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Effects of Yarrow (Achillea millefolium) Extract on Acute Lung Injury: An Experimental Study

Civanperçemi (Achillea millefolium) Ekstresinin Akut Akciğer Hasarı Üzerindeki Etkileri: Deneysel Bir Çalışma

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Abstract

Introduction: Pro-inflammatory/anti-inflammatory cytokine and oxidant/antioxidant imbalances develop in acute respiratory distress syndrome cases, a significant cause of morbidity and mortality in childhood. This study aims to investigate whether Achillea millefolium, with its antioxidant and anti-inflammatory effects, can be used in the treatment of acute lung injury.

Methods: A total of 36 male Wistar rats were divided into four groups. Acute lung injury was induced by cecal ligation and puncture (CLP). Serum samples were analyzed for TNF-α, IL-10, native thiol, total thiol, and disulfide levels. Lung samples were examined using hematoxylin-eosin staining.

Results: A significant difference was observed in TNF-α values among groups (p=0.003). CLP group showed higher TNF-α values compared to the control group (50.88±5.21 vs. 34.13±9.89 pg/mL, p=0.002), and histologically demonstrated increased scores of lymphocytes, fibroblasts, histiocytes, neutrophils, hemorrhage, and congestion (p=0.006, p<0.001, p=0.007, p=0.001, and p=0.001, respectively). TNF-α values in the CLP+AM group showed a statistically significant decrease compared to the CLP group (50.88±5.21 vs. 38.59±11.65 pg/mL, p=0.035), and histologically, scores of lymphocytes, fibroblasts, histiocytes, neutrophils, hemorrhage, and congestion were reduced (p=0.017, p=0.005, p=0.007, p=0.001, and p=0.02, respectively). CLP+D group also showed a non-significant decrease in TNF-α values compared to the CLP group (50.88±5.21 vs. 39.31±5.09 pg/mL, p=0.055), but histologically, congestion, fibroblast, and histiocyte scores were significantly reduced (p=0.015 and p=0.002,

Öz

Giriş: Çocukluk çağı için önemli bir morbidite ve mortalite nedeni olan akut solunum sıkıntısı sendrom tablolarında pro-enflamatuvar/anti-enflamatuvar sitokin ve oksidan/anti-oksidan dengesizlikleri gelişmektedir. Bu çalışmada antioksidan ve anti-enflamatuvar etkileri olan Achillea millefolium'un akut akciğer hasarı tedavisinde kullanılıp kullanılamayacağını test etmektir.

Yöntemler: Toplam 36 adet erkek Wistar cinsi rat 4 gruba bölündü. Akut akciğer hasarı çekal ligasyon ve punksiyon (CLP) ile oluşturuldu. Serumdan alınan örneklerde TNF-α, IL-10, native tiyol, toplam tiyol ve disülfit seviyeleri çalışıldı. Akciğerden alınan örnekler hematoksilen-eozin boyası ile boyanarak incelendi.

Bulgular: Gruplar arasında TNF-α değerleri açısından anlamlı bir fark saptandı (p=0,003). CLP grubunda kontrol grubuna göre yüksek TNF-α değerleri saptandı (50,88±5,21 vs. 34,13±9,89 pg/mL, p=0,002), histolojik olarak da bu tablolarda lenfosit, fibroblast ve histiosit, nötrofil, hemoraji ve konjesyon skorlarının arttığı gösterilmiştir (sırasıyla p=0,006, p<0,001, p=0,007, p=0,001 ve p=0,001). TNF-α değerleri CLP grubuna göre CLP+AM grubunda istatistiksel açıdan anlamlı bir azalma göstermekteydi (50,88±5,21 vs. 38,59±11,65 pg/mL, p=0,035), histolojik olarak da bu tablolarda lenfosit, fibroblast ve histiosit, nötrofil, hemoraji ve konjesyon skorlarının düşürülebildiği gösterilmiştir (sırasıyla p=0,017, p=0,005, p=0,007, p=0,001 ve p=0,02). Yine CLP grubuna göre CLP+D grubunda da TNF-α değerlerinde istatistiksel açıdan anlamlı olmayan bir azalma vardı (50,88±5,21 vs. 39,31±5,09 pg/mL, p=0,055), ancak histolojik olarak bu tablolarda konjesyon, fibroblast ve histiosit

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Abstract

respectively). There was no statistically significant difference among other groups (p>0.05).

Conclusion: This study suggests that Achillea millefolium, with its anti-inflammatory effect, may be used in the treatment of acute lung injury. However, clinical studies are needed to support these findings.

Keywords: Achillea millefolium, acute lung injury, TNF-α

Öz

skorları istatistiksel olarak anlamlı oranda düşürebildi (sırasıyla p=0,015 ve p=0,002). Diğer gruplar arasında ise istatistiksel olarak anlamlı bir farklılık yoktu (p>0,05).

Sonuç: Bu çalışma Achillea millefolium'un enflamasyonu baskılayıcı etkisi ile akut akciğer hasarının tedavi edilmesinde kullanılabileceğini düşündürmektedir. Bu sonucun klinik çalışmalarla desteklenmesi gerektiğini düşünmekteyiz.

Anahtar Kelimeler: Achillea millefolium, akut akciğer hasarı, TNF-α

Introduction

Acute respiratory distress syndrome (ARDS) is a clinical picture characterized by hypoxia, impaired gas exchange, decreased lung compliance and increased physiological dead spaces as a result of direct or indirect damage to the alveolar structure due to many different causes.[1] ARDS can be seen in all age groups and studies have shown that the incidence in childhood is 2-12.8/100,000.[2,3] ARDS, which is an important cause of morbidity in childhood, also has a serious mortality rate, which has been shown to be 24% in some studies.[4]

There is no specific treatment for ARDS yet; current treatments can be summarized as ventilation or supportive therapies to protect the patient from hypoxia. The high morbidity/mortality rates of ARDS and the lack of a definitive treatment are the main reasons for numerous studies on the issue.

Yarrow [Achillea millefolium (AM)] is a plant from the asteraceae family. Its sesquiterpene and flavonoid compounds give AM antioxidant, anti-inflammatory, antibacterial, antiviral and analgesic properties,[5] for which it is used in traditional medicine for the treatment of hypertension, diabetes mellitus, hepatobiliary disorders, gastrointestinal disorders and wound healing.[6] The aim of this study was to test whether AM, which has antioxidant and anti-inflammatory effects, could be used in the treatment of acute lung injury (ALI).

Materials and Methods

Ethical approval was obtained from Bolu Abant İzzet Baysal University Experimental Animals Ethics Committee (no: 2021/35).

Preparation of AM Extract

Yarrow flowers collected in season were purchased from herbalists. The extraction of AM flowers was carried out by maceration method. Each 100 g of dry powder of AM flower was mixed with 600 mL ethanol (95%) and kept at 25 °C in the dark for two days. After two days, the extract mixture was filtered and the liquid part (ethanol) was transferred into a glass vial.[7] The solvent was then removed using a rotary evaporator set at 40 °C, resulting in AM extract in the vial. This extract was stored in the refrigerator at +4 °C until use.

Establishment of the Experiment Model

Male Wistar rats weighing 200-250 grams were purchased from the Experimental Animal Research and Application Center of our university. The rats were maintained under standard laboratory conditions (relative humidity 50-70%, room temperature 19±2 °C, 12-h light, 12-h dark cycle) and fed a standard diet and water ad libitum. The care of the rats and all surgical procedures were planned in accordance with the Universal Declaration of Animal Rights.

A total of 36 rats were randomly divided equally into 4 groups;

Rats in the sham group underwent laparotomy under xylazine/ketamine anesthesia and the abdominal incision was closed.

Cecal ligation and puncture (CLP) group rats were anesthetized with xylazine/ketamine anesthesia, and after laparotomy, the cecum was exposed and ligated under the ileocecal valve. The distal three-quarters of the cecum was punctured twice with a 20-gauge needle and a small amount of feces was extruded to ensure patency of the punctures. The exposed cecum was returned to the abdomen, after which the abdominal wall was closed in layers using sterile 6-0 surgical sutures. 1 mL of sterile 0.9% NaCl was administered subcutaneously.[8]

Rats in the CLP+AM group received 400 mg/kg AM extract intraperitoneally 14 hours after CLP was performed by the same method.[7]

Rats in the CLP + dexamethasone+AM (CLP+D) group received 400 mg/kg AM extract and 1.5 mg/kg dexamethasone intraperitoneally 14 hours after CLP was performed with the same method.[9]

Blood Collection and Sacrification

All rats were left in wire cages at room temperature and in a brightly lit environment 18 hours after the procedure, 90/10 mg/kg xylazine/ketamine IM anesthesia was administered[10], 5 mL of blood was collected intracardiacly, then the right lung was removed by opening the thorax and fixed in 10%

buffered formaldehyde for histological evaluation. The blood samples were centrifuged at 4000 rpm for 10 min and stored in ependorf tubes at -80 °C until the study.

Biochemical Tests

Blood samples were analyzed for inflammatory and anti-inflammatory cytokines TNF-α and IL-10 as well as thiol-disulfide levels. TNF-α and IL-10 (SinoGeneClon Biotech Co. Ltd., China) levels were determined by micro ELISA method according to the kit instructions. Serum thiol-disulfide homeostasis was analyzed by the new automated measurement method developed by Erel et al.[11], which measured native thiol (NT), total thiol (TT) and disulfide levels.

Histological Assessment

For histomorphologic examination, lung tissues obtained from rats were fixed in 10% formalin. The lung tissues were cut and traced in such a way that the widest surfaces were seen and then embedded in paraffin. Sections with 3 µm thickness were taken from the prepared paraffin blocks and stained with hematoxylin-eosin stain. The sections were evaluated by a pathologist under a LEICA DM 2000 LED light microscope. On histopathological examination of the lung tissue, the presence of lymphocytes around the bronchi and vessels, polymorphonuclear leukocyte infiltration, abscess formation, clustering of lipid-laden macrophages in the alveoli, alveolar hemorrhage, vascular congestion and alveolar wall thickening (fibroblasts and histiocytes in the alveolar wall) were evaluated and scored semiquantitatively from 0 to 4 (0: minimal damage, 1: mild damage, 2: moderate damage, 3: severe damage and 4: maximum damage).[12-14] Hematoxylin eosin stained sections were photographed at different magnifications with the Infinity 3 Analyze Release 6.5 imaging system.

Statistical Analysis

SPSS program was used for statistical analysis. Data were expressed as mean ± standard deviation. The Kolmogorov-Smirnov test was used to analyze the conformity of the groups to normal distribution, and One-Way Analysis of Variance followed by Bonferroni tests were used for biochemical data that showed normal distribution. The Kruskal-Wallis analysis was employed to evaluate the differences in histopathologic scores that did not show normal distribution, and the Mann-Whitney U test was used to compare significant changes between the groups. The value of $p<0.05$ was considered significant.

Results

Biochemical Evaluations

There was no significant difference in TT values between the groups (p=0.089). Lower TT values were found in the CLP group compared to the control group, but this change was not statistically significant (p=0.99). TT values showed a statistically insignificant increase in the CLP+AM group compared to the CLP group (p=1) (Table 1).

There was no significant difference between the groups in terms of NT values (p=0.17). Lower NT values were found in the CLP group compared to the control group, but this difference was not statistically significant (p=0.75). NT values showed a non-statistically significant increase in the CLP + AM group compared to the CLP group (p=1) (Table 1).

There was no significant difference between the groups in terms of disulfide values (p=0.075). Higher disulfide values were found in the CLP group compared to the control group, but this difference was not statistically significant (p=0.1). Disulfide values showed a statistically insignificant decrease in the CLP + AM group compared to the CLP group (p=1) (Table 1).

There was a significant difference between the groups in terms of TNF-α values (p=0.003). Higher TNF-α values were found in the CLP group compared to the control group (50.88±5.21 vs. 34.13±9.89 pg/mL, p=0.002). TNF-α values showed a statistically significant decrease in the CLP + AM group compared to the CLP group (50.88±5.21 vs. 38.59±11.65 pg/mL, p=0.035), and there was also a decrease in TNF-α values in the CLP+D group compared to the CLP group, but this decrease was not statistically significant (50.88±5.21 vs. 39.31±5.09 pg/mL, p=0.055) (Table 1, Figure 1).

There was no significant difference in IL-10 values between the groups (p=0.25). Lower IL-10 values were found in the CLP group compared to the control group, but this change was not statistically significant (p=0.51). IL-10 values showed

Table 1. Variation of biochemical parameters between groups

	Control	CLP	CLP + AM	CLP + D	p
TT (µmol/L)	341.08±61.36	317.65±57.15	324.09±37	275.09±59.78	0.089
NT (µmol/L)	281.99±62.78	243.16±50.75	249.9±24.83	228.52±56.76	0.17
Disulfide (µmol/L)	29.91±13.76	37.24±13.16	37.09±13.06	22.91±10.72	0.075
TNF-α (pg/mL)	34.13±9.89	50.88±5.21	38.59±11.65	39.31±5.09	**0.003**
IL-10 (pg/mL)	11.49±7.5	7.16±2.48	8.27±3.24	10.82±5	0.25

CLP: Cecal ligation and puncture, AM: Achillea millefolium, D: Dexametazon + AM, TT: Total thiol, NT: Native thiol, TNF: Tumor necrosis factor

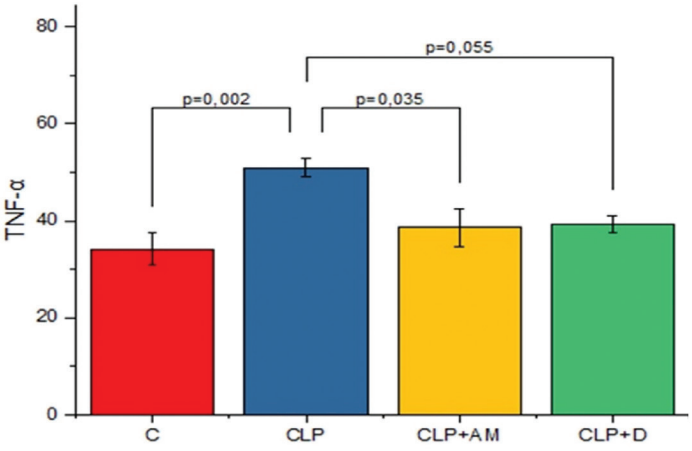

Figure 1. Variation of TNF-α values between groups
C: Control, CLP: Cecal ligation and puncture, AM: Achillea millefolium, D: Dexamethasone + AM, TNF: Tumor necrosis factor

a non-statistically significant increase in the CLP + AM group compared to the CLP group (p=1) (Table 1).

Histological Evaluations

There was a significant difference in lymphocyte scores between the groups (p<0.001). There was a significant difference in lymphocyte scores between CLP and control groups (1.87±0.99 vs. 0.55±0.52, p=0.006) (Figure 2 a-c). Lymphocyte scores were significantly lower in the CLP + AM group compared to the CLP group (1.87±0.99 vs. 0.55±0.52, p=0.017) (Figure 2e), but no such difference was found in the CLP + dexamethasone + AM group (1.87±0.99 vs. 1.77±0.44, p=0.95) (Table 2, Figure 2g, Figure 3a).

There was a significant difference in neutrophil scores between the groups (p=0.004). A significant difference was found in lymphocyte scores between CLP and control groups (1±0.92

Table 2. Comparison of lung histologic scores according to groups

Characteristics	Control	CLP	CLP + AM	CLP + D	p
Lymphocyte*	0.55±0.52	1.87±0.99	0.88±0.33	1.77±0.44	<0.001
Neutrophil*	0.0±0.51	1±0.92	0.0±0.0	0.33±0.7	0.004
Macrophage*	0.0±0.0	0.12±0.35	0.0±0.0	0.33±0.5	0.093
Fibroblast and histiocyte*	0.22±0.44	2.12±0.64	0.77±0.83	0.66±0.7	0.001
Hemorrhage*	0.22±0.44	1.62±0.51	0.33±0.5	1±0.7	<0.001
Congestion*	0.66±0.5	2.62±0.91	1.66±0.5	1.55±0.52	<0.001
Abscess*	0.0±0.0	0.12±0.35	0.0±0.0	0.0±0.0	0.33

CLP: Cecal ligation and puncture, AM: Achillea millefolium, D: Dexametazon + AM, *: Mann-Whitney U

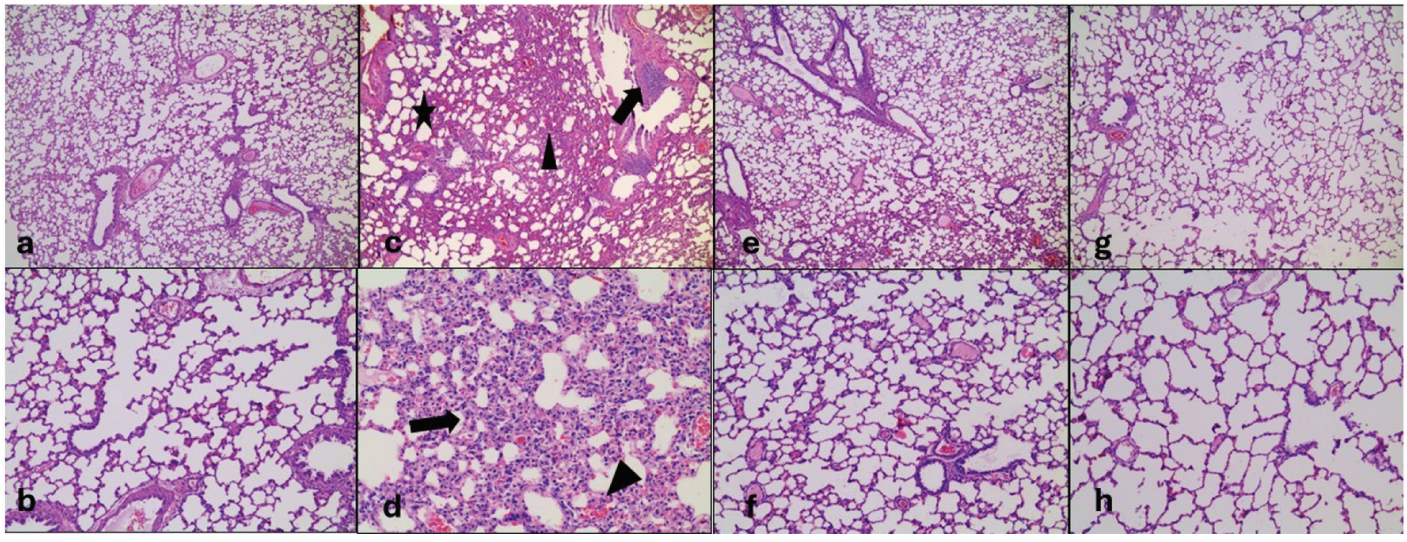

Figure 2. H&E stained sections showing histologic changes in lung tissue (a) Control group: No obvious inflammation and alveolar wall thickening, HEX40; b) Control group: No alveolar hemorrhage and vascular congestion, HEX100; c) CLP group: Peribronchial lymphocytic inflammation is evident (black arrow), alveolar wall thickening (black arrowhead) and areas of alveolar hemorrhage (black asterisk) are present, HEX40; d) CLP group: Alveolar wall thickening and neutrophilic inflammation (black arrow), vascular congestion (black arrowhead), HEX100; e) CLP + Achillea millefolium group: No alveolar wall thickening and inflammation, HEX40; f) CLP+Achillea millefolium group: No alveolar wall thickening and inflammation, mild congestion present, HEX100; g) CLP + dexamethasone + Achillea millefolium group: No significant alveolar damage and inflammation, HEX40; h) CLP + dexamethasone +Achillea millefolium group: No significant alveolar damage and inflammation, HEX100)
H&E: Hematoxylin and eosin, CLP: Cecal ligation and puncture

vs. 0.0±0.51, p=0.007) (Figure 2 a,b,d). Lymphocyte scores were significantly lower in the CLP + AM group compared to the CLP group (1±0.92 vs. 0.0±0.0, p=0.007) (Figure 2f), but not in the CLP + dexamethasone + AM group (1±0.92 vs. 0.33±0.7, p=0.1) (Table 2, Figure 2h, Figure 3c).

There was a significant difference in fibroblast and histiocyte scores between the groups (p=0.001) and a significant difference in fibroblast and histiocyte scores between CLP and control groups (2.12±0.64 vs. 0.22±0.44, p<0.001). Fibroblast and histiocyte scores were significantly lower in the CLP + dexamethasone +AM group compared to the CLP group (2.12±0.64 vs. 0.66±0.7, p=0.002). Fibroblast and histiocyte scores were also significantly lower in the CLP+AM group compared to the CLP group (2.12±0.64 vs. 0.77±0.83, p=0.005) (Table 2, Figure 3b).

There was a significant difference in hemorrhage scores between the groups (p<0.001). There was a significant difference in hemorrhage scores between CLP and control groups (1.62±0.51 vs. 0.22±0.44, p=0.001). Hemorrhage scores were also significantly lower in the CLP + AM group compared to the CLP group (1.62±0.51 vs. 0.33±0.5, p=0.001), but no such difference was found in the CLP + dexamethasone+AM group (1.62±0.51 vs. 1±0.7, p=0.064) (Table 2, Figure 3d).

There was a significant difference in congestion scores between the groups (p=0.001) and a significant difference in congestion scores between the CLP and control groups (2.62±0.91 vs. 0.66±0.5, p=0.001). Congestion scores were significantly lower in the CLP + dexamethasone + AM group compared to the CLP group (2.62±0.91 vs. 1.55±0.52, p=0.015). Congestion scores were also significantly lower in the CLP+AM group compared to the CLP group (2.62±0.91 vs. 1.66±0.5, p=0.02) (Table 2, Figure 2f, Figure 3e).

There was no significant difference in macrophage and abscess scores between the groups (p=0.093 and p=0.33, respectively) (Table 2, Figure 2e).

Discussion

AM has a significant suppressive effect on inflammation, suggesting that it can be used in conditions such as ALI/ARDS.

Sepsis is a high mortality organ dysfunction that develops after immune dysfunction in the organism. In many cases, ALI/ARDS develops due to the cytokine storm during sepsis, which occurs directly or indirectly due to alveolar damage and lung epithelial damage.[1] Mortality in ARDS caused by sepsis has been found to be 30-40% higher than other causes.[15] For this reason, we used sepsis induced by the CLP method in rats in our study.

Cytokines released by different cells are messenger molecules that play an active role in the regulation of biological processes and are essential for the regulation of both local

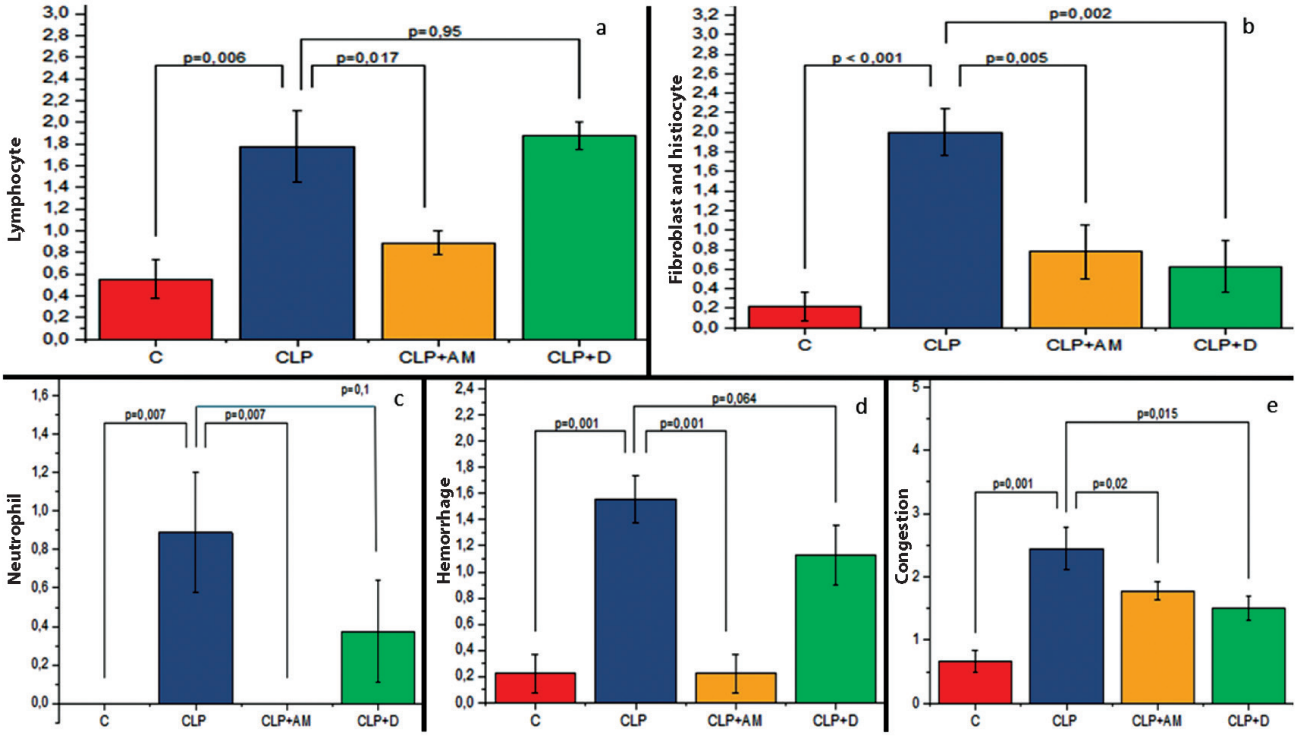

Figure 3. Change in histologic scores between groups
C: Control, CLP: Cecal ligation and puncture, AM: Achillea millefolium, D: Dexamethasone + AM

and systemic inflammatory responses. The main production goal of cytokines is to eliminate the harmful agent, but sometimes the response results in damage to the organism due to exaggerated levels of cytokines produced and the effect of other cells stimulated, the most important example of this is ARDS, which is characterized by a cytokine storm.[1] One of the most important cells involved in these conditions is macrophages. Macrophages, which have an important role in the protection of the human body from harmful organisms and pathogens, release various pro-inflammatory cytokines such as TNF-α, IL-1, IL-6, IL-8, etc. when activated to stimulate other defense mechanisms.[16] TNF-α, one of the most important pro-inflammatory mediators, is a cytokine that normally plays an active role in mechanisms such as cell proliferation, cellular differentiation and apoptosis. In inflammatory conditions, TNF-α stimulates neutrophil migration to the site of inflammation and the release of other pro-inflammatory cytokines.[17] M1 macrophages stimulated by the NF-κB pathway have been shown to take an active role in TNF-α production in addition to free oxygen radicals and IL-6.[18] TNF-α levels, which increase in the initial stages of ARDS in which macrophages play an active role, decrease in the recovery periods. Therefore, methods to decrease TNF-α levels, which is the most important cytokine that causes alveolar destruction in the initial stage of ARDS, have been investigated, and as a result of these investigations, it has been shown that inhibition of M1 macrophages decreases TNF-α levels, and this effect contributes to the decrease in the severity and mortality of the clinical picture of ARDS.[19] In our study, it was also shown that TNF-α levels increased in AM caused by sepsis, and histologically, this change was supported by the increase in lymphocyte, fibroblast and histiocyte, neutrophil, hemorrhage and congestion scores. Sesquiterpenes in the structure of AM are known to suppress this inflammation by inhibiting arachidonic acid metabolism.[20] In an experimental study, AM was shown to reduce inflammation by 35%.[21] In our study, it was shown that TNF-α values decreased in rats with CLP, which were given AM, and histologically this change was supported by the decrease in lymphocyte, fibroblast and histiocyte, neutrophil, hemorrhage and congestion scores.

An important molecule known to suppress inflammatory processes is dexamethasone. One of the most important cytokines affected by dexamethasone in inflammatory processes is TNF-α.[22] In our study, TNF-α values were found to decrease in the dexamethasone-treated group compared to the other groups, although not statistically significant. However, histologically, the decrease in congestion, fibroblast and histiocyte scores in this group was statistically significant.

The immune system is responsible for protecting the host from pathogenic microorganisms by triggering inflammatory processes. Cells that play an active role in inflammation secrete pro-inflammatory cytokines that mediate the activation of other cells and the release of other cytokines, and can also produce anti-inflammatory cytokines to suppress these inflammatory processes when necessary. In cases where the inflammatory response is excessive, the host is also harmed. Therefore, the presence of anti-inflammatory processes is very important. The most important cytokine involved in balancing anti-inflammatory processes is IL-10.[23,24] This cytokine is produced by M2 macrophages, monocytes, neutrophils and T helper cells.[25] Studies on ARDS have shown that IL-10 levels, which decrease in the initial stages of the condition, increase during recovery periods.[26] Increased IL-10 levels inhibit inflammatory processes and restore the disturbed inflammatory-anti-inflammatory balance. In cases where the expected increase in IL-10 levels is not achieved, inflammatory processes get out of control, leading to an increase in the severity of lung injury and thus increasing the morbidity and mortality.[27] Studies have found that AM shows anti-inflammatory activity by increasing IL-10 levels.[21] In our study, the increase in IL-10 levels provided by AM administration was not statistically significant.

One aspect of the damage that occurs in ALI/ARDS following sepsis is the disruption of oxidant-antioxidant metabolism in favor of oxidative stress. SORs in ALI/ARDS pave the way for damage to the alveolar endothelium.[27] Cysteine plays an important role in preventing oxidative damage with its functional thiol group. Thiol compounds are oxidized by the sulfhydryl groups in them to form reversible disulfide bonds, thus playing an active role in maintaining the thiol-disulfide balance.[28] Studies on ALI/ARDS have found a relative decrease in antioxidant molecules, but an increase in the oxidized forms of these molecules and disulfide values.[29]

Studies have shown that AM is a potent antioxidant.[30] In our study, NT and TT values decreased in the CLP group compared to controls, but this decrease was not statistically significant. Again, although these values increased in the CLP group given AM, these increases were not statistically significant.

Conclusion

This study suggests that AM may be used in the treatment of ALI/ARDS due to its biochemical and histologic suppressive effect on inflammation.

Ethics

Ethics Committee Approval: Ethical approval was obtained from Bolu Abant İzzet Baysal University Experimental Animals Ethics Committee (no: 2021/35).

Informed Consent: N/A.

Footnotes

Authorship Contributions

Concept: B.Y., M.B., Design: B.Y., A.Ç., A.B.Y., Data Collection or Processing: S.E.D., M.A., Analysis or Interpretation: S.E.D., M.A., Literature Search: B.Y., M.B., M.F.B., Writing: B.Y., M.B., M.F.B.

Conflict of Interest: No conflict of interest was declared by the authors.

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