mg and 17.91±1.13 nmol/mg,

Table 1. *Effects of Peganum harmala extract treatments on changes in serum levels of interleukin-1β (IL-1β) , interleukin-10 (IL-10) and tumor necrosis factor-α (TNF-α) in sera of rats*

Tablo 1. *Peganum harmala ekstraktı uygulamalarının rat serumlarındaki interlökin-1β (IL-1β), interlökin-10 (IL-10) ve tümör nekrosis faktör-α (TNF-α) üzerine etkileri*

Groups	IL-1β (pg/ml)	IL-10 (pg/ml)	TNF-α (pg/ml)
Sham	34.53±3.06	18.70±4.36	2.95±0.62
CLP	84.40±10.40*	494.33±79.28*	18.46±5.33*
CLP + PH1	52.60±4.24#	236.20±182.15	24.96±1.88*
CLP + PH2	49.84±17.95#	141.70±70.18#	12.16±6.00*#
Sham + PH2	40.79±2.15	20.22±3.73	3.96±0.24

** Significantly different from Sham rat group (P<0.05), # Significantly different from CLP rat group (P<0.05)*

Table 2. *Effects of Peganum harmala extracts treatments on changes in levels of malondialdehyde (MDA), activities of superoxide dismutase (SOD) and total glutathione (GSH) in lung tissues of rats*

Tablo 2. *Peganum harmala ekstraktı uygulamalarının rat akciğer dokularında malondialdehit (MDA), süperoksit dismutaz (SOD) aktivitesi ve total glutatyon (GSH) düzeyleri üzerine etkileri*

Groups	MDA (nmol/mg)	SOD (U/mg)	GSH (nmol/mg)
Sham	12.93±1.19	129.79±5.77	4.29±0.41
CLP	17.00±0.58*	110.05±7.56*	2.30±0.37*
CLP + PH1	15.85±0.79*	150.79±5.58*#	4.52±0.56#
CLP + PH2	13.98±0.69#	166.14±5.50*#	4.79±0.53#
Sham + PH2	13.16±0.81	130.73±7.68	3.89±0.27

** Significantly different from Sham rat group (P<0.05), # Significantly different from CLP rat group (P<0.05)* respectively. On the contrary, the SOD and GSH levels in kidney tissue decreased in the CLP group (SOD:103.14±8.64 U/mg protein and GSH: 2.44±0.22 nmol/mg protein), when compared to the sham operated rats that the SOD and GSH levels were 126.35±10.13 U/mg protein and 3.37±0.19 nmol/mg protein, respectively. However, the levels of SOD and GSH were significantly increased in kidney tissues of PH1+CLP and PH2+CLP group, respectively (SOD: 118.39±8.31 U/mg protein, GSH: 2.95±0.25 nmol/mg protein and SOD: 173.39±7.64 U/mg protein and GSH: 4.08±0.48 nmol/mg protein).

Histopathologic Findings

No histopathologic changes were observed in sham group *(Fig. 1)*. There were hyperemia, vasculitis, severe alveolar and bronchiolar exudation *(Fig. 2a)*, glomerulonephritis, tubular degeneration and severe hyaline cast formation *(Fig. 2b)* in the septic group. Similar histopathologic changes were observed in smaller areas in PH treated groups. There were no exudation within the alveoli and bronchiolar lumina in these groups *(Fig. 3a, Fig. 4a)* but vasculitis in some lung sections was observed. Nephrosis with hyaline casts was observed in many areas in group III *(Fig. 3b)*. However, hyaline formation in limited areas was observed in CLP + 180 mg/kg PH *(Fig. 4b)*. Inflammation scoring was demonstrated in *Table 4*.

DISCUSSION

Sepsis is an important health problem with high

Table 3. *Effects of Peganum harmala extracts treatments on changes in levels of malondialdehyde (MDA), activities of superoxide dismutase (SOD) and total glutathione (GSH) in kidney tissues of rats*

Tablo 3. *Peganum harmala ekstraktı uygulamalarının rat böbrek dokularında malondialdehit (MDA), süperoksit dismutaz (SOD) aktivitesi ve total glutatyon (GSH) düzeyleri üzerine etkileri*

Groups	MDA (nmol/mg)	SOD (U/mg)	GSH (nmol/mg)
Sham	18.81±0.71	126.35±10.13	3.37±0.19
CLP	25.26±2.06*	103.14±8.64*	2.44±0.22*
CLP + PH1	20.33±1.84#	118.39±8.31#	2.95±0.25#
CLP + PH2	17.91±1.13#	173.37±7.64*#	4.08±0.48*#
Sham + PH2	19.75±1.33	131.33±9.38	3.76±0.16

** Significantly different from Sham rat group (P<0.05), # Significantly different from CLP rat group (P<0.05)*

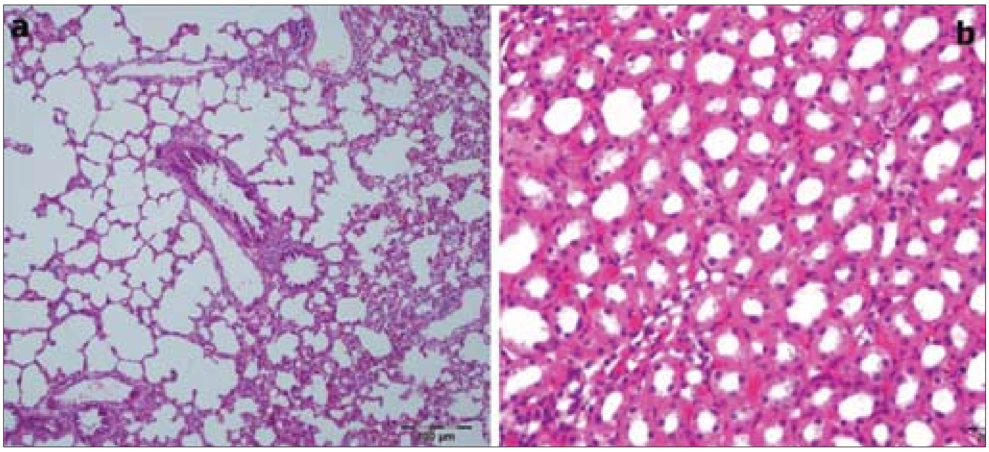

Fig 1. Group I (Sham). Normal histology of lung and kidney tissues in control group. There was no alveolar or bronchiolar exudate accumulation in lung (a) and no hyaline casts within the tubuli (b); HE

Şekil 1. Grup I (Sham). Kontrol grubunda akciğer ve böbrek dokularının normal histolojisi. Alveol ve bronşiol lümenlerinde eksudat yok (a) ve tubülüs lümenlerinde hiyalin kastları yok (b); HE

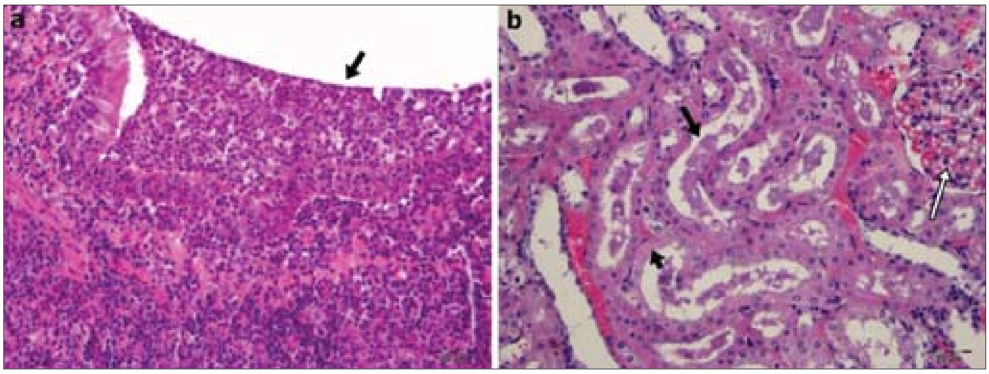

Fig 2. Group II (CLP). Severe bronchopneumonia and alveolar and bronchiolar exudate accumulation *(arrow in a)*, Glomerulitis *(white arrow in b)*, Hyaline casts *(long black arrow in b)* and tubulary degeneration *(short arrow in b)*; HE

Şekil 2. Grup II (CLP). Şiddetli bronkopnömoni, alveolar ve bronşiolar eksudat *(ok, a)*, Glomerulitis *(beyaz ok, b)*, Hyalin kastları *(uzun siyah ok, b)* ve tubüler dejenerasyon *(kısa ok, b)*; HE

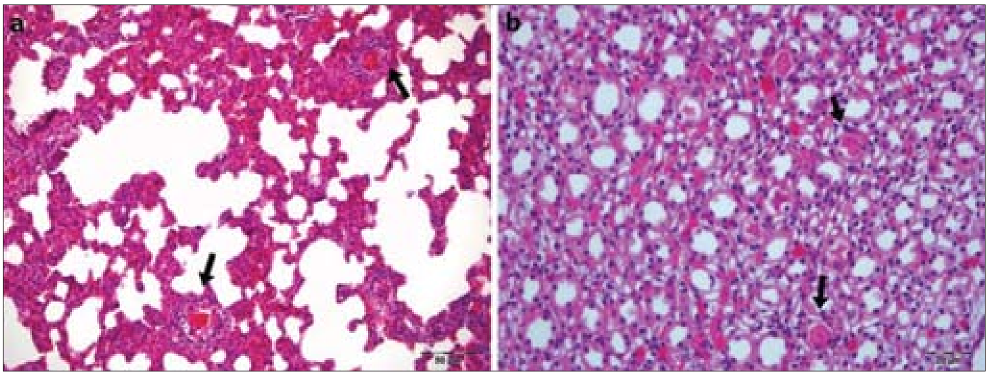

Fig 3. Group III (CLP + 90 mg/kg PH). Prominent decreasing of alveolar and bronchiolar exudate accumulation with vasculitis *(arrow in a)*, Hyaline casts *(arrows in b);* HE

Şekil 3. Grup III (CLP + 90 mg/kg PH). Alveolar ve bronşiolar eksudat birikiminde belirgin azalma görülürken vaskülitis devam etmekte *(ok, a)*, Hiyalin kastları *(oklar, b)*; HE

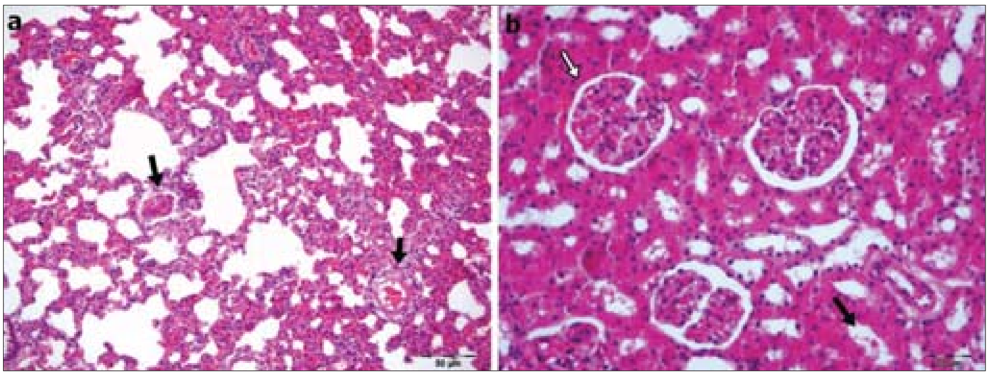

Fig 4. Group IV (CLP +180 mg/kg PH). Prominent decreasing of alveolar and bronchiolar exudate accumulation with vasculitis *(arrows in a)*, Glomerulitis *(white arrow in b)* and decreasing in hyaline cast formation *(black arrow in b);* HE

Şekil 4. Grup IV (CLP + 180 mg/kg PH). Alveolar ve bronşiolar eksudat birikiminde belirgin azalma ile birlikte devam eden vaskülitis *(oklar, a)*, Glomerulitis *(beyaz ok, b)* ve hiyalin kast oluşumu *(siyah ok, b);* HE

***Table 4.** Inflammation scoring in lung and kidney tissues*
***Tablo 4**. Akciğer ve böbrek dokularında inflamasyon skorları*

Tissue Lesions	Group I (Sham)	Group II (CLP)	Group III (CLP +90 mg/kg PH)	Group IV (CLP +180 mg/kg PH)	Group V (Sham+180 mg/kg PH)
Lung					
Hyperemia	-	++	++	++	-
Alveolar exudation	-	+++	+	+	-
Bronchiolar exudation	-	+++	+	+	-
Desquamation of bronchiolar epithelium	-	++	++	++	-
Vasculitis	-	++	++	++	-
Kidney					
Hyperemia	-	+++	++	++	-
Glomerulitis	-	+++	++	++	-
Degeneration of tubulary epithelium	-	++	++	+	-
Hyaline casts	-	+++	+++	+	-
Interstitial cellulary infiltration	-	+	+	+	-

mortality and morbidity. Therefore, most of studies have been focused on prevention and treatment of sepsis by researchers. In the present study, the effects of PH extract were investigated on tissues (lung and kidney) and cytokines in sepsis induced by CLP in rats. Two different doses of PH were evaluated.

In light of literatures, the CLP is commonly used as a model in animals. Sepsis resulting from it in animals is described the clinical situation of bowel perforation and bacterial infection during both early and late phases. Wang et al.[29] reported that the period of 16-20 h is the late phase of sepsis induced by CLP in intra- abdominal sepsis model. In the current study, the late phase of sepsis was used (after 16 h) for experimental model.

As is known, microorganisms and their toxins cause the activation of inflammatory systems and the release of cytokines in sepsis syndrome [30]. Cytokines are small cell-signaling protein molecules that play major roles in immune system response to inflammation and multi organ deficiencies. These cytokines are predominantly pro-inflammatory (TNF-α and IL-1ß) and the releasing of these pro-inflammatory mediators are characterized as the initial phase of sepsis. However, other cytokines are called anti-inflammatory (IL-10) and they leads to the compensatory antagonistic mechanism and the development of a balanced state of immunity [11,31-34]. TNF-α is produced primarily by activated macrophages, although it can be produced by many other cell types such as lymphoid, mast, endothelial cells, cardiac myocytes, adipose tissue, fibroblasts, and neurons [35,36]. It has an important role in coordinating of the inflammatory response and the releasing of other cytokines [37]. In previous studies, it was reported that the TNF-α infusion caused pulmonary hypertension, hypoxemia, decreased lung compliance, and increases in pulmonary micro-vascular permeability [38,39]. IL-1β is also produced by activated macrophages as a pro-protein, which is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis [40]. Endo et al.[41] reported an increase in TNF-α and IL-1β levels of plasma in septic shock. In the present study, IL-1ß, IL-10 and TNF-α levels significantly increased in the serum of the CLP group when compared to the sham group. These results are in accordance with previous studies [7,42,43]. However, PH1 and PH2 treated CLP groups decreased the cytokine (IL-1ß, IL-10 and TNF-α) levels when compared to the CLP group. The current data suggests that the PH has an ability to produce less inflammatory cytokines in response the CLP-induced sepsis in rats and in part, it can prevent the cytokine-related organ injury. Previous studies have shown that PH has anti-inflammatory and antioxidant effects [19,23].

Oxidative stress is the imbalance between oxidants and antioxidants at the cell. This imbalance can cause oxidative damage [44]. Malondialdehyde (MDA) is resulted from lipid peroxidation of polyunsaturated fatty acids and used as a marker for oxidative stress. The degree of lipid peroxidation can be estimated by the amount of malondialdehyde in tissues. Superoxide dismutase (SOD) and Glutathione (GSH) are important antioxidants in the intracellular protective mechanisms caused by reactive oxygen species such as free radicals and peroxides [45]. The SOD shows the antioxidant effect by converting toxic superoxide radicals into nontoxic hydroxyl peroxide and molecular oxygen [46]. Previous studies reported that, the level of GSH decreased in septic shock [47-49]. Ritter et al.[50] showed that MDA and SOD levels are markers of early mortality in septic rats. It has been reported that in CLP-induced sepsis, increasing oxidative stress in tissue in parallel with plasma are important mechanisms due to the output of free radicals [51]. In addition to, endotoxin administration caused to increase in cytokines along with lipid peroxide formation and membrane damage in animals [52,53]. Starkopf et al.[54] reported an increase in lipid peroxidation levels and a decrease in serum antioxidant capacity in sepsis. Our study showed increased tissue MDA level and decreased GSH and SOD levels after CLP, consistent with the literatures [7,43,55]. We observed a significant decrease in MDA and an increase in SOD and GSH in the PH-treated CLP rats compared to the sham groups. These results show the protective capacity of PH on lung and kidney tissues of septic rats.

In the present study, any histopathologic changes were not observed in sham group. However, we found significant histopathological changes in lung and kidney after the CLP-induced sepsis. There were dense inflammatory cell infiltrations with diffuse and nodular forms displayed remarkable findings at first glance. When the histopathological changes were evaluated in both PH1+CLP and PH2+CLP application groups, the inflammatory cell infiltrations decreased when compared to the CLP group. It was observed an inhibition of exudation in lung that in both PH1 and PH2 treated CLP groups, but the kidney had limited hyaline in only PH2 treated CLP group. According to our histopathological analysis, significant differences were determined in terms of inflammation scores between the sepsis group and the other groups. The CLP+PH1 and the CLP+PH2 groups had the same inflammation score in lung, finally, the effect of PH is not depend on dosage in lung, however, the high dose of PH had the lowest inflammation score in kidney.

As a result, the PH is a highly protective agent in preventing lung and kidney damage caused by CLP-induced sepsis via maintenance of alteration in the tissue levels of SOD, GSH, and MDA and alteration in serum levels of inflammatory cytokines such as TNF-α, IL-1β, and IL-10. Moreover, the administration of PH in CLP-induced septic rats has prevented the oxidative stress, cytokine response and the inflammation along with the protection of vital organ tissues.

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