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RESEARCH ARTICLE

Effects of Platelet-activating Factor Receptor Antagonist (PAFRA) on Selected Inflammatory and Biochemical Parameters in Lipopolysaccharide-Induced Rat Endotoxemia Model [1]

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Summary

Platelet-activating factor (PAF) is a significant phospholipid mediator of the immune system produced by a veriety of cells involved in inflammatory reactions in sepsis. In this experimental study, our aim was to investigate the role of PAF receptor antagonist (PAFRA) on biochemical and inflammatory disturbances in lipopolysaccharide (LPS)-treated rats. A total of 32 adult male Wistar rats were divided into four equal groups: Group 1 (control group, C) was treated with 0.9% saline. Group 2: LPS was injected intravenously (1.6 mg/100 g), Group 3 received PAFRA treatment (10 mg/kg) 2 min before 0.9% saline injection, Group 4 received PAFRA treatment 2 min before endotoxin treatment. Blood samples were collected 6 h after treatment. LPS (Group-II) caused statistically significant increases in serum TNF-α, IL-6 and IL1β levels, CRP, LDH, AST, ALT, creatinine, BUN, cholesterol, triglyceride concentration, and caused statistically significant decreases in platelet count, glucose, total protein and albumin levels. Also, when compared to control group leukopenia and significant changes in the leukocyte differential were evident. In group 4, PAFRA inhibited serum TNF-α and IL1β levels, leukopenia compared with the group 2 ($P<0.05$). However, there were no significant differences in the other parameters between the two groups. The results demonstrate that at the administered dose and route, PAFRA has a slight effect in the pathogenesis of endotoxemia.

Keywords: *Endotoxin, Cytokines, Biochemical parameters, Platelet-activating factor Receptor antagonist, Rat*

Lipopolisakkarit ile İndüklenen Rat Endotoksemi Modelinde Bazı Yangısel ve Biyokimyasal Parametreler Üzerine Platelet Aktive Edici Faktör Reseptör Antagonisti (PAFRA)'nin Etkileri

Özet

Platelet Aktive edici Faktör (PAF), sepsisde yangısel reaksiyonlara karışan birçok hücre tarafından üretilen immun sistemin önemli bir fosfolipid mediyatörüdür. Bu deneysel çalışmada amacımız, lipopolisakkarit (LPS) uygulanan sıçanlarda biyokimyasal ve yangısel bozukluklar üzerine PAF reseptör antagonisti (PAFRA)'nın rolünü araştırmaktı. Total 32 adet yetişkin erkek sıçan dört eşit gruba ayrıldı: grup 1 kontrol (C) olarak hizmet etti. Grup 2'deki hayvanlara intravenöz LPS (1.6 mg/100 g, *Escherichia Coli*, 0.111:B4) verildi. Grup 3'de 0.9% serum fizyolojik enjeksiyonundan 2 dak. önce PAFRA (10mg/kg) intraperitoneal olarak enjekte edildi. Grup 4'de, LPS uygulamasından 2 dak. önce PAFRA uygulandı. Kan örnekleri uygulamadan sonraki 6.saatte toplandı. LPS (grup 2), serum TNF-α, IL-6 ve IL1β seviyesi, CRP, LDH, AST, ALT, kreatinin, BUN, kolesterol, trigliserit konsantrasyonunu önemli düzeyde artırdı, platelet sayısı, glikoz, total protein ve albumin seviyesini önemli oranda düşürdü. Ayrıca kontrol grupla karşılaştırıldığında LPS grupta lökopeni ve diferensiyal lökosit sayısında önemli değişiklikler mevcuttu ($P<0.05$). Grup 2 ile karşılaştırıldığında grup 4'de PAFRA, TNF-α ve IL1β seviyelerini ve lökopeniyi inhibe etti ($P<0.05$). Buna rağmen iki grup arasındaki diğer parametrelerde önemli değişiklikler yoktu. Mevcut sonuçlar; uygulanan doz ve yolda PAFRA'nın endotokseminin patogenezinde hafif bir etkiye sahip olduğunu göstermektedir.

Anahtar sözcükler: *Endotoksin, Sitokin, Biyokimyasal parametreler, Platelet aktive edici faktör reseptör antagonisti, Sıçan*

INTRODUCTION

Sepsis from gram-negative bacterial infections such as some enteric disease, septisemia, metritis, mastitis, and pneumonia may be complicated by a variety of conditions characterized by fever, tachycardia, tachypnea, hypotension,

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disseminated intravascular coagulation (DIC), multiple organ failure, and even death [1,2] . Despite the potent antimicrobial treatments, improved levels of monitoring and intensive supportive care in the last decade, sepsis increasingly remains one of major causes of death, and the mortality rate (60%) in animals [3,4]. Sepsis causes a generalized inflammatory reaction including the concurrent activation of several endogenous mediator systems such as immune system, endothelium, and coagulation system [5]. Endotoxin (LPS), a cell wall constituent of gram-negative bacteria, is involved in the patogenesis of endotoxic shock, coagulopathy. Administration of LPS to experimental animals leads to the production of the pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 from monocytes, macrophages and endothelium [6]. In recent years, it has become apparent that the mediators of inflammation have critical roles in sepsis. After intravenous endotoxin challenge, rapid production and release of proinflammatory cytokines (e.g. TNF-α, IL-1β, IL-6) from monocytes, macrophages and endothelium were detected [7]. Release of these pro-inflammatory cytokines determines the development and incidence of tissue damage, multi organ failure (MOF) or even death [8]. In recent years, some therapeutic strategies for human and animal septic shock have been designed to neutralize the inflammatory mediators. Especially, anti-cytokine strategies such as anti-inflammatory cytokines (IL-10, IL-13), IL-1 receptor antagonist (IL-1Ra), knock-out of TNFR (p55), and anticytokine antibodies has gained increasing importance endotoxemia studies [2,6,9].

Platelet-activating factor (PAF; 1-O-alkyl-2-acetyl-sn-glyceryl - 3-phosphonocholine) is a natural phospholipid synthesized by several different cells including basophils, macrophages, neutrophils and platelets, in response to various stimuli including lipopolysaccharide (LPS), and tissue factors released after endothelial disruption [10]. The administration of PAF to experimental animals causes diverse pathophysiological changes very similar to those observed during endotoxaemia such as hypotension, increased vascular permeability, thrombocytopenia and gastrointestinal damage [10,11]. LPS affects the expression of both PAF and its receptor [12]. The effects of PAF are mediated through specific PAF receptors (PAF-R) [13]. PAF-R is a G-protein coupled receptor and it exists in various cells such as platelet, neutrophil. Engagement of the PAFR by PAF activate a variety of intracellular signaling cascades and, induces functional responses of PAFR-bearing cells that then initiate or amplify inflammatory and thrombotic events [14,15]. Early observations indicated that additive or synergistic activities of PAF and cytokines may have key pathologic effects in the pathogenesis of lethal septicemia, and showed that interactions between PAF, tumor necrosis factor a (TNF-α), and IL-1 signaling cascades are particularly important [14]. PAF is an important mediator in experimental models. The effects of PAF can be inhibited both *in vivo* and *in vitro* with PAF receptor antagonists in LPS-induced sepsis [16]. Multiple studies have shown that complete protection against LPS-induced sepsis can be achieved if the agent is administered prior to the onset of the experimental intervention causing sepsis [12]. Ginkgolide B (BN52021) is a specific PAF-R antagonist and It is able to antagonize binding of PAF and its receptor (PAF-R) competitively, and thus PAF is unable to activate effector enzyme through G-protein transduction to block signal transduction of PAF-R. PAFRA may inhibit platelet aggregation, antagonize inflammation and shock, and protect blood vessels of heart and brain [15].

The present study was planned to determine whether administration of PAFRA attenuates the cytokine response and biochemical disturbances due to LPS-induced inflammation in rat endotoxemia model.

MATERIAL and METHODS

In our study, thirty two healthy adult male wistar rats (weight range: 200-250 g, Kobay experimental animal laboratory, Ankara) were acclimated at a constant temperature of 20ºC for at least a week. The animals were fed a standard pellet diet, and tap water was available *ad libitum*. All rats were in excellent physical condition prior to the experiments. This study was conducted according to the guidelines approved by the local ethics committee of the Faculty of Veterinary Medicine (University of Selcuk, Konya, Turkey, report no. 2011/005). Lipopolysaccharide (*Escherichia Coli*, 0.111:B4, SIGMA Cat.no: L4130) was dissolved in physiological saline immediately before use.

A total of 32 adult male Wistar rats were randomly divided into four equal groups: Group 1, Control group (C) was treated with 0.9% saline (0.2 ml iv). Group 2 (LPS): lipopolysaccharide (LPS) was dissolved in physiological saline immediately before use. LPS (*Escherichia coli* lipopolysaccharide, 0.111:B4 serotype, Sigma L4130) was injected intravenously (1.6 mg/100 g, into the tail vein). Group 3 (PAFRA): the rats in this group received PAFRA treatment alone (10mg/kg, Sigma G6910) 2 min prior to a single injection of saline solution (0.2 ml, iv.) instead of LPS. Group 4 (LPS + PAFRA): these rats received 10mg/kg IP PAFRA 2 min before endotoxin challenge (1.6 mg/100 g). Blood samples (2-3 ml) were collected by cardiac puncture 6 h after treatment. At the end of experiment, rats were sacrificed under deep anesthesia with high doses of thiopental sodium (Pental® sodium inj., IE Ulagay Ilac Sanayi, Istanbul, Turkey).

The levels of serum tumor necrosis factor-*α* (TNF-*α*) (eBioscience International, Inc. rat TNFα kit, Nivelles, Belgium), interleukin-1β (IL-1β) (eBioscience International, Inc. rat IL-1β kit), interleukin-6 (IL-6) (eBioscience International, Inc. rat IL-6 kit) and, C-reactive protein (CRP) (Alpha Diagnostic International Rat CRP kit) were determined by enzyme-linked immunosorbent assay (ELISA) using an ELISA reader (Anthos Labtec Instruments, A5022, Salzburg). For biochemical analyses, serum concentrations of cholesterol, triglycerides,

creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), glucose, total protein (TP), albumin (Alb) were determined by an autoanalyser (Siemens Dimension RxL Max otoanalizatör) using commercial kits (Dade Behring). The leukocyte count and platelet count (PLT) were determined by a haemocytometer method using Turk and Rees-Ecker solution, respectively. Selected blood smears were stained with May-Grünwald and Giemsa solution, and then used to determine the percentage values of different leukocytes.

Values are reported as mean ± standard error and were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's test, in the SPSS-15.0. In all cases, probability of error of less than 0.05 was selected as the criterion for statistical significance. To calculate the true concentration, raw data from ELISA array were multiplied by the appropriate dilution factor (x2 for cytokines and x 20K for CRP).

RESULTS

The effects of PAFRA on inflammatory and biochemical parameters of groups including control, LPS, PAFRA and PAFRA+LPS-treated rats are presented in *Table 1*.

When compared with the control group, there were no significant changes in any of the measured parameters in only PAFRA-treated rats (group 3) (P>0.05).

As compared to the control group, LPS injection displayed statistically significant increases in serum TNF-a, IL-6 and IL-1β levels, CRP, AST, ALT, LDH, creatinine, BUN, cholesterol, triglyceride concentration, and caused statistically significant decreases in platelet count, glucose, total protein and albumin levels. LPS administration (group 2) caused a decrease in leukocyte count with a significant neutrophilia and lymphopenia. In group 4, PAFRA inhibited serum TNF-α and IL1β levels compared with the group 2 (P<0.05). Additionally, the diminution observed in leukocyte count, changes in the percentage of neutrophils and lymphocytes following endotoxin administration was suppressed by PAFRA (P<0.05). However, the other parameters were not suppressed by the administration of PAFRA.

Table 1. *Effect of PAFRA on selected serum cytokine levels in a rat endotoxemic model (mean±SE)*

Tablo 1. *Rat endotoksemi modelinde belirli serum sitokin düzeyleri üzerine PAFRA'nın etkileri (mean±SE)*

Investigated Parameters	Control (n=8)	LPS (n=8)	PAFRA (n=8)	PAFRA+LPS (n=8)
TNF-a (pg/ml)	BDL	2404±333^{a}	BDL	1683±253^{b}
IL-6 (pg/ml)	BDL	4158±514^{a}	BDL	3727±415^{a}
IL-1β (pg/ml)	BDL	2781±334^{a}	BDL	2080±195^{b}

a,b,c,d: *Differences in the same row are statistically significant when the values are marked with different letters (P<0.05),* ***LPS;*** *Lipopolysaccharide,* ***PAFRA;*** *Platelet-activating factor receptor antagonist,* ***BDL;*** *below the detection limit*

Table 2. *Effect of PAFRA on some haematological parameters in endotoxaemic rats (mean ± SE)*

Tablo 2. *Rat endotoksemi modelinde bazı hematolojik parametreler üzerine PAFRA'nın etkileri (mean±SE)*

Investigated Parameters	Control (n=8)	LPS (n=8)	PAFRA (n=8)	PAFRA+LPS (n=8)
CRP (µg/ml)	214±50^{b}	2506±497^{a}	208±36^{b}	2371±392^{a}
PLT (x10^{9} / L)	629±45^{a}	120±12^{b}	659±26^{a}	175±38^{b}
Leukocyte (mm^{3})	6452±890^{a}	1512±195^{c}	6129±682^{a}	3988±391^{b}
Neutrophil (%)	24.4±3.2^{c}	71.5±3.9^{a}	27.5±3.6^{c}	45.4±4.3^{b}
Lymphocyte (%)	67.3±3.9^{a}	24.6±3.0^{c}	64.1±3.6^{a}	49.8±4.8^{b}

a,b,c,d; *Differences in the same row are statistically significant when the values are marked with different letters (P<0.05).* ***LPS;*** *Lipopolysaccharide,* ***PAFRA;*** *Platelet-activating factor receptor antagonist*

Table 3. *Effects of PAFRA on some biochemical parameters in endotoxaemic rats (mean ± SE)*

Tablo 3. *Rat endotoksemi modelinde bazı biyokimyasal parametreler üzerine PAFRA'nın etkileri (mean ± SE)*

Investigated Parameters	Control (n=8)	LPS (n=8)	PAFRA (n=8)	PAFRA+LPS (n=8)
AST U/L	132±22^{b}	795±162^{a}	112±13^{b}	728±124^{a}
ALT U/L	69.9±7.6^{b}	249.4±33.3^{a}	62.6±6.5^{b}	213.0±20.4^{a}
LDH (U/L)	349±31^{b}	1321±227^{a}	299±35^{b}	1129±192^{a}
Creatinine (mg/dL)	0.26±0.03^{b}	0.75±0.12^{a}	0.30±0.03^{b}	0.66±0.11^{a}
BUN (mg/dL)	13.9±0.9^{b}	40.9±3.1^{a}	14.4±1.4^{b}	38.6±3.7^{a}
T. Protein (g/dL)	5.28±0.21^{a}	3.94±0.10^{b}	5.44±0.20^{a}	4.11±0.29^{b}
Albumin (g/dL)	2.94±0.17^{a}	2.24±0.15^{b}	3.09±0.13^{a}	2.38±0.20^{b}
Triglyceride (mg/dL)	76.1±9.4^{bc}	123.5±16.9^{a}	70.4±6.3^{c}	107.8±10.7^{ab}
Cholesterol (mg/dL)	51.9±5.3^{b}	87.0±7.7^{a}	57.0±5.2^{b}	91.3±8.5^{a}
Glucose (mg/dL)	126.6±9.5^{b}	92.1±6.9^{a}	118.3±5.4^{b}	97.0±6.1^{a}

a,b,c,d; *Differences in the same row are statistically significant when the values are marked with different letters (P<0.05).* ***LPS;*** *Lipopolysaccharide,* ***PAFRA;*** *Platelet-activating factor receptor antagonist*

DISCUSSION

In experimental studies on laboratory animals, LPS-induced endotoxemia are well used to mimic the clinical features observed in animals with sepsis [18]. In endotoxemia, cytokines such as TNF-a, IL-1β and IL-6 are central mediators of pathological processes. LPS stimulates cytokine secretion from macrophages and induces endothelial cell damage. In earlier experimental and clinical trials with sepsis, PAFRA effectively exhibited potent protective effect on LPS-induced antioxidant and antiinflammatory disturbances [14,19,20], but PAFRA administration on the levels of serum proinflammatory cytokines and biochemical parameters in endotoxemia is as yet unclear.

In our study, PAFRA (10mg/kg IP, ginkgolide B Sigma Cat No G6910) and LPS (1.6 mg/100g IV) were administrated simultaneously. The dose of PAFRA used in this study was chosen from those previously reported [10,21].

In our work the selected LPS dose (*Escherichia Coli*, 0.111:B4 1.6 mg/100 g) is a sufficient dose to reach a high concentration of plasma cytokines during endotoxemi in rat [4]. Various researchers have reported the release of LPS-induced proinflammatory cytokines in rat endotoxemic models [3,4,22,23]. Mathiak et al.[24] have determined that LPS-induced IL-6 has the highest plasma concentration peak around 4-6 h. Earlier investigation reported that the increase of IL-6 concen-tration correlates with the severity of septic patients [6]. In this study, serum TNF-α, IL-1β and IL-6 were undetectable in control group (C), there were a marked elevation of serum TNF-α, IL 1β and IL-6 levels at 6 h after LPS administration (group II) ($P<0.05$) *(Table 1)*. In group IV PAFRA significantly inhibited LPS-induced increases in the levels of serum TNF-α, IL-1β when compared with LPS- group II ($P<0.05$) *(Table 1)*. In a study the over expression of the PAFR increases lethality in response to LPS administration in mice [25]. Moreover, during lethal CLP sepsis, there was a dysregulated elevation of systemic TNF-α and IL-6 levels and that PAFR blockade significantly reduced the levels of these cytokines [20]. PAFRA has been shown to reduce TNF-α production by 40% compared to that in placebo-treated animals in studies of endotoxin-induced sepsis [26]. On the other hand, in a stud carried out by Suputtamongkol et al.[13], levels in blood of the proinflammatory cytokines TNF-α, IL-6 and IL-8 were very high on admission and remained elevated in patients who developed multi organ failure with sepsis, but PAFRA (lexipafant) did not lower the levels of any of these cytokines significantly compared to the placebo treatment. Han et al.[19] have investigated the molecular mechanisms underlying the biphasic activation of NF-$_{\kappa}$B in response to LPS. They have showed that PAF, which is released in response to LPS injection, activates the early phase of NF-$_{\kappa}$B activation. This NF-$_{\kappa}$B activity leads to induction of proinflammatory cytokines (TNF and IL-1) expression, which leads to another stimulus for the synthesis of PAF, resulting in the second phase of NF-$_{\kappa}$B activation. Additionally, pretreatment with the PAF antagonist BN50739 or CV 6209 prior to LPS injection resulted in abrogation of the early peak of NF-$_{\kappa}$B. Ogata et al.[27] postulate that PAFRA block the biological effects of endo- genous PAF induced by bacteria or bacterial toxins. Therefore, PAFRA may attenuate the synergism between endogenous PAF and bacterial toxins, ultimately inhibiting inflammatory cytokine signal transduction. In a study, PAFRA inhibited LPS-induced TNF mRNA expression [28]. Also, Ishii et al.[29] reported that that the PAF receptor is not an LPS receptor but plays an important role in LPS-induced transcriptional change and calcium ion signaling. It has been reported that PAF itself activates NF-$_{\kappa}$B, inducing cytokine production and PAFR expression [30,31]. Our results show that, there was a significant elevation of systemic cytokine levels and that PAFR blockade significantly reduced the levels of these cytokines. The mechanism of the PAFRA action on LPS- induced cytokine inhibiton may be due to these effects.

In the present experiment, endotoxin injection caused statistically significant increases in serum CRP, AST, ALT, creatinine, BUN, LDH, cholesterol, triglyceride concentration *(Table 1)*, however, it caused statistically significant decrease in platelet count, total protein, albumin and glucose levels compared to control group. Serum CRP markedly increased after LPS infusion. PAFRA administration was not effective on serum CRP levels at 6 hour when compared to endo-toxemic animals receiving LPS alone ($P>0.05$) *(Table 1)*. Jeschke et al.[8] showed that serum CRP levels significantl increased in endotoxemic rats. Diaz Padilla et al.[32] concluded that rat CRP, similarly to human CRP, could activate autologous comlement, supporting that opsonization of ligands with complement is an important biological function of CRP. As has been previously demonstrated in endotoxaemic animal models by several authors [1,3,33,34], liver damage and loss of organ integrity, with subsequently the increases in plasma AST and ALT levels occur during endotoxemia as a consequence of LPS damage. We determined that LPS significantly increased hepatic enzymes AST and ALT which are markers of hepatic injury. PAFRA administration didn't exhibit protective effects on the liver, kidney and lipid metabolism of rats as judged from biochemical profile in this endoto-xaemia model. A number of studies have reported that PAF is involved in inflammatory tissue alterations associated with acute liver injury [21,35]. Earlier studies demonstrated PAF is one of the key mediators of a variety of liver injuries and that inhibition of PAF through the use of its receptor anta-gonists attenuates the extended injury [36,37]. Grypioti et al.[38] have previously reported that PAF was increased almost at the same time with all biochemical parameters (AST, ALT, ALP) indicative of liver injury in acetaminophen-induced liver toxicity in rats. Also, Grypioti et al.[10] has demonstrated that PAF-R antagonist (ginkgolide B, BN52021) attenuates liver damage and can provide important means of improving liver function following APAP intoxication. Our observation contradicts that of Grypioti et al.[10] who showed a significant improvementin the plasma levels of AST and ALT. In harmony with earlier findings [4,22,39], In this study the endo toxin increased serum cholesterol and triglycerid levels. Previous studies reported that LPS and TNF-α infusion stimulated hepatic lipogenesis with subsequent incerase cholesterol and triglycerides. This increase may be related to increased hepatic production of VLDL [40,41]. Al-Dughaym [42] reported that in endotoxaemia the decreases in TP, albumin level at 6 h may be attributed to hypovoloemia due to increased capillary permeability and reduced liver synthesis or decrease intestinal absorption which is in agreement with our observations. In harmony with earlier

findings [2,8,42], In the present study, a significant decrease in glucose concentration was observed in the endotoxaemic animals as compared to the controls. This hypoglycaemia was not suppressed by the administration of PAFRA.

Platelet count determined at 6 h after LPS injection displayed significant decreases, In endotoxaemia, the decreases in platelet count is thought to be a consequence of platelet aggregation in the lungs and other capillary beds, and of shortened platelet survival. The LPS-induced thrombocytopenia in rats is not directly mediated by PAF, because rat platelets are devoid of specific PAF receptors [43]. Thus, PAF seems to produce thrombocytopenia in rats through TNF-α production [44]. The endotoxin-induced leukopenia related to an increased adherence of activated neutrophils (expressing adhesion molecules) to endothelial cells is mainly mediated by TNF-α [45]. In our study, PAFRA significantly suppressed disturbances in leukocyte count, neutrophil and lymphocyte percentage associated with endotoxaemia. The neutropenia is followed by significant neutrophilia over the next several hours due to increased levels of activated complement products due to granulocyte colony-stimulating factor (G-CSF) and proinflammatory cytokines. Platelet-activating factor (PAF) stimulates leukocyte-endothelial cell (EC) adhesion through its effects either on leukocytes or on ECs [46]. The platelet activating factor (PAF) has been shown to play a significant role in endotoxin-induced leukocyte adherence. In harmony with our findings, The PAF receptor antagonist BN52021 attenuated the leukocyte adherence [47]. Beyer et al.[48] examined the effect of intra-abdominal contamination induced by cecal ligation and puncture (CLP) on neutrophil infiltration into the gastrointestinal tract. They found that CLP significantly increased the infiltration and a PAF receptor antagonist, WEB-2086, significantly attenuated it. In a recent study In endotoxin-induced uveitis models of rats PAF inhibitors, antagonize LPS induced leukocyte accumulation [49]. The mechanisms involved in the impairment of neutrophil migration may be due to the reduction in the levels of proinflammatory cytokines by PAFRA [50]. Leukocyte adhesion to vascular endothelium during endotoxemia was suppressed by a PAFRA in rats [51]. PAFRA blocked development of LPS-induced rat neutropenia [51,52]. Consistently vascular hyper permeability was inhibited by PAFRA [53]. This effects on hematological variables may be ascribed to the inhibiting effect of PAFRA on leukocyte migration.

In conclusion, in the current study, at the administered dose and route, PAFRA has a partial effect on inflammatory and haematological parameters; however, it has no useful effect as required by treatment with PAFRA on biochemical disturbances. Further experimental studies including administration route and the combination of PAFRA with other antiinflammatory agents are necessary to clarify its effects in endotoxaemia.

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