The effect of \textit{p}-coumaric acid and ellagic acid on the liver and lungs in a rat model of sepsis

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\textbf{Background:} Sepsis pathophysiology includes oxidative stress and inflammatory processes.

\textbf{Objectives:} To investigate the antioxidant and anti-inflammatory effects of \textit{p}-coumaric acid (PC) and ellagic acid (EA) in a rat model of sepsis.

\textbf{Methods:} Wistar rats were assigned to 5 groups: control (C), sepsis (S), and S with treatment by PC, EA, or PC and EA combined (PCE). We determined the liver and lung tissue levels of pro- and anti-inflammatory cytokines, oxidative stress, and antioxidant markers.

\textbf{Results:} In the model of sepsis, proinflammatory cytokine levels increased, anti-inflammatory cytokines decreased, oxidative stress markers increased, and activity of antioxidant enzymes decreased significantly. In the liver of rats treated with PC, EA, or PCE, TNF-\textgreek{a} levels were reduced significantly, whereas IL-1\textgreek{b} and IL-6 levels were reduced significantly in rats treated with EA or PCE. Despite an increase in anti-inflammatory cytokines in the liver and lungs in all the treatment groups compared with S, an increase in IL-10 was only found in the liver of rats treated with PCE. The levels of malondialdehyde decreased significantly in the liver and lungs in rats in all treatment groups. The catalase and superoxide dismutase activity increased significantly in rats treated with PCE. While glutathione peroxidase activity in the liver only increased significantly in rats treated with PCE, it increased in the lungs of rats in all treatment groups.

\textbf{Conclusions:} PC and EA treatment had antioxidant and anti-inflammatory effects, which were stronger when these treatments were combined. Combined treatment with these substances may be beneficial in the treatment of sepsis.

\textbf{Keywords:} Antioxidant, anti-inflammatory, polyphenol, hydroxycinnamic acid, sepsis

Sepsis is defined as a harmful systemic inflammatory response to infection leading to organ dysfunction. Despite the development of interventions against infection, sepsis is still a serious event that leads to high mortality rates \cite{1}. Because the clinical signs of sepsis are nonspecific, and clinicians may not immediately suspect this condition, and diagnosis is often delayed \cite{2}. To diagnose patients, the high expenses involved—for the laboratory tests used in intensive care, imaging, drugs administered, intravenous support therapies, and the cost of healthcare personnel—can also be a problem \cite{3}. Despite an increasingly improved understanding of sepsis pathogenesis and treatment protocols, including immunomodulatory methods for interventions against infection, scant progress has been recorded in this field \cite{4}.

Polyphenols, which occur naturally in nutrients of plant origin, are used as preservatives in the food industry because of their particular antioxidant and anti-inflammatory properties. Among the phenolic acid derivatives, a subgroup of polyphenols, \textit{p}-coumaric acid (PC) and ellagic acid (EA) are well-known compounds with these properties. PC is found in edible plants such as groundnuts, tomatoes, carrots, sage, and garlic, as well as in beverages such as wine, vinegar, tea, coffee, and beer \cite{5, 6}. In addition, PC

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has antimicrobial properties [6-8]. EA is found in abundance in fruit and nuts, such as pomegranates, raspberries, strawberries, grapes, almonds, and walnuts. The oxygen radical-clearing and immunomodulatory properties of EA have been recognized [9-11].

In the pathophysiology of sepsis, the combination of oxidative stress and inflammatory processes has been widely recognized [12]. The target organ in sepsis is the vascular endothelium, and almost all mediators have an effect on the vasculature. The first response to endothelial damage by microorganisms is mediated by neutrophils, which initiate phagocytosis by activating proteolytic enzymes, and the production of free oxygen radicals (FOR). A large amount of FOR are produced by increased NADPH oxidase activity that produces superoxide (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$) in the respiratory burst. Infection can be reduced through transformation of chloride and hydrogen peroxide (H$_2$O$_2$) into hypochlorous acid (HOCl$^-$) by myeloperoxidase (MPO) activated in neutrophils.

At the same time, proinflammatory cytokines from monocytes and macrophages (which are secondary responders in inflammation), platelet activating factor (PAF), and nitric oxide (NO) are expressed, and, finally, with the proinflammatory mediators primarily affecting tumor necrosis factor (TNF-$\alpha$) and interleukin (IL)-1$\beta$, a more complex status is reached as arachidonic acid metabolites activate the coagulation and complement systems [13, 14]. To prevent damage to the cells by these changes, anti-inflammatory cytokines produced against proinflammatory cytokines and antioxidant enzymes against reactive oxygen species (ROS) are activated. Consequently, either balance is restored by eliminating the disease agent, e.g. through HOCl$^-$, and clearance of oxygen radicals and antioxidant effects, or sepsis symptoms result because of excessive exposure to inflammatory and oxidative stress factors; and multiorgan failure may develop [15].

An understanding of the role of mitochondrial dysfunction in sepsis, together with elucidation of the inflammatory processes in the pathogenesis of sepsis, has popularized the use of antioxidant agents in its treatment [16]. In experimental models of inflammation, PC was shown to decrease levels of proinflammatory cytokines (primarily TNF-$\alpha$), mediators such as prostaglandin (PGE$_2$) and cyclooxygenase (COX$_{2}$), as well as to inhibit phagocytosis by macrophages [8, 17]. In other models of inflammation, PC displayed an immunomodulatory effect on both humoral and cellular immunity, and this has been shown to contribute to its antioxidant effects [18, 19]. There is even stronger evidence for the anti-inflammatory properties of EA. Notably, Liu et al. reported that EA significantly decreases levels of proinflammatory cytokines such as TNF-$\alpha$, IL-1$\beta$, and IL-6, and increases the level of anti-inflammatory cytokines such as IL-10 [20]. In a model of inflammation Mo et al. showed anti-inflammatory and analgesic effects of EA leading to inhibition of TNF-$\alpha$ and IL-1$\beta$ [21]. In other models of inflammation in vivo and in vitro, EA had anti-inflammatory effects by suppressing leukocyte migration and neutrophil count [22], and by lowering the TNF-$\alpha$, IL-1$\beta$, PGE$_2$, and COX$_{2}$ levels with neutrophil inhibition [23].

In addition to the anti-inflammatory effect of PC in several models of ischemia–reperfusion and inflammation, antioxidant activity can be increased by reducing lipid peroxidization [6, 24]. Similarly, EA has an antioxidant effect and reduces oxidative stress by its radical-clearing properties [10, 11]. As a result, while malondialdehyde (MDA) levels are reduced with a reduction in lipid peroxidation, glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT) antioxidant enzyme activities increase.

Because phenolic acid derivatives are effective against oxygen radical-clearing and immunomodulatory processes, the use of these derivatives together with conventional antimicrobial treatments may be beneficial. If so, the combination of treatments might reduce the costs of treatment and mortality related to sepsis. To our knowledge, no previous study of the direct use of PC and EA on sepsis has been reported in the literature. To determine the potential utility of PC and EA we administered these compounds to rats in which a model of sepsis had been induced by intraperitoneal administration of lipopolysaccharide (LPS) and determined potential antioxidative and anti-inflammatory effects by assay of lung and liver tissue samples for proinflammatory mediators of sepsis: TNF-$\alpha$, IL-1$\beta$, and IL-6, as well as anti-inflammatory mediators IL-4 and IL-10. GSH-Px, SOD, CAT, and MPO activities, and MDA levels were determined as markers of antioxidant activity.

**Materials and methods**

**Animals**

Approval for the study was granted by the Animal Research Local Ethics Committee of Kahramanmaraş.
p-Coumaric acid and ellagic acid in sepsis

Sütçü Imam University (KSU; Protocol No. 2014/03-67). All the procedures in the Experimental Animals Laboratory of KSU were performed in conformity with the regulations and guidelines for the use of experimental animals specified in the 8th edition of the Guide for the Care and Use of Laboratory Animals by the Institute of Laboratory Animal Research, Washington, DC, 2011 (The National Academies Press publisher).

We used 30 adult male Wistar albino rats, each weighing 300 ± 50 g. The animals were acclimatized to the laboratory housing environment for a week: room temperature 22 ± 2°C, humidity 60 ± 5%, under a 12-hour light–dark cycle using white fluorescent lights, and allowed a diet of standard food pellets (Bil-Yem, Ankara, Turkey). Throughout the experimental period, all the animals had free access to food and drinking water.

**Biochemical agents**

p-Coumaric acid ((E)-3-(4-hydroxyphenyl)-2-propenoic acid or 4-hydroxycinnamic acid), ellagic acid (2,3,7,8-tetrahydroxy-chromeno[5,4,3-cde]chromene-5,10-dione), and lipopolysaccharides from *Escherichia coli* O111:B4 (LPS) were obtained from Sigma-Aldrich.

**Experimental design**

The rats were assigned without any special selection to 5 groups of 6 animals each: a control (C) group, a sepsis (S) group, a p-coumaric acid (PC)-treated group, an ellagic acid (EA)-treated group, and a PC and EA combination (PCE)-treatment group. Rats in the C and S groups were fed standard rat food and administered normal saline (1–2 mL) by gavage for 7 days. At the same time, the rats in the PC, EA, and PCE groups were allowed standard food with the addition of 100 mg/kg PC, 50 mg/kg EA, or 100 mg/kg PC + 50 mg/kg EA, respectively, by gavage. The doses of PC and EA were selected according to similar studies on this and related topics [25, 26]. The doses were achieved by dissolving the PC in 5% dimethyl sulfoxide (DMSO) and the EA in normal saline (1–2 mL/dose). After 7 days of treatment, a model of intra-abdominal sepsis was induced in the rats of the S, PC, EA, and PCE groups with an intraperitoneal (ip.) injection of 10 mg/kg LPS. The rats in the control group were administered the same volume of saline intraperitoneally [27].

After inducing sepsis and until the surgical procedures, the same doses of PC and EA were again administered by gavage daily to rats in the PC, EA, and PCE groups in addition to the standard food allowed freely. At 24 h after LPS administration, each rat was deeply anesthetized with 50 mg/kg ketamine ip. (Ketalar, Eczacibasi, Istanbul, Turkey). When a sufficient level of anesthesia was reached, the abdominal skin of each rat was shaved and treated with povidone–iodine, and an entry incision was made on the midline. Tissue samples were taken from the lungs and liver for biochemical assays, and the rats were killed by exsanguination.

We assayed lung and liver tissue sample homogenate supernatants for GSH-Px, SOD, CAT, MPO, and MDA, and cytokines TNF-, IL-1, IL-6, IL-4, and IL-10.

**Tissue homogenates**

All tissue specimens were washed with 0.9% saline to remove hematomas and blood, and excess saline was removed. The specimens were stored in plastic bottles at –20°C awaiting biochemical analyses. Tissues were weighed, placed in a 0.1 M phosphate buffer at pH 7.0 in conical 10 mL polystyrene tubes and homogenized using a Miccra D-8 homogenizer tool (ART, Germany). Aliquots of homogenates were then centrifuged at 10,000 ×g for 30 min and the supernatant of aspirated for assay. Tissue protein levels in the supernatants were assayed according to the Lowry method [28] using bovine serum albumin (Sigma catalogue No. A9418) as the protein standard.

**Biochemical analyses**

**Determination of GSH-Px, SOD, CAT, and MPO activity, and MDA levels.** GSH-Px activity in homogenate supernatants of the tissue samples was determined using the method described by Rotruck et al. [29]. SOD activity was determined using the method described by Sun et al. [30], CAT activity was determined using the method described by Sinha [31], and MPO activity was determined using the method described by Stucchi et al. [32]. MDA levels in the tissue homogenate supernatants were measured using the spectrophotometric method described by Ohkawa et al. [33].

**Determination of TNF-α, IL-1β, IL-4, IL-6, and IL-10 levels.** Serum TNF-α levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit for rat TNF-α (catalog No. KRC3011,
Novex, Thermo Fisher Scientific). IL-1β, IL-4, IL-6, and IL-10 levels in the tissue homogenate supernatants were measured using ELISA kits for rat IL-1β, IL-4, IL-6, and IL-10 (Novex catalog Nos. KRC0011, KRC0041, KRC0061, KRC0102).

Statistical analyses
Statistical analyses of the data were conducted using IBM SPSS Statistics for Windows software (version 22.0; IBM Corp). Numerical variables are presented as means ± standard deviation (SD). Comparisons between the groups were made using an ANOVA, and within-group variance was determined using a Turkey post hoc honest significant difference test. $P < 0.05$ was accepted as significant.

Results

GSH-Px activity
By comparison with untreated rats in which sepsis was induced, significantly higher levels of GSH-Px activity were found in the liver tissue of control rats without sepsis ($P < 0.01$) and rats with sepsis treated with PCE ($P < 0.05$), and in the lung tissue of control rats ($P = 0.003$), and rats with sepsis treated with PC ($P = 0.036$), EA ($P = 0.022$), and PCE ($P = 0.003$; Figures 1 and 2).

SOD activity
The SOD activity levels in both the liver tissue and lung tissue of control rats and rats with sepsis treated with PCE were significantly higher than in rats in which sepsis was induced without treatment ($P = 0.002$, $P = 0.017$, and $P = 0.003$, $P = 0.048$, respectively). Despite a tendency for the liver tissue levels of SOD activity to be higher in liver tissues from rats with sepsis treated with EA, and the lung tissue levels of SOD to be higher in rats with sepsis treated with PC or EA, the differences were not significant ($P > 0.05$; Figures 1 and 2).

CAT activity
By comparison with the rats in which sepsis was induced without treatment, the levels of CAT activity in both the liver tissue and lung tissue of control rats and rats with sepsis treated with PCE were significantly higher ($P < 0.05$ for both; Figures 1 and 2).

Figure 1. Rat liver tissue levels of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT), and myeloperoxidase (MPO) antioxidative enzymes, and malondialdehyde (MDA) marker of lipid peroxidation. C, Control group; S, LPS-induced sepsis group, PC, rats with sepsis treated with p-coumaric acid; EA, ellagic acid; and PCE, combination of PC and EA ($*P < 0.05$, $**P < 0.01$).
**MPO activity**

By comparison with the rats in which sepsis was induced without treatment, the levels of MPO activity in the liver tissue were significantly lower than that in control rats \((P = 0.01)\) and rats treated with EA \((P = 0.043)\). No significant difference was seen in the levels of MPO activity in the lung tissue of the control or any treatment group \((P > 0.05\) for all; Figures 1 and 2).

**MDA levels**

By comparison with rats in which sepsis was induced without treatment, the MDA levels were significantly lower in the liver tissue of rats with sepsis, but treated with PC \((P = 0.007)\), EA \((P = 0.003)\), or PCE \((P = 0.002)\), and in the lung tissue of control rats \((P < 0.01)\), and rats with sepsis, but treated with PC \((P < 0.05)\) or PCE \((P < 0.01\); Figures 1 and 2).

**TNF-\(\alpha\) levels**

The levels of TNF-\(\alpha\) in the liver of rats with LPS-induced sepsis, were significantly higher than in control rats. Such an increase was prevented by any of the PC, EA, or PCE treatments, and the levels of TNF-\(\alpha\) in the liver of rats with sepsis were significantly lower in all of the treatment groups than in the group without treatment (Table 1). Although the levels of TNF-\(\alpha\) in the lungs of rats from the control group and all treatment groups tended to be lower than in the lungs from the group without treatment, we did not find any significant differences \((P > 0.05\) for all; Table 2).

**IL-1\(\beta\) levels**

The levels of IL-1\(\beta\) in the liver of the rats with LPS-induced sepsis were significantly higher than in control rats \((P = 0.002)\) and rats with sepsis treated with EA \((P = 0.009)\) or PCE \((P = 0.037\); Table 1). The IL-1\(\beta\) levels in the lungs of the rats with sepsis were significantly higher than in control rats \((P = 0.008)\). Although all of the various treatments tended to reduce the IL-1\(\beta\) levels in the lungs of the rats with sepsis, we did not find any significant differences (all \(P > 0.05\); Table 2).

**IL-4 levels**

The levels of IL-4 levels in the liver of the rats with LPS-induced sepsis were significantly higher than in control rats \((P = 0.004)\). Although the levels of IL-4 in the liver of septic rats with any of the treatments tended to be higher than in the group without treatment, we did not find any significant differences (all \(P > 0.05\); Table 1). The levels of IL-
4 levels in the lungs of the rats with LPS-induced sepsis were significantly higher than in control rats \((P<0.01)\). Although the levels of IL-4 in the lungs of septic rats with any treatment tended to be higher than in the group without treatment, we did not find any significant differences \((all P > 0.05)\); Table 2).

**IL-6 levels**

The levels of IL-6 in the liver of rats with LPS-induced sepsis were significantly higher than in control rats \((P = 0.002)\) and rats with sepsis treated with EA \((P = 0.007)\) or PCE \((P = 0.005)\). Although rats with sepsis treated with PC tended to have to have lower levels of IL-6 than the group without treatment, we did not find any significant difference (Table 1). The levels of IL-6 in the lungs of rats with sepsis were significantly higher than in control rats \((P = 0.006)\) and rats with sepsis treated with the PCE combination \((P = 0.023)\). Although septic rats treated with PC or EA alone tended to have to have lower levels of IL-6 than septic rats without treatment, we did not find any significant differences (Table 2).

**IL-10 level**

The levels of IL-10 in the liver of rats with LPS-induced sepsis were significantly lower than in control rats \((P = 0.001)\) and rats with sepsis treated with the PCE combination \((P = 0.031)\). Although septic rats treated with PC or EA alone tended to have to have higher levels of IL-10 than septic rats without treatment, we did not find any significant differences (Table 1). The levels of IL-10 in the lungs of rats with LPS-induced sepsis were significantly lower than in control rats \((P = 0.01)\). Although the levels of IL-10 in the lungs tended to be higher in septic rats with any treatment compared with septic rats that were untreated, we did not find any significant differences \((all P > 0.05)\); Table 2).

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**Table 1. Rat liver tissue levels of proinflammatory and anti-inflammatory cytokines**

<table>
<thead>
<tr>
<th></th>
<th>C (n = 6)</th>
<th>S (n = 6)</th>
<th>PC (n = 6)</th>
<th>EA (n = 6)</th>
<th>PCE (n = 6)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α (pg/mg protein)</td>
<td>645 ± 37</td>
<td>949 ± 78*</td>
<td>647 ± 127</td>
<td>650 ± 100</td>
<td>632 ± 133</td>
<td>0.001</td>
</tr>
<tr>
<td>IL-1β (pg/mg protein)</td>
<td>2910 ± 184</td>
<td>3716 ± 403d,e</td>
<td>3366 ± 232</td>
<td>3018 ± 460</td>
<td>3132 ± 280</td>
<td>0.002</td>
</tr>
<tr>
<td>IL-4 (pg/mg protein)</td>
<td>1216 ± 149</td>
<td>866 ± 143a</td>
<td>907 ± 30</td>
<td>922 ± 53</td>
<td>972 ± 263</td>
<td>0.004</td>
</tr>
<tr>
<td>IL-6 (pg/mg protein)</td>
<td>2010 ± 708</td>
<td>3620 ± 359ace</td>
<td>2855 ± 521</td>
<td>2238 ± 872</td>
<td>2187 ± 606</td>
<td>0.001</td>
</tr>
<tr>
<td>IL-10 (pg/mg protein)</td>
<td>1453 ± 222ab</td>
<td>816 ± 295e</td>
<td>902 ± 161</td>
<td>938 ± 33</td>
<td>1257 ± 14</td>
<td>0.001</td>
</tr>
</tbody>
</table>

C, control group; S, LPS-induced sepsis group, PC, rats with sepsis treated with p-coumaric acid, EA, ellagic acid; and PCE, PC and EA combined; TNF, tumor necrosis factor; IL, interleukin. Data are expressed as mean ± SD, unless otherwise noted. ANOVA post hoc Tukey honest significant difference or Dunnnett test

* S vs C; \(P < 0.01\); *PC vs S, \(P < 0.01\); *EA vs S, \(P < 0.05\); *EA vs S, \(P < 0.01\); *PCE vs S, \(P < 0.05\); *PCE vs S, \(P < 0.01\); *C vs PC, \(P < 0.05\); *PC vs S, \(P < 0.05\).

**Table 2. Rat lung tissue levels of proinflammatory and anti-inflammatory cytokines**

<table>
<thead>
<tr>
<th></th>
<th>C (n = 6)</th>
<th>S (n = 6)</th>
<th>PC (n = 6)</th>
<th>EA (n = 6)</th>
<th>PCE (n = 6)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α (pg/mg protein)</td>
<td>88 ± 10</td>
<td>103 ± 20</td>
<td>93 ± 17</td>
<td>92 ± 8</td>
<td>92 ± 7</td>
<td>0.38</td>
</tr>
<tr>
<td>IL-1β (pg/mg protein)</td>
<td>251 ± 86</td>
<td>837 ± 86a</td>
<td>530 ± 75</td>
<td>480 ± 90</td>
<td>481 ± 96</td>
<td>0.02</td>
</tr>
<tr>
<td>IL-4 (pg/mg protein)</td>
<td>8 ± 7</td>
<td>110 ± 25a</td>
<td>121 ± 38</td>
<td>124 ± 30</td>
<td>139 ± 15</td>
<td>0.37</td>
</tr>
<tr>
<td>IL-6 (pg/mg protein)</td>
<td>9 ± 6</td>
<td>115 ± 91ab</td>
<td>38 ± 24</td>
<td>36.4 ± 19</td>
<td>25 ± 19</td>
<td>0.007</td>
</tr>
<tr>
<td>IL-10 (pg/mg protein)</td>
<td>67 ± 41</td>
<td>28 ± 17a</td>
<td>35 ± 19</td>
<td>33 ± 15</td>
<td>52 ± 26</td>
<td>0.08</td>
</tr>
</tbody>
</table>

C, control group; S, LPS-induced sepsis group; PC, rats with sepsis treated with p-coumaric acid; EA, ellagic acid; PCE, combination of PC and EA; TNF, tumor necrosis factor; IL, interleukin. Data are expressed as mean ± SD, unless otherwise noted. ANOVA post hoc Tukey honest significant difference or Dunnnett test

* S vs C; \(P < 0.01\); *PCE vs S, \(P < 0.05\).
Discussion

As expected, we found an increase in the levels of proinflammatory cytokines TNF-α, IL-1β, IL-6 in the liver and lungs of the rats with the LPS-induced model of sepsis, together with a reduction in putative anti-inflammatory cytokines IL-4 and IL-10, an increase in the oxidative stress marker MDA, and a decrease in GSH-Px, SOD, and CAT antioxidant enzyme activity. When the rats with sepsis were treated with PC or EA, and especially when PC and EA were combined, proinflammatory cytokine levels were reduced, and the levels of anti-inflammatory cytokines were increased, although, for IL-10, this increase was limited to treatment with PC and EA combined. The combined use of PC and EA significantly reduced lipid peroxidization by oxidative stress in all the treatment groups. Increases in the activity of antioxidant enzymes GSH-Px, SOD, and CAT were seen with both PC and EA treatment, especially in the lung tissue.

Consistent with similar findings in other models [8, 17-21, 23] the data generated in our rat model of sepsis showed that PC and EA treatment alone and together significantly reduced levels of proinflammatory cytokines TNF-α, IL-1β, and IL-6 in the liver tissues. Moreover, EA applied alone and together with PC significantly reduced levels of IL-1β and IL-6 in lung tissues. Although the levels of all the proinflammatory cytokines in the lungs of the treated groups tended to be lower than those in rats with untreated sepsis, we did not find significant differences. When the anti-inflammatory cytokines were determined, despite a tendency for increases in the levels of IL-4 and IL-10 in the liver tissues in all the treatment groups, compared with the untreated sepsis group, a significant increase was only observed in the IL-10 level with combined EA and PC treatment. In the lung tissues, although the levels of IL-4 and IL-10 tended to be increased in all the treatment groups, we did not find significant differences. Some studies have found that HOCl, formed by MPO enzymes when neutrophils interact with a microorganism, is inhibited by both EA and PC [17, 34]. In the present study, a reduction of MPO activity was only found with EA treatment, and this reduction was only significant in the liver.

Consistent with similar findings in other models [6, 24, 10, 11], in the present study, a significant reduction of the elevated MDA levels in the livers and lungs of the rats with induced sepsis was found in all the treatment groups. When antioxidant enzyme activity was determined, we found that while CAT and SOD activities were significantly reduced in the liver and lung tissues of the rats with induced sepsis, they were increased significantly by treatment with EA and PC combined. While a significant increase in GSH-Px activity was only found in the liver of rats with induced sepsis treated with EA and PC together, in the lung tissues, a significant increase was found in all the treatment groups.

Although our data demonstrate the potential of PC and EA as anti-inflammatory and antioxidative compounds for the adjunct treatment of sepsis, our present study has a series of limitations as follows. To our knowledge, no previous studies used PC and EA directly in rat models of sepsis, and so there was no basis for defining the optimal doses of PC and EA to be used for the rats, and selection of the number of rats to provide adequate statistical power to observe differences. Because of ethical constraints to limit the number of rats used, we used only 6 rats per group, which was considered to be suitable for these experiments. Another limitation of the present study was the lack of an appropriate positive control substance—for example, vitamin C or E, which are well known to have antioxidant effects. To limit the use rats (reduction) and maximize the number in the treatment groups, no additional group was used to study a positive control substance. The control group was given saline, but no additional control group given 5% DMSO was used. Certain concerns about DMSO need to be addressed in future studies. DMSO may affect oxidative and other enzyme levels in the liver and lungs, and has been shown to have anti-inflammatory and antioxidative properties in vitro and in vivo [35-37]. The extent of the effect of DMSO likely depends on the dose, route of administration, and the particular animal or in vitro model used [37, 38]. Nevertheless, DMSO is commonly used in vehicles for test substances [37, 39]. We consider that we have minimized the volume to mitigate its possible effect on the measured markers, and that EA, in the vehicle without DMSO, generally had a greater effect than PC administered in DMSO-containing vehicle. The effects of DMSO at the minimal doses given in the present model remain to be determined in future studies to abate doubts about its use as a drug vehicle and its possible interference in evaluation of substances using this model.
**Conclusion**

Polyphenolic substances PC and EA have significant antioxidant and anti-inflammatory effects on sepsis, particularly when used together. Because of these properties, their use may be beneficial as a support for conventional antimicrobial therapy in the treatment of sepsis.

**Author contributions**

AU, FMY, and HÖ substantially contributed to the conception and design of the study; AU, FMY, BB, and FIT acquired the data; and AU, ÖFB, and GÖ contributed to its analysis and interpretation. AU, FIT, BB, and GÖ drafted the article; and HÖ, FMY, and ÖFB critically revised it. All authors approved the version submitted for publication and take responsibility for the statements made in the article.

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**Conflicts of interest**

The authors declare no conflict of interest.

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