Türk Tarım ve Doğa Bilimleri Dergisi 10(1): 196–202, 2023

https://doi.org/10.30910/turkjans.1209593

TÜRK
TARIM ve DOĞA BİLİMLERİ
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TURKISH
JOURNAL of AGRICULTURAL
and NATURAL SCIENCES

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Araştırma Makalesi

Ashwagandha kök ekstresi ACE ve MAPK sinyal yollarının inhibisyonu yoluyla Oleik asit kaynaklı AAH/ASSS sıçan modelinde inflamasyonu hafifletir

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Geliş Tarihi: 24.11.2022 Düzeltme Geliş Tarihi: 12.01.2023 Kabul Tarihi: 15.01.2023

ÖZ

Ashwagandha (*Withania somniferous*), halk hekimliğinin en önemli bitkilerinden biridir ve çeşitli hastalıkları tedavi etmek için yaygın olarak kullanılmaktadır. Akut akciğer hasarı (AAH) ve akut solunum sıkıntısı sendromu (ASSS), yoğun inflamasyona sekonder alveoler hasar ile birlikte hipoksemiye bağlı olarak ani gelişen solunum yetmezliği olarak tanımlanır. Mevcut çalışma, Ashwagandha'nın Oleik Asit kaynaklı AAH/ASSS'na karşı aktivitesini sıçan modelinde değerlendirmeye odaklanmıştır. Bu amaçla hayvanlar, aşağıdaki üç gruba ayrılmıştır: Kontrol, Oleik asit (50 µl kg^{-1}, i.v. enjeksiyon), Ashwagandha (500 mg/kg, oral) + Oleik asit. Ashwagandha, tek Oleik asit dozundan önce iki hafta boyunca günlük olarak verilmiştir. Son uygulamadan 24 saat sonra gruptaki tüm hayvanlar sevofluran ile sakrifiye edilerek akciğerleri değerlendirilmiştir. Mitojenle-etkinleşen protein kinazlar (MAPK)'ın seviyeleri ve miyeloperoksidaz (MPO), glutatyon (GSH), süperoksit dismutaz (SOD), total oksidan durum (TOS) ve anjiyotensin dönüştürücü enzim (ACE) aktiviteleri akciğer dokularıda ELISA ile belirlenmiştir. Model grubu ile karşılaştırıldığında, Ashwagandha uygulanan grupta MAPK, MPO ve TOS seviyelerinde önemli bir düzelme olmuştur. Ayrıca Ashwagandha, GSH ve SOD aktivitelerini önemli ölçüde arttırırken, ACE aktivitesini azaltmıştır. Bu nedenle Ashwagandha, Oleik asitin neden olduğu akut akciğer hasarını hafifletmek için potansiyel bir doğal kaynak olarak kullanılabilir.

Anahtar kelimeler: Ashwagandha, akciğer hasarı, TOS, MAPK, ACE aktivitesi

Ashwagandha root extract attenuates inflammation in Oleic acid induced-ALI/ARDS rat model via inhibition of ACE and MAPK signaling pathways

ABSTRACT

Ashwagandha (*Withania somniferous*) is one of the most important plants of folk medicine and is widely used to treat various diseases. Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are defined as a respiratory failure that abruptly develops due to hypoxemia with alveolar injury secondary to intense inflammation. The present study was focused on evaluating the activity of Ashwagandha against Oleic Acid-Induced ALI/ARDS in a rat model. For this purpose, the animals were divided into the following three groups: Control, Oleic acid (50 µl kg^{-1}, i.v. injection), Ashwagandha (500 mg/kg, orally) + Oleic acid. Ashwagandha was given daily for two weeks before a single dose of the Oleic acid. 24 hours after the last application, all the group animals were sacrificed by sevoflurane, and their lung was evaluated. The levels of Mitogen-activated protein kinases (MAPK), and the activities of myeloperoxidase (MPO), glutathione (GSH), superoxide dismutase (SOD), total oxidant status (TOS), and angiotensin-converting enzyme (ACE) were determined in lung tissues by ELISA. Compared with the model group, there was a significantly improving in the levels of MAPK, MPO, and TOS in the Ashwagandha administration group. Moreover, Ashwagandha markedly increased the activities of GSH and SOD, and decreased the activity of ACE. Therefore, Ashwagandha may be used as a potential natural resource for mitigating acute lung injury caused by Oleic acid.

Key words: Ashwagandha, lung injury, TOS, MAPK, ACE activity

INTRODUCTION

Medicinal plants are attractive sources of new drugs for preventing and treating various diseases (Anand et al., 2019). Ashwagandha (*Withania somniferous*) is one of the most important herbals of traditional Indian medicine and is distributed in deserts and open fields in the Mediterranean region up to South-East Asia (Paul et al., 2021). Ashwagandha and its extracts are widely used in folk medicine to treat various diseases such as asthma, weight loss, arthritis, constipation, insomnia, goiter, mood disorders, memory loss, and non-neurodegenerative diseases (Ale et al., 2021). Ashwagandha root extracts have multiple pharmacological properties, including anti-ulcerogenic, anti-stress, aphrodisiac, narcotic, neuroprotective, diuretic, anticancer, immunomodulatory, anti-inflammatory, and antioxidant activities, etc. (Ale et al., 2021). The extracts also show an antidepressant effect and improve the functioning of the reproductive system. Ashwagandha improves immunity naturally without any adverse effects (Mishra and Kumar, 2021). In addition to these pharmacological properties, the protective effects of the plant root extracts have been reported in lung injury. For example, the root powder significantly improves oxidative stress, inflammation, and endothelial dysfunction in monocrotaline-induced pulmonary hypertension, and attenuates apoptotic resistance and proliferative marker in rat lungs, as well as reduces the markers of right ventricular hypertrophy and right ventricular pressure (Kaur et al., 2015). It also reduces cough attacks and the frequency of breathing troubles in patients with chronic obstructive pulmonary disease (Singh, 2015). The root extracts have been also proven to be beneficial against pro-inflammatory enzymes involved in the progression of lung cancer (Nile et al., 2021). Previous studies reveal that Ashwagandha is to have safe compounds. Ashwagandha extract contains multiple compounds, such as alkaloids, saponins, and withanolides. Withaferin-A, Withanolide D, Withanoside I–VII, and Withanolide Glycosides isolated from its roots contribute primarily to its pharmacological activities (Tong et al., 2011). Bioactive constituents of Ashwagandha root have a wide range of antiinflammatory and antioxidant effects against various pulmonary diseases (Daneshvar et al., 2021).

Acute lung injury and acute respiratory distress syndrome (ALI and ARDS) are defined as a respiratory failure that abruptly develops due to hypoxemia with alveolar defect secondary to intense inflammation (Raghavendran and Napolitano, 2011). ALI/ARDS can result from extra-pulmonary sources such as sepsis, trauma, drowning, fat embolism, massive transfusion, inhalation of toxic fumes, drug overdose, and pancreatitis, as well as pulmonary infection or aspiration (Zambon and Vincent, 2008). ALI/ARDS is also a symptom of coronavirus disease 2019, a transmissible infectious disease that nowadays seriously threatens human health. (Qiu et al., 2020). Unfortunately, there is currently no specific medication or other therapy available for the treatment of ALI/ARDS. Ashwagandha could provide overall protection against lung injury in looking at previous experimental studies and its traditional use. However, there are limited clinical trial experience about the benefits of Ashwagandha in acute lung injury. Therefore, the aim of the present study was focused on evaluating the activity of Ashwagandha against Oleic Acid-Induced ALI/ARDS in a rat model.

MATERIAL and METHODS

Animals and ALI/ARDS model

Male Sprague Dawley rats (300 ± 20 g) were used for the study and fed a standard diet and water ad libitum. The work was performed according to the Guide for the Care and Use of Laboratory Animals (US National Institutes of Health). The protocols for the experiments were endorsed by the Animal Ethics Committee of Kastamonu University (Number: 2022,19). All rats were randomly assigned to three groups (n = 6 each): I) Control II) Oleic acid III) Ashwagandha + Oleic acid. Ashwagandha root extract was purchased from Organic Traditions Store and dissolved in distilled water. Ashwagandha at 500 mg/kg dose was given orally daily for two weeks before a single dose i.v. injection of the Oleic acid (50 µl kg^{-1}) (Singh 2019). 24 hours after the last application, all the group animals were sacrificed by sevoflurane, and their lungs were removed. The lung tissues were rinsed in PBS (pH 7.4) to remove excess blood thoroughly and preserved at –20° C until analyses.

Determination of Biochemical Parameters

All lung samples taken from each group were ground with liquid nitrogen in porcelain mortars and homogenized in PBS (tissue weight (g): PBS (mL) volume=1:9). The homogenates were then centrifuged for 15 minutes at 12,000 RPM at 4°C to get the supernatant. Freeze/thaw cycles were avoided. The obtained supernatants were assayed for Myeloperoxidase (MPO), Glutathione (GSH), Superoxide Dismutase (SOD), Mitogen-activated protein kinases (MAPK), Angiotensin-converting enzyme (ACE) (BT LAB Company, China), and Total oxidant status (TOS) (Rel Assay Diagnostics, Gaziantep, Turkey) levels in rat lung tissue by enzyme-linked

immunosorbent assay (ELISA) available kits in accordance with the manufacturer's manuals. A standard curve was generated for each set of samples assayed.

Statistics

Graph Pad Prism software (Version 6.0) was used to analyze the results. For comparisons, unpaired Student's t-tests were used. All data was expressed as the mean ± SEM.

RESULTS and DISCUSSION

To explore the role of Ashwagandha root extract as a potential pulmonary inflammatory mediator in ARDS, we examined the levels of MPO, and MAPK in rat lungs. As shown in Figure 1, the MPO levels in the lung were significantly increased in the Oleic acid group as compared with the control group ($p<0.001$). However, pre-administration of 500 mg/kg Ashwagandha significantly reduced MPO levels compared to the Oleic acid group ($p<0.01$). Furthermore, MAPK level in the lung was significantly elevated in the Oleic acid group compared to the control group, while treatment with Ashwagandha significantly normalized the elevated levels of MPO in the lung ($p < 0.05$).

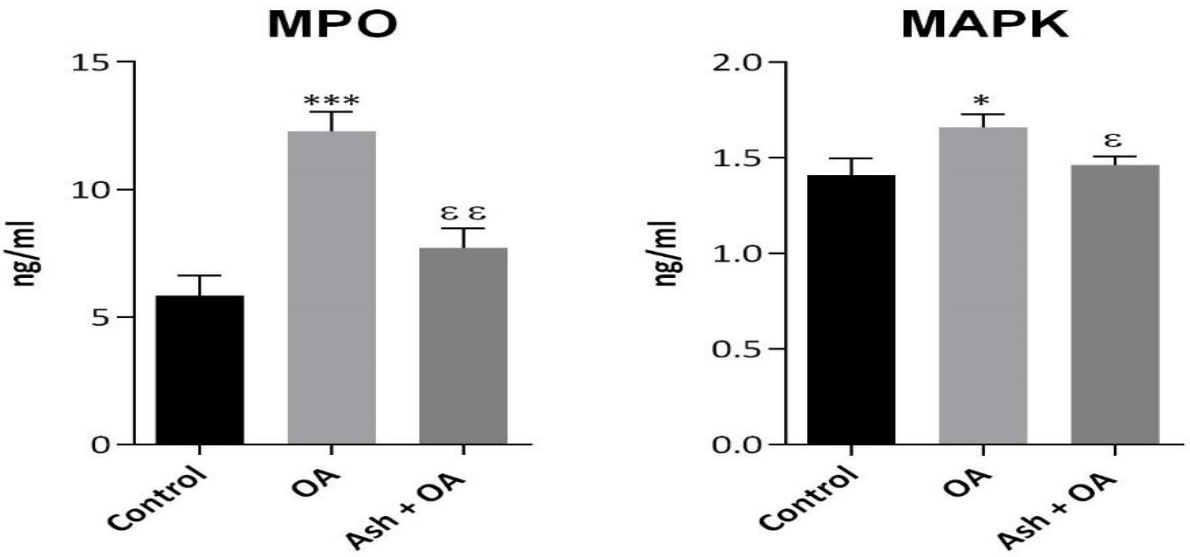

Figure 1. The effects of Ashwagandha on the MPO and MAPK levels on lung in ALI/ARDS.
***** denotes significant differences between other studied groups and control (*****: $p<0.05$, *******: $p<0.001$), ε denotes significant differences between other studied groups and the OA group (ε: $p<0.05$, εε: $p<0.01$). Abbreviation used: OA: Oleic acid, Ash: Ashwagandha.

To assess whether Ashwagandha pre-treatment has a protective role on lung injury with Oleic acid, we assessed the degree of oxidative stress by measuring the level of GSH and the activity of SOD in rat lung tissues. As shown in Figure 2, the levels of SOD and GSH on the lung were reduced in the Oleic acid group as compared to control group rats ($p<0.05$, $p<0.01$, respectively). These levels were reverted by Ashwagandha pre-treatment ($p<0.05$). Furthermore, TOS levels were significantly increased in the lung in the Oleic acid group as compared to the control group ($p<0.05$). On the other hand, administration of Ashwagandha markedly reduced TOS level as compared to the Oleic acid group rat lungs ($p<0.01$). In Figure 3, ACE level in the lung was markedly increased in the Oleic acid group rat the control group. In contrast, Ashwagandha pre-treatment significantly blocked the elevation of ACE level in the lung compared to the Oleic acid group rats ($p < 0.05$).

Oleic acid is a type of unsaturated fatty acid, and increased serum fatty acid levels lead to fatty acid toxicity in organs, especially in the lungs (Gonçalves-de-Albuquerque et al., 2016). Oleic acid-induced lung injury is a useful animal model to study mechanisms of ARDS/ALI, mimicking human ARDS/ALI (Akella et al., 2014). Therefore, our study used the intravenous Oleic acid 50 µl kg^{-1} method to model ALI/ARDS in animals. Consistent with earlier findings, after an administration of Oleic acid for 24 h, lung injuries were significantly increased in rats, in contrast, pre-treatment with Ashwagandha at a dose of 500 mg/kg markedly improved the lung injury. In ARDS/ALI, neutrophils are the key cells migrating to the lung tissues and contribute to initiating inflammation and tissue damage. Neutrophils release proteases, chemokines, and cytokines, and generate ROS (reactive

oxygen species) (Gonçalves-de-Albuquerque et al., 2016). Myeloperoxidase (MPO) is one of the enzymes most abundantly released from the neutrophils during ARDS/ALI; therefore, it plays a main role in acute injuries (Zhang et al., 2007). In the present experiment, MPO was significantly higher in rats treated only with Oleic acid. As mentioned in the present results, MPO levels reversed in the Ashwagandha + Oleic acid group. This result is consistent with the study showing that the level of MPO increases with the effect of Oleic acid (Ito et al., 2005). Moreover, our finding confirmed the study that the inhibitory activity of Withanolides found in the Ashwagandha roots against the MPO in chronic obstructive pulmonary disease patients (Singh et al., 2022).

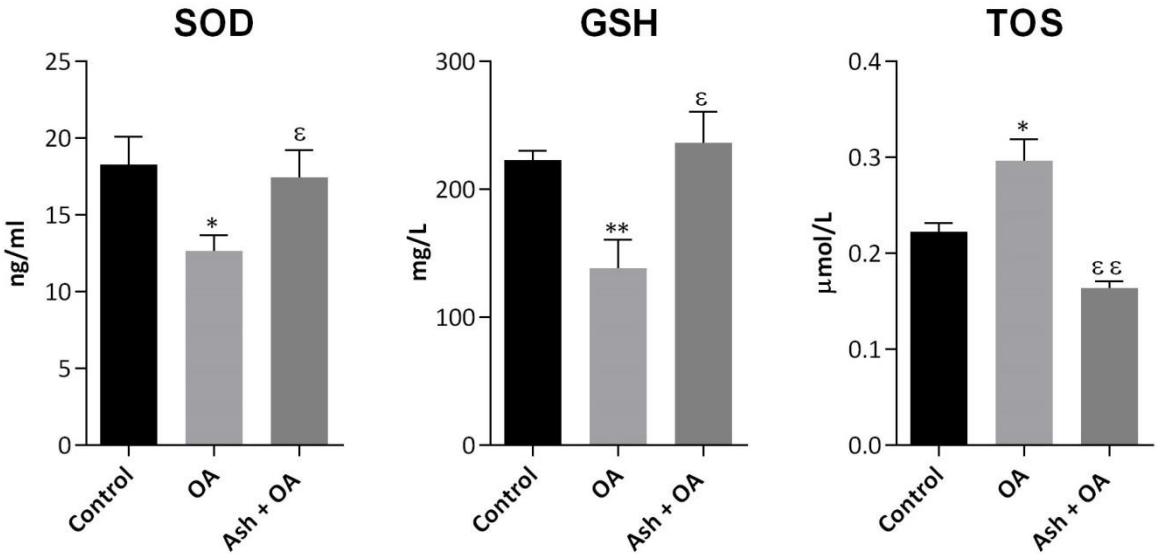

Figure 2. The effects of Ashwagandha on the SOD, GSH, and TOS levels on lung in ALI/ARDS.
***** denotes significant differences between other studied groups and control (*****: p<0.05, ******: p<0.01), ε denotes significant differences between other studied groups and the OA group (ε: p<0.05, εε: p<0.01). Abbreviation used: OA: Oleic acid, Ash: Ashwagandha.

In addition, the elevated MPO is known to affect the generation of ROS (Zhang et al., 2007). MPO activity or ROS overproduction generates oxidative stress which is an imbalance between oxidant and antioxidant substances, and this plays a central role in inflammation (Valavanidis et al., 2013). It is known that the natural defense against ROS forms antioxidant enzymes and antioxidant scavengers (Brieger et al., 2012). The enzymes SOD and GSH prevent the accumulation of ROS, and are therefore considered a primary defense mechanism protecting tissues against oxidative damage (Oktyabrsky and Smirnova, 2007). In the present study, we found that SOD and GSH levels in rats were highly decreased by the stimulation with OA and Ashwagandha pre-treatment significantly increased their activities. Our results suggested that Ashwagandha restores the antioxidant enzymes. On the other hand, an increase in TOS levels reflects the degree of oxidative stress in tissue damage (Tayman et al., 2021). In the present study, rats treated with 500 mg/kg of Ashwagandha showed decreased TOS levels, which is evidence of the antioxidant defense. Therefore, we suggest that the extract of Ashwagandha was effective in reducing the production of ROS caused by Oleic acid in lung tissue, possibly due to its antioxidant activity. Previous studies revealed that Ashwagandha root extract has an efficient antioxidant activity. In the investigations of Sankar et al. (2007), Ashwagandha root extract significantly mitigated the oxidative stress induced by MPTP in the midbrain of mice. The findings of Birla et al. showed that Ashwagandha significantly alleviates the level of Bisphenol A intoxicated oxidative stress and memory impairment in mice. Moreover, Khan et al. suggested that the extract of Ashwagandha root exhibited anti-arthritic activity by ameliorating oxidative stress in collagen-induced arthritic rats. Sabina et al. (2013) reported that Ashwagandha possesses hepatoprotective effects due to its antioxidant activity in acetaminophen-intoxicated rats. The findings of Elhadidy et al. (2018) showed the anti-inflammatory effects of Ashwagandha against aluminum neurotoxicity through its potential antioxidant activity.

In addition, this study found that high levels of MAPK were positively correlated with levels of oxidative stress in lung tissues, indicating that activation of MAPK is involved in the inflammatory response to ARDS/ALI. MAPKs are all very important pathways in the development of inflammatory diseases since they regulate the release of cytokines, and control immunity and inflammation. Thus, inhibition of MAPKs efficiently attenuates

the pulmonary inflammatory response (Zhang et al., 2017). In the present study, the results revealed that Oleic acid significantly increased the level of MAPK; however, treatment with Ashwagandha significantly inhibited the MAPK pathways in rats with lung injury.

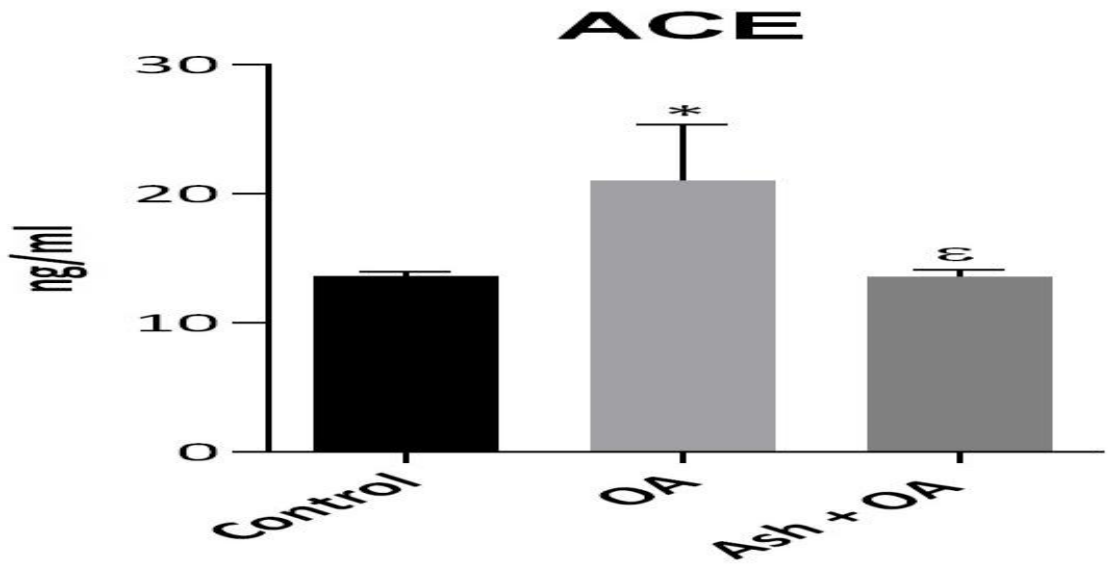

Figure 3. The effects of Ashwagandha on the ACE activity on lung in ALI/ARDS.
* denotes significant differences between other studied groups and control (*: p<0.05), ε denotes significant differences between other studied groups and the OA group (ε: p<0.05). Abbreviation used: OA: Oleic acid, Ash: Ashwagandha.

Another possible mechanism underlying the beneficial effects of Ashwagandha in our study is its Angiotensin-converting enzyme (ACE) inhibitory effects. The ACE and its homolog ACE2 are is a key function in the renin–angiotensin system, that plays a very important role the pulmonary circulation and the inflammatory response in lung injury. ACE converts angiotensin I to angiotensin II whereas ACE2 inactivates angiotensin II as a negative regulator of the system. Thus, ACE promotes lung injury in ALI/ARDS while ACE2 protects from the injury by blocking the renin-angiotensin pathway (Imai et al., 2005). ACE also functions as a critical severe acute respiratory syndrome (SARS) receptor. In addition, it appears that SARS Spike protein-mediated downregulation of ACE2 contributes to the severity of lung injury (Imai et al., 2008). So, ACE inhibition has been proven to be a novel approach to the treatment of acute lung damage (Imai et al., 2005). In our experiment, exposure to Oleic acid led to increased ACE activity whereas Ashwagandha treatment effectively reduced the activity of ACE in the lung tissue. Therefore, another protective mechanism of Ashwagandha in Oleic acid-induced acute lung injury may be mediated through the inhibition of ACE activity. Numerous studies have demonstrated that ACE inhibition exhibits protective effects against lung injury (Wang et al., 2018). Recent studies also revealed that Ashwagandha exhibited the best potential inhibitors against protease of ACE and coronavirus 2019. A molecular docking study showed that docked compounds obtained from Ashwagandha may be served as potential inhibitors of SARS main protease with their significant binding affinity (Shree et al., 2022). Another study suggested that Withanoside V from constituents of Ashwagandha is a strong inhibitor of the main protease of SARS-CoV-2 (Tripathi et al., 2021). It has been suggested that compounds of Ashwagandha can bind to the key targets including Spike protein and ACE-2 of SARS-CoV-2 (Kashyap et al., 2020). The present study showed inhibition of ACE activity in the group treated with Ashwagandha, which agrees with the results in previous reports on lung injury

CONCLUSION

This is the first report to reveal that Ashwagandha could inhibit ACE activity and reduce oxidative stress in the Oleic acid-induced ALI/ARDS rats model. Thus, our findings offer new insight into the role of Ashwagandha in attenuating inflammation and oxidative stress for ALI/ARDS treatment.

Acknowledgments: I thank Assoc. Prof. Hüseyin Serkan EROL from the Faculty of Veterinary Medicine of Kastamonu University for his support with the experimental model.

Conflict of Interest Declaration: The author has no conflict of interest concerning this work.

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