MELLIFERA RESEARCH ARTICLE

Investigation of Protective Effects of Apilarnil Against Lipopolysaccharide Induced Lung Injury in Rats

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A B S T R A C T

Apilarnil is a bee product that has attracted attention due to its beneficial biological properties recently. This study aimed to investigate the effect of apilarnil (API) on endotoxin-induced lung injury. For the study, 64 adult male Sprague dawley rats were divided into eight groups; control, 0.2, 0.4 and 0.8 g / kg API treated groups by gavage for 10 days, 30 mg / kg lipopolysaccharide (LPS) administered intraperitoneally (single dose), LPS + 0.2, LPS + 0.4 and LPS + 0.8 g / kg API applied groups. In histopathological evaluation, hyperemia, intra-alveolar hemorrhage, cellular infiltration, and increased cellular abnormal proliferation were observed in the lung samples of the LPS group. It was found that the lung samples of LPS + 0,4 and LPS + 0,8 API groups decreased statistically significant compared to the LPS group. The number of TUNEL positive cells observed in both LPS and API treated groups showed a statistically significant decrease compared to the LPS group. In comet test, 0,8 API group was found to be reduced more in tail % DNA and tail length when LPS + API treated groups were compared with LPS group. In conclusion, the API applied to rats can prevent LPS-induced lung injury.

Keywords: Apilarnil, LPS, TUNEL method, Comet test, rat

Introduction

Sepsis is a medical condition that describes the systemic immunological response of the body to an infectious process that can lead to end-stage organ dysfunction and death [1]. The annual incidence of severe sepsis and septic shock in the United States is 300 cases per 100,000 people [2]. It is estimated that more than 30 million people worldwide are affected by sepsis each year, resulting in 6 million deaths per year [3]. The highest

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organ damage in sepsis seems in the lungs, liver, kidneys, heart, intestines, and brain [4, 5]. Awareness in the pathogenesis of sepsis is very important for new developments in diagnosis, follow-up, and treatment. The sepsis triad is inflammation, coagulation, and irregular fibrinolysis. Sepsis may be caused by bacteria, viruses, fungi or parasites or may develop in other events such as severe trauma, pneumonia, pancreatitis, and urinary tract infection [6]. Many mediators such as proinflammatory cytokines, chemokines, and free radicals are known to be involved in the process of sepsis [7, 8]. Therefore, compounds with anti-inflammatory properties are thought to be useful in the treatment of sepsis.

LPS obtained as a lyophilized powder is obtained from a large number of Gram (-) bacteria such as *Escherichia coli (E. coli), Salmonella typhimurium, Klebsiella pneumoniae, Pseudomonas aeruginosa*. However, experimental septic shock studies have focused on LPSs obtained from *E. coli*. Reducing inflammation induced by *E. coli* may be a potential therapeutic method for the treatment of sepsis [9].

API is a bee product that has attracted attention in recent years, and it is obtained by lyophilized male bee larvae. However, API is known to have antiviral, immune system enhancer, anabolic stimulant, body energy, vitality and regenerative potentiator properties [10]. Scientific studies related to API are mostly made in farm animals [10-12].

In this study, we aimed to determine the effect of apilarnil on endotoxin-induced lung injury, LPS was used to perform a septic shock model. For this purpose, histopathological analysis of lung samples, TUNEL analysis for the evaluation of apoptotic cells, and Comet assay to determine DNA damage after application were performed.

Materials and Methods

Chemicals

Lipopolysaccharide (Escherichia coli LPS, serotype 0127: B8) was obtained from Sigma Aldrich and Lyophilized Apilarnil is Nutral Therapy Ltd. Doses used for this study were determined from previous studies [10, 13].

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Experiment Groups

Ethics committee approval was received from Erciyes University Animal Experiments Local Ethics Committee (HAYDEK) for this study (Protokol No: 18/063). Animals were housed at 22–24 °C in a continuously ventilated environment with a lighting period of 12 h dark and 12 h light. Throughout the study, the animals were provided with ad libitum rat feed and drinking water. Sixty-four adult male Sprague dawley rats weighing approximately 200-250 g were randomly divided into 8 groups (Table 1).

Six hours after the administration, the rats were anesthetized (ketamine hydrochloride; 50 mg/kg i.m/i.p ve % 2 xylazine hydrochloride; 10 mg/kg i.m/i.p) and lung tissues were removed.

Table 1. Experimental groups and treatment methods

Experimental Groups	Applied Chemical Agent	Dose Amount	Procedure of administration
Control	Saline	1 ml (0.9% NaCl)	i.p.
LPS	LPS	30 mg / kg / bw	i.p.
0.2 g/kg API	API	0.2 g / kg / bw	oral gavage for 10 days
0.4 g/kg API	API	0.4 g / kg / bw	oral gavage for 10 days
0.8 g/kg API	API	0.8 g / kg / bw	oral gavage for 10 days
0.2 g/kg API + LPS	API + LPS	0.2 g / kg API, 30 mg / kg LPS	API oral gavage for 10 days, after 60 min LPS single dose i.p.
0.4 g/kg API + LPS	API + LPS	0.4 g / kg API, 30 mg / kg LPS	API oral gavage for 10 days, after 60 min LPS single dose i.p.
0.8 g/kg API + LPS	API + LPS	0.8 g / kg API, 30 mg / kg LPS	API oral gavage for 10 days, after 60 min LPS single dose i.p.

Histologic Analysis

At the end of the study, rats were sacrificed, and the lung tissues were removed. After routine paraffin embedding, 5 μm thick paraffin sections were stained with Harris hematoxylin and eosin and examined under a light microscope for histopathological evaluation. Hyperemia/congestion, intra-alveolar hemorrhage, cellular infiltration,

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and cellular abnormal proliferation were evaluated in the lung tissues [14]. Histopathological results in each category were scored as follows: 0 = none, 1 = mild, 2 = moderate, 3 = severe.

Tunel Analysis

Apoptotic cells in incisions, obtained from the subjects, were determined by using the Roche brand In Situ Cell Detection Apoptosis Fluorescein Kit. The staining operation was conducted in line with kit procedure. After tissue incisions, taken in 5 µm thickness, were first deparaffinized and then rehydrated, they were washed twice with PBS for 5 minutes, they were later kept at 0.01 M in 5% sodium citrate buffer in microwave oven at 350 W for 5 minutes for antigen recovery, and it was left for cooling in room temperature for 10 minutes. Tissues, which were washed twice with PBS for 5 minutes, were incubated in drying-oven for 60 minutes after they were placed into a moisture chamber at 37 ^{0}C with TUNEL reaction mixture which came out of the kit. Reverse staining was administrated with 4',6-diamidino-2-phenylindole (DAPI) to tissues, which were washed twice for 5 minutes, and the DAPI solution was used as closer for nuclear staining. Tissues, which were closed with glycerol closure solution, were displayed in Olympus BX51 model fluorescent microscope. Apoptotic cells were counted in the image J program from the images taken at 40X lens from each incision from fifteen different sites, to calculate the apoptotic index.

Evaluation of DNA Structure by COMET Assay

The lungs extracted from rats are centrifuged at 5000 rpm for 30 minutes and the resulting supernatant is mixed with low melting agarose (0.65%) and 75 µl of the prepared suspension is transferred onto slides that is coated with low melting agar (0.05%). Electrophoresis is carried out in buffer at 200 V for 4 min. The preparations are transferred to cold lysis solution for 1 hour after the electrophoresis buffer. The preparations from the lysis solution are washed in distilled water for 5 minutes. After washing, the preparations are stained with 80 µl ethidium bromide. Post-staining image analysis is performed using BS 200 ProP (BS 200 ProP, BAB Imaging System). A 40x objective is used on a fluorescent microscope to observe DNA damage. The result of imaging is to determine the percentage of tail DNA, tail length and tail

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moment for 50 comet cells, and the differences between the groups are calculated statistically.

Statistical analysis

Experimental data were statistically analyzed in GraphPad Prism (version 6.0, GraphPad Software Inc., San Diego, California) and presented as mean ± SD. Data were analyzed using one-way ANOVA with Tukey's post hoc tests for multiple comparisons. P<0.05 was considered significant.

Results and Discussion

Histopathological evaluation

According to histopathologic evaluation, the normal histological structure was observed in the lung tissues of the control group and the groups receiving API in increasing doses. Hyperemia, intra-alveolar hemorrhage, cellular infiltration, and increased cellular abnormal proliferation were observed in the lung samples of the LPS group (Figure 1A). It was found that the lung samples of LPS + 0,4 and LPS + 0,8 API groups' injury score were decreased statistically significant compared to the LPS group (Figure 1B).

TUNEL Results

In TUNEL analysis, the number of TUNEL positive cells observed in the LPS group in the lung samples showed a statistically significant increase compared to the control and apilarnil treated groups (Figure 2). The number of TUNEL positive cells observed in both LPS and API treated groups showed a statistically significant decrease compared to the LPS group (Table 2).

Table 2. Numerical data of TUNEL analysis in the lung tissues of experimental groups

Gruplar	Kontrol	0.2	0.4	0.8	LPS	LPS+ 0.2	LPS+ 0.4	LPS+ 0.8	*p*
TUNEL	0.12±0.13[a]	0.13±0.33[a]	0.04±0.20[a]	0.24±0.52[a]	0.64±0.81[b]	0.14±0.35[a]	0.08±0.27[a]	0.0.5±0.21[a]	0.001

Data are expressed as mean ± standard deviation. There is no significant difference between the groups containing the same letter (a, b, c). *P* <0,05 was considered significant.

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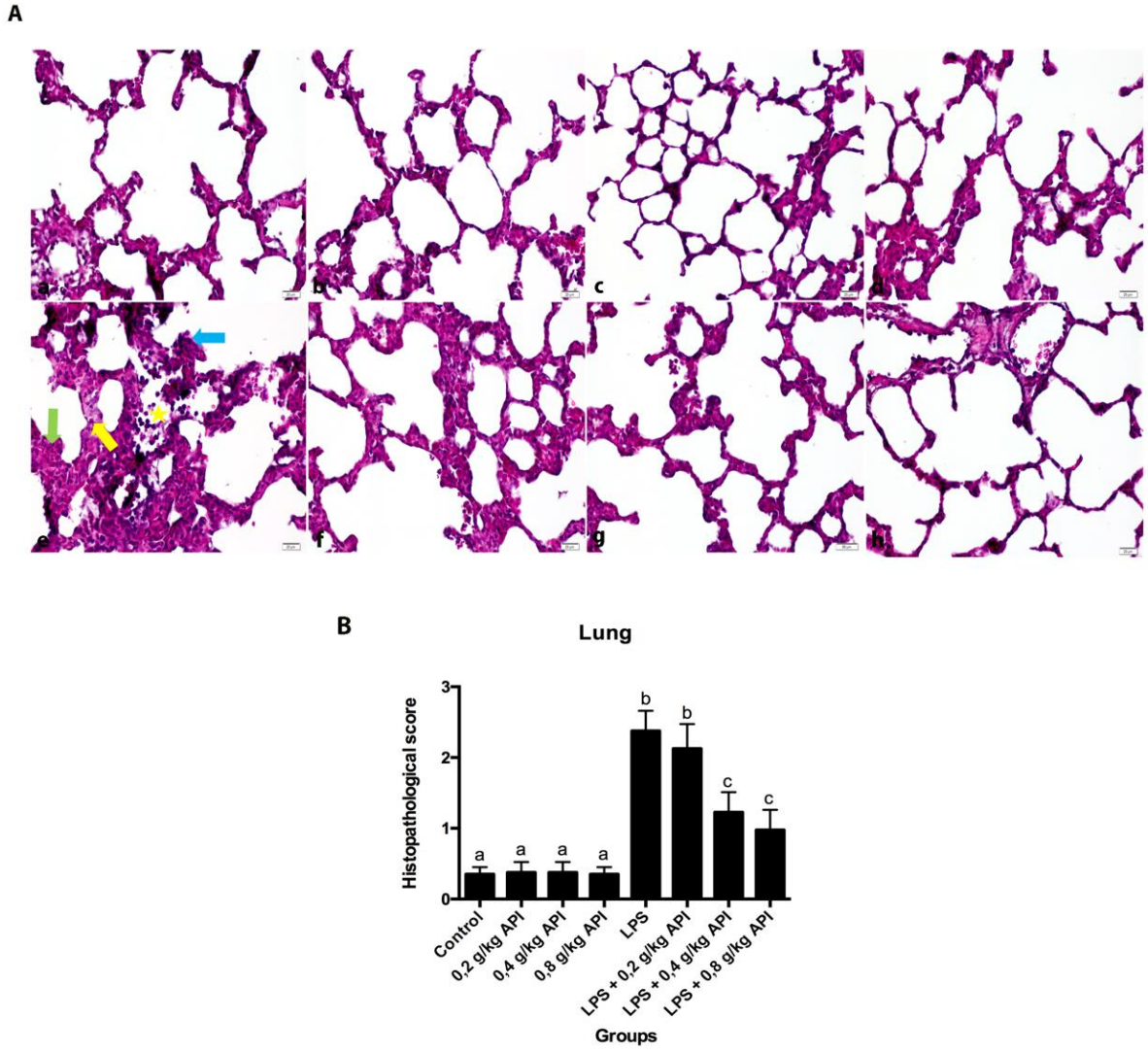

Figure 1. Histopathological evaluation of rat lung tissues of experimental groups by H&E staining method. **A.** Control (a), 0,2 g/kg body weight (bw) apilarnil (API) (b), 0,4 g/kg/ API (c), 0,8 g/kg/ bw API (d), LPS (e), LPS+ 0,2 g/kg/bw API (f), LPS+ 0,4 g/kg/bw API (g), LPS+ 0,8 g/kg bw (h) Magnification 40X, bar = 20µm (yellow arrow: congestion; star: intra alveolar hemorrhage; blue arrow: cellular infiltration; green arrow: cellular abnormal proliferation). **B.** The bar graph data are expressed as mean ± SD and compared by one-way ANOVA and TUKEY's multiple comparisons test. There is no significant difference between the groups containing the same letter (a, b, c). $P<0.05$ was considered significant.

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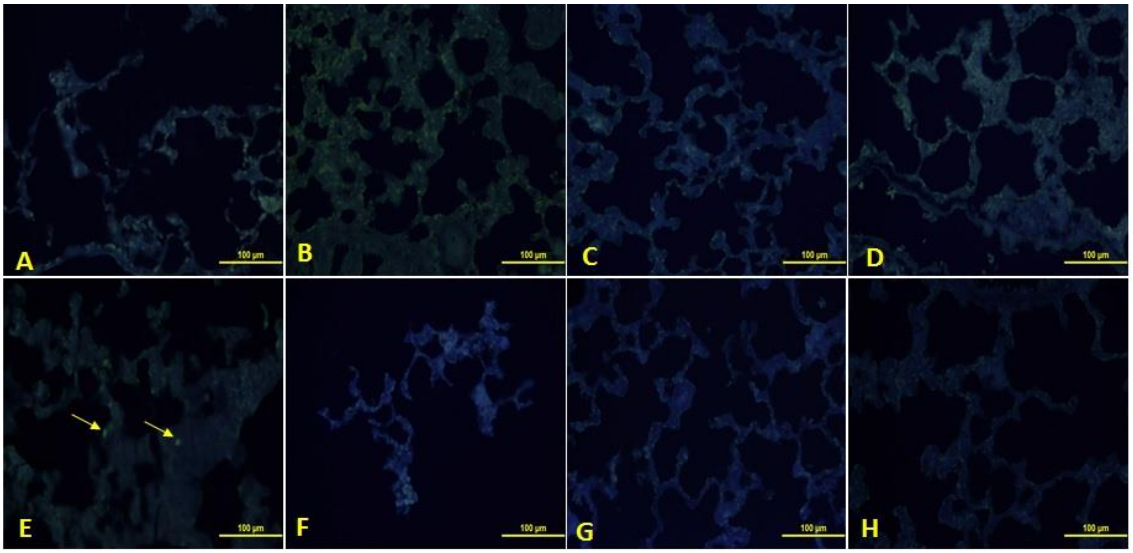

Figure 2. Evaluation of rat lung tissues of experimental groups by TUNEL method. Control (A), 0,2 g/kg/bw apilarnil (API) (B), 0,4 g/kg/bw API (C), 0,8 g/kg/bw API (D), LPS (E), LPS+ 0,2 g/kg/bw API (F), LPS+ 0,4 g/kg/bw API (G), LPS+ 0,8 g/kg/bw (H) Magnification: 40X, bar = 100μm.

Evaluation of Comet Assay Results

At the end of 6 hours LPS and/or API groups of rat lung tissues were taken for the comet method and tail %DNA, tail length and tail moment changes were determined (Figure 3 and Table 3).

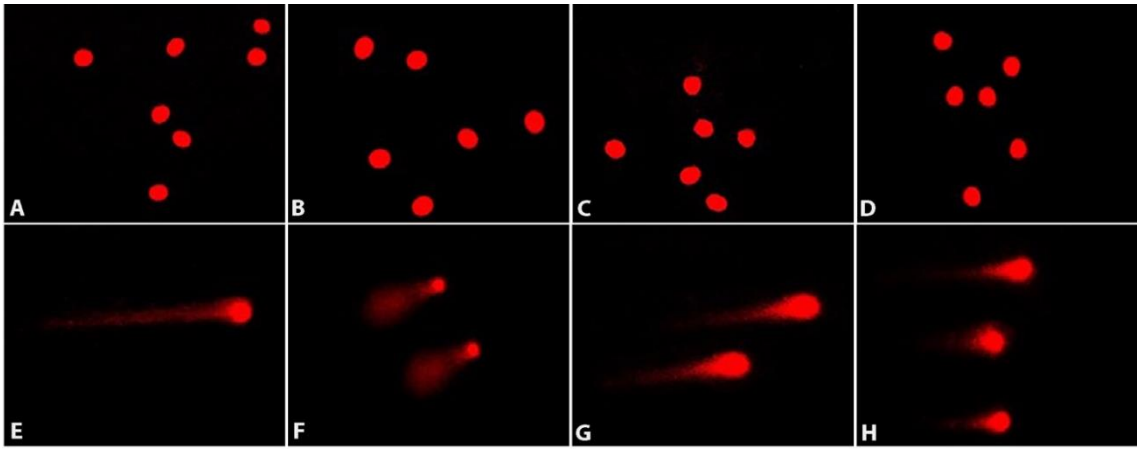

Figure 3. Determination of DNA damage caused by LPS administered to rat lungs cells and increasing doses of apilarnil. Control and API-treated group, (A-D); LPS-treated group (E), LPS + 0.2 g/kg API-administered group, (F) LPS + 0.4 g/kg API-administered group (G), LPS + 0.8 g/kg API-administered group (H).

No statistically significant difference was found in the DNA parameters of lung cells of rats in LPS-treatment groups and only increasing doses of API. Tail % DNA, tail

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length and tail moment changes were determined in the LPS treated groups and it was found that there was a statistically significant increase in the studied parameters when the LPS treated groups and the control groups were compared. When LPS+API treated groups were compared, a statistically significant ($p < 0.05$) decrease was found in the studied parameters due to increasing concentrations of API. When 0.8 g/kg API+LPS applied group compared with 0.2 and 0.4 g/kg API+LPS, 0.8 g/kg API was found to be reduced more in tail % DNA and tail length according to 0.2 and 0.4 g/kg API applied group (Figure 3 and Table 3).

Table 3. Tail DNA%, Tail length and Tail moment values of lung cells exposed to LPS and increasing doses of API

	Tail DNA% Mean ± SD	**Tail Length Mean ± SD**	**Tail moment Mean ± SD**
Control	25.56±1.96[a]	16.40±1.22[a]	4.19±0.02[a]
0.2 g/kg API	24.48±3.25[a]	17.30±5.40[a]	4.23±0.17[a]
0.4 g/kg API	24.05±2.04[a]	16.79±2.02[a]	4.03±0.04[a]
0.8 g/kg API	23.55±2.31[a]	15.15±3.22[a]	3.56 ±0.07[a]
LPS	90.39±10.20[b]	82.94±8.15[b]	74.96±0.8[b]
LPS+0.2 g/kg API	70.82±6.53[c]	61.34±5.22[c]	43.44±0.34[c]
LPS+0.4 g/kg API	58.09±2.25[c]	25.12±4.85[c]	14.59±0.10[c]
LPS+0.8 g/kg API	39.06±3.12[d]	37.98±1.62[d]	14.83±0.05[d]

Data are expressed as mean ± standard deviation. There is no significant difference between the groups containing the same letter (a, b, c). $P < 0,05$ was considered significant.

Although many strategies have been developed to understand and treat the pathophysiological mechanisms of endotoxemia induced by endotoxins [15]. It is still a major problem in intensive care units. In recent years, alternative treatment options have gained increasing importance in addition to medical treatment, especially in the field of human medicine. API is one of the natural products that can be used for this purpose and contains many biologically active compounds. API related studies are very limited, but there are studies showing that API has positive effects on reproductive functions [10-12]. Besides, Meda et al. [16] reported that API has been

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used successfully in South Africa (Burkina Faso) for gastrointestinal diseases, respiratory diseases, vertigo, ophthalmic diseases, toothache, muscle fatigue, wounds, burns and back pain, in particular, male infertility [16]. In this study, the effect of API on endotoxin-induced lung injury was investigated.

LPS application has been shown to cause tissue damage in many studies. Demiralay et al. [17] showed that LPS application in lung tissue increased inflammation, alveolar damage, vascular occlusion and the number of TUNEL positive bronchiolar and alveolar epithelial cells increased and LPS application resulted in induction of apoptotic cells characteristic of apoptotic cell death [17]. Wang et al. [18], investigated the role of bone marrow-derived mesenchymal stem cell (BMSC) transplantation on LPS-induced acute lung injury (ALI) in rats and they showed that LPS causes edema, severe damage to the alveolar wall, cellular abnormal proliferation, hyperemia and cellular infiltration [18]. Liu et al. [19] also showed that LPS administration causes serious damage to the lungs (a large amount of neutrophil and macrophage infiltration in the alveolar cavity, infiltration of the alveoli, edema, thickening of the alveolar wall and pulmonary interstitium), pulmonary alveoli, terminal bronchioles and whole lung tissue structure and in addition, LPS administration has been shown to significantly increase the number of apoptotic cells [19]. In the present study, it was observed that the lung samples of LPS + 0,4 and LPS + 0,8 API groups histopathological score were decreased statistically significant compared to the LPS group (Figure 1B). The data obtained at the end of the study are consistent with the existing literature.

LPS application increases the number of apoptotic cells in tissues [17-19]. As far as we investigate that there are no studies on the effects of API on increased apoptosis. The studies are related to other bee products. Cağlı et al. [20] investigated the effect of caffeic acid phenethyl ester (CAPE) on experimentally induced myocardial ischemia-reperfusion (I / R) injury and apoptotic changes and showed that pretreatment with CAPE reduced apoptosis in rat myocardium induced by I / R [20]. Yuluğ et al. [21] examined the effects of propolis on cisplatin-induced renal injury in mice and showed that tissue damage and increased apoptotic cell counts

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due to CP were reduced due to the antioxidant and antiapoptotic effects of propolis [21, 22]. Kamijo et al. [22] showed that Cernitin pollen-extract (CN-009) significantly reduced tissue damage and apoptotic cell counts in the chronic nonbacterial prostatitis model [22]. As a result of TUNEL test in our study, we found that apilarnil has positive effects on increased apoptosis after LPS.

Comet test is one of the quick and easy test methods to determine the effects of various chemical agents that produce clastogenic effects in DNA [23, 24]. This method is used as a rapid and sensitive genotoxicity test to show DNA damage directly as well as single chain fractures and incomplete DNA repair sites [25]. The appearance of DNA migration generated by an electric current applied in the art is similar to a comet because it resembles a head and tail [26].

Agents with genotoxic effects cause damage to the DNA of prokaryotic and eukaryotic organisms. This damage is usually seen in the single and/or double helix in the DNA. Agents with genotoxic effects may cause carcinogenesis or chromosomal aberrations leading to sister chromatid changes, micronucleus, cell death and mutations [27]. In some studies, API has been reported to have antioxidant, antimicrobial and antiproliferative potential [28-30]. In this study, single and/or double-stranded DNA breaks were determined by comet test after LPS and/or API exposure time. Single and/or double-stranded DNA breaks were measured as tail% DNA, tail length and tail moment. Toxic effects of LPS on lungs cells of rats were evaluated by co-administration of API (0.2, 0.4 and 0.8 g/kg bw). There is no study showing the protective effect of API on DNA damage in lungs cells of rats in vivo. The toxic effects of LPS were determined by looking at tail DNA and tail length of lungs cells. At the end of 6 hours, it was determined that tail% DNA and tail length were increased in groups treated with LPS-treatment group compared to control group. In addition, in groups exposed to different concentrations of API, 0.8 g/kg API was found to be more protective than 0.2 and 0.4 g/kg API in lungs cells of rats.

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Conclusion

In conclusion, we think that API prevented LPS-induced lung injury. We believe that our study will contribute to the literature in terms of demonstrating apilarnil's efficacy at tissue level. However, more comprehensive studies are needed.

Sıçanlarda lipopolisakkarit ile oluşturulan akciğer hasarına karşı apilarnilin koruyucu etkilerinin araştırılması

Öz: Apilarnil son yıllarda faydalı biyolojik özellikleri nedeniyle dikkat çeken bir arı ürünüdür. Bu çalışmanın amacı, apilarnil (API)' in, endotoksin ile indüklenen akciğer hasarı üzerine etkisini araştırmaktır. Çalışma için 64 adet Sprague dawley cinsi yetişkin erkek sıçan sekiz gruba ayrılmıştır; Kontrol, 10 gün boyunca gavaj ile 0.2, 0.4 ve 0.8 g/kg apilarnil (API) uygulan gruplar, intraperitoneal olarak 30 mg/kg lipopolisakkarit (LPS) uygulanan grup (tek doz), LPS + 0.2, LPS + 0.4 ve LPS + 0.8 g/kg API uygulanan gruplar. Histopatolojik değerlendirmede LPS uygulanan gruptaki ratların akciğer dokularında kanama, intra-alveolar hemoraji, hücresel infiltrasyon ve hücresel anormal proliferasyonda artış gözlendi. Oluşan doku hasarının özellikle LPS + 0.4 g/kg ve LPS + 0.8 g/kg API uygulanan gruplardaki ratların akciğer dokularında sadece LPS uygulanan grupla kıyaslandığında, istatistiksel olarak anlamlı derecede azaldığı tespit edildi. LPS ile birlikte API uygulanan gruplarda gözlenen TUNEL pozitif hücre sayısı ise LPS grubuna göre önemli oranda azaldı. Komet testinde, LPS+API ile tedavi edilen gruplar LPS grubu ile karşılaştırıldığında 0,8 g/kg API grubunun kuyruk % DNA'sının ve kuyruk uzunluğunun daha çok azaldığı bulundu. Sonuç olarak sıçanlara uygulanan API, LPS ile oluşturulan akciğer hasarını önleyebilmektedir.

Anahtar kelimeler: Apilarnil, LPS, TUNEL metodu, Komet testi, Sıçan

R E F E RE N C E S

[1] KAUKONEN, K M; BAILEY, M; SUZUKI, S, PILCHER, D; BELLOMO, R (2014), Mortality related to severe sepsis and septic shock among critically ill patients in Australia and New Zealand, 2000-2012, JAMA, 311(13), 1308-1316.

[2] GYAWALI, B; RAMAKRISHNA, K; DHAMOON, A S (2019), Sepsis: The evolution in definition, pathophysiology, and management, SAGE Open Med, 7, 2050312119835043.

Cite as: DOĞANYİĞİT, Z; KAYMAK, E; OKAN, A; PANDIR, D; SİLİCİ, S (2020), Investigation of Protective Effects of Apilarnil Against Lipopolysaccharide Induced Lung Injury in Rats, Mellifera, 20(1):3-15.

[3] LEVY, M M; ARTIGAS, A; PHILLIPS, G S; RHODES, A; BEALE, R; OSBORN, T; VINCENT, J L; TOWNSEND, S; LEMESHOW, S; DELLINGER, R P (2012), Outcomes of the Surviving Sepsis Campaign in intensive care units in the USA and Europe: a prospective cohort study, Lancet Infect Dis, 12(12):919-24.

[4] POLAT, G; UGAN, R A; CADIRCI, E; HALICI, Z (2017), Sepsis and Septic Shock: Current Treatment Strategies and New Approaches, Eurasian J Med, 49(1):53-58.

[5] ADAM, N; KANDELMAN, S; MANTZ, J; CHRETIEN, F; SHARSHAR, T (2013), Sepsis-induced brain dysfunction, Expert Rev Anti Infect Ther, 11(2): 211-21.

[6] COHEN, J (2002), The immunopathogenesis of sepsis, Nature, 420: 885-91.

[7] NDUKA, O O; PARRILLO, J E (2009), The pathophysiology of septic shock, Crit Care Clin, 25, 677-702, vii.

[8] CINEL, I; OPAL, S M (2009), Molecular biology of inflammation and sepsis: a primer, Crit Care Med, 37: 291-304.

[9] POP-BEGAN, V, PAUNESCU, V, GRIGOREAN, V, POP-BEGAN, D; POPESCU, C (2014), Molecular mechanisms in the pathogenesis of sepsis, J Med Life, 7 Spec No, 2:38-41.

[10] ALTAN, O; YUCEL, B; ACIKGOZ, Z; SEREMET, C; KOSOGLU, M; TURGAN, N; OZGONUL, A M (2013), Apilarnil reduces fear and advances sexual development in male broilers but has no effect on growth, Br Poult Sci, 54:355-61.

[11] BOLATOVNA, K S; RUSTENOV, A; ELEQALIEVA, N; OMIRZAK, T; AKHANOV, U K (2015), Improving reproductive qualities of pigs using the drone brood homogenate, Biol Med (Aligarh), 7, BM-091-15.

[12] YÜCEL, B; AÇIKGÖZ, Z; BAYRAKTAR, H; SEREMET, Ç (2011), The effects of apilarnil (drone bee larvae) administration on growth performance and secondary sex characteristics of male broilers, J Anim Vet Adv, 10: 2263-2266.

[13] DOGANYIGIT, Z; KUP, F O; SILICI, S; DENIZ, K; YAKAN, B; ATAYOGLU, T (2013), Protective effects of propolis on female rats' histopathological, biochemical and genotoxic changes during LPS induced endotoxemia, Phytomedicine, 20: 632-9.

[14] HIRANO, Y; AZIZ, M; YANG, W L; WANG, Z; ZHOU, M; OCHANI, M; KHADER, A; WANG, P (2015), Neutralization of osteopontin attenuates neutrophil migration in sepsis-induced acute lung injury, Crit Care, 19:53.

[15] WILHELM, E A; JESSE, C R; ROMAN, S S; NOGUEIRA, C W; SAVEGNAGO, L (2009), Hepatoprotective effect of 3-alkynyl selenophene on acute liver injury induced by D-galactosamine and lipopolysaccharide, Exp Mol Pathol, 87: 20-6.

[16] MEDA, A; LAMIEN, C E; MILLOGO, J; ROMITO, M; NACOULMA, O G (2004), Therapeutic uses of honey and honeybee larvae in central Burkina Faso, J Ethnopharmacol, 95: 103-7.

[17] DEMIRALAY, R; GURSAN, N; ERDEM, H (2006), Regulation of sepsis-induced apoptosis of pulmonary cells by posttreatment of erdosteine and N-aceylcysteine, Toxicology, 228: 151-61.

[18] WANG, J; QIN, Y; MI, X (2016), The protective effects of bone marrow-derived mesenchymal stem cell (BMSC) on LPS-induced acute lung injury via TLR3-mediated IFNs, MAPK and NF-kappaB signaling pathways, Biomed Pharmacother, 79: 176-87.

[19] LIU, W A; PANG, G; WANG, S; SUN, A (2017), Protective effect of ulinastatin on severe pulmonary infection under immunosuppression and its molecular mechanism, EXPERIMENTAL AND THERAPEUTIC MEDICINE, 14:3583-3588.

[20] CAGLI, K; BAGCI, C; GULEC, M; CENGIZ, B; AKYOL, O; SARI, I; CAVDAR, S; PENCE, S; DINCKAN, H (2005), In vivo effects of caffeic acid phenethyl ester on myocardial ischemia-reperfusion injury and apoptotic changes in rats, Ann Clin Lab Sci, 35:440-8.

[21] YULUG, E; TUREDI, S; YILDIRIM, O; YENILMEZ, E; ALIYAZICIOGLU, Y; DEMIR, S; OZER-YAMAN, S; MENTESE, A (2019), Biochemical and morphological evaluation of the effects of propolis on cisplatin induced kidney damage in rats, Biotech Histochem, 94:204-213.

[22] KAMIJO, T; SATO, S; KITAMURA, T (2001), Effect of cernitin pollen-extract on experimental nonbacterial prostatitis in rats, Prostate, 49:122-31.

[23] SINGH, N P; MCCOY, M T; TICE, R R; SCHNEIDER, E L (1988), A simple technique for quantitation of low levels of DNA damage in individual cells, Exp Cell Res, 175:184-91.

Cite as: DOĞANYİĞİT, Z; KAYMAK, E; OKAN, A; PANDIR, D; SİLİCİ, S (2020), Investigation of Protective Effects of Apilarnil Against Lipopolysaccharide Induced Lung Injury in Rats, Mellifera, 20(1):3-15.

[24] FAIRBAIRN, D W; OLIVE, P L; O'NEILL, K L 81995), The comet assay: a comprehensive review, Mutat Res, 339: 37-59.

[25] TICE, R R; VASQUEZ, G (1998), Protocol for the application of the pH>13 alkaline sigle cell gel (SCG) assay to the detection of DNA damage in mammalian cells, ILS: The pH>13 Alkaline SCG Assay,Available from: http://cometassay.com/Tice%20and%20Vasques.pdf (Data of access)

[26] KLAUDE, M; ERIKSSON, S; NYGREN, J; AHNSTROM, G (1996), The comet assay: mechanisms and technical considerations, Mutat Res, 363: 89-96.

[27] HIROSE, H; SAKUMA, N; KAJI, N; SUHARA, T; SEKIJIMA, M; NOJIMA, T; MIYAKOSHI, J (2006), Phosphorylation and gene expression of p53 are not affected in human cells exposed to 2.1425 GHz band CW or W-CDMA modulated radiation allocated to mobile radio base stations, Bioelectromagnetics, 27: 494-504.

[28] CAPUCHO, C; SETTE, R; DE SOUZA PREDES, F; DE CASTRO MONTEIRO, J; PIGOSO, A A; BARBIERI, R; DOLDER, M A; SEVERI-AGUIAR, G D (2012). Green Brazilian propolis effects on sperm count and epididymis morphology and oxidative stress, Food Chem Toxicol, 50: 3956-62.

[29] RIZK, S M; ZAKI, H F; MINA, M A (2014), Propolis attenuates doxorubicin-induced testicular toxicity in rats, Food Chem Toxicol, 67: 176-86.

[30] DOGANYIGIT, Z; SILICI, S; DEMIRTAS, A; KAYA, E; KAYMAK, E (2019), Determination of histological, immunohistochemical and biochemical effects of acute and chronic grayanotoxin III administration in different doses in rats, Environ Sci Pollut Res Int, 26: 1323-1335.

Cite as: DOĞANYİĞİT, Z; KAYMAK, E; OKAN, A; PANDIR, D; SİLİCİ, S (2020), Investigation of Protective Effects of Apilarnil Against Lipopolysaccharide Induced Lung Injury in Rats, Mellifera, 20(1):3-15.