International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2023.623.631

Research Article
Effect of Taxifolin on Lung Injury Induced by *Staphylococcus aureus* in Albino Wistar-Type Rats: A Biochemical and Histopathological Evaluation

[1]Bulent Dabanlioglu, [2]Bahadir Suleyman, [2]Renad Mammadov, [2]Bulent Yavuzer, [2]Durdu Altuner, [1]Onder Akkas, [3]Behzad Mokhtare, [4]Taha Abdulkadir Coban and [2]Halis Suleyman

[1]Department of Medical Microbiology, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan 24100, Turkey
[2]Department of Pharmacology, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan 24100, Turkey
[3]Department of Pathology, Faculty of Veterinary Medicine, Ataturk University, Erzurum 25240, Turkey
[4]Department of Medical Biochemistry, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan 24100, Turkey

Abstract

Background and Objective: *Staphylococcus aureus* is considered the main cause of pneumonia. Pneumonia is the most common cause of acute lung injury. Taxifolin is a flavonol with antioxidant, anti-inflammatory, antimicrobial, antiviral, antifungal, anti-hyperglycemic, anti-hyperlipidemic, anti-psoriatic and pulmono-protective activities. The present study aimed to biochemically and histopathologically investigate the protective effect of taxifolin against possible acute lung oxidative and inflammatory damage caused by *Staphylococcus aureus* (*S. aureus*) infection in rats. **Materials and Methods:** The rats were divided into three groups 6 rats each, healthy control (HG), *S. aureus* inoculated (SaG) and *S. aureus* inoculated+taxifolin treated (SaT). Animals were euthanized. Malondialdehyde (MDA) total glutathione (tGSH), Nuclear Factor kappa B (NF-κB), Tumor Necrosis Factor-alpha (TNF-α) and Interleukin One Beta (IL-1β) levels were measured in the excised lung tissues and also examined histopathologically. **Results:** *S. aureus* inoculation significantly increased MDA, NF-κB, TNF-α and IL-1β levels and decreased tGSH levels in lung tissue. Treatment with taxifolin significantly suppressed the increase in MDA, NF-κB, TNF-α and IL-1β levels and the decrease in tGSH levels. Histopathologically, *S. aureus* inoculation led to severe mononuclear cell infiltration in interstitial areas, lymphoid hyperplasia in bronchial-associated lymphoid tissue and desquamation in bronchial epithelium. Treatment with taxifolin attenuated these histopathological findings. **Conclusion:** Current experimental results suggested that taxifolin may be beneficial in the treatment of oxidative and inflammatory lung injury associated with *S. aureus* infection.

Key words: Lung injury, oxidative and inflammatory injury, rats, *Staphylococcus aureus*, taxifolin

Citation: Dabanlioglu, B., B. Suleyman, R. Mammadov, B. Yavuzer and D. Altuner *et al*., 2023. Effect of taxifolin on lung injury induced by *Staphylococcus aureus* in albino Wistar-type rats: A biochemical and histopathological evaluation. Int. J. pharmacol., 19: 623-631.

Corresponding Author: Bulent Dabanlioglu, Department of Medical Microbiology, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan 24100, Turkey Tel: +90 532 4205430, Fax: +90 446 2261819

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a human pathogen that can cause a wide range of diseases and has been identified in the literature since the 1800s[1,2]. Nearly 40% of the healthy human population carries *S. aureus*, with the nose, throat, skin and intestinal tract being the most frequently detected sites and carriers are typically asymptomatic[2]. *Staphylococcus aureus* is an important cause of nosocomial and community-acquired infections[3] and can lead to many infectious diseases, including mild skin and soft tissue infections, infective endocarditis, osteomyelitis, bacteremia and fatal pneumonia[4]. *Staphylococcus aureus* is considered the main cause of pneumonia[5]. Pneumonia, on the other hand, is the most common cause of acute lung injury (ALI) or Acute Respiratory Distress Syndrome (ARDS) with its new definition[6,7]. In the initial stage of an *S. aureus* infection, phagocytes (neutrophils and macrophages) infiltrate the infection site and the production of lysosomal enzymes and reactive oxygen species (ROSs) is subsequently induced in these cells[8]. Increased ROS production in cells and tissues alters the oxidant antioxidant balance in favor of oxidants and leads to oxidative stress[9]. Koç *et al.*[10] reported that ROSs cause oxidative stress oxidize lipids in the cell membrane (LPO) and create toxic products such as malondialdehyde (MDA) from lipids. In addition, through Toll-like Receptor 2 (TLR2)-dependent signaling molecules, including Mitogen-Activated Protein Kinases (MAPKs) and NF-κB, *S. aureus* has been reported by Jiang *et al.*[11] to promote the inflammatory response in mice. Activation of MAPKs triggers inflammatory reactions[12]. The role of proinflammatory cytokines including Nuclear Factor kappa B (NF-κB), Tumor Necrosis Factor-alpha (TNF-α), Interleukin-1β (IL-1β) and Interleukin-6 (IL-6) in the pathogenesis of inflammatory damage induced by *S. aureus* has been reported by Jiang *et al.*[11].

The aim of the present study was to investigate the protective effect of taxifolin, an antioxidant flavonol, against acute lung oxidative and inflammatory damage induced by an *S. aureus* infection[13]. Taxifolin, derived from the bark of Douglas fir trees, is widely found in plants such as water safflower, larch, camphor pine, milk thistle, French maritime bark and Smilacis Glabrae Rhizoma (Yang) and in foods such as grapes, citrus fruits, green tea, wine, onions and olive oil[14,15]. Taxifolin was reported to have antioxidant, anti-inflammatory, antimicrobial, antiviral, antifungal, anti-hyperglycemic, anti-hyperlipidemic, anti-psoriatic and pulmono-protective activities[16]. Taxifolin reportedly exhibited its antioxidant activity by chelating with Fe^{2+}, reducing Fe^{3+} and Cu^{2+}, inhibiting membrane LPO and scavenging ROSs[17,18]. Its anti-inflammatory activity has been reported to function by suppressing NF-κB and protein kinase activation and decreasing TNF- α, IL-1β and IL-6 levels[19]. To the best of our knowledge, no study has examined the protective effects of taxifolin against acute lung oxidative and inflammatory damage induced by an *S. aureus* infection. Therefore, the aim of the present study was to investigate the biochemically and histopathologically protective effect of taxifolin against possible acute lung oxidative and inflammatory damage caused by an *S. aureus* infection in rats.

MATERIALS AND METHODS

Study area: The present study was carried out in Erzincan Binali Yıldırım University at Animal Experiments Laboratory between 10-01-2022 and 24-01-2022 in two weeks.

Animals: Albino Wistar-type male rats were obtained from Atatürk University Medical Experimental Research and Application Center. A total of 18 male albino Wistar-type rats (body weight, 270-287 g) were used for the experiment. Before the experiment, animals were housed in a suitable laboratory environment at normal room temperature (22°C) and fed *ad libitum* with animal feed and tap water. The experiments were conducted in accordance by the Turkey Regulation of Animal Research Ethics. In addition, this study was carried out in accordance with the principles of the Declaration of Helsinki. The protocols and procedures were approved by the Atatürk University Animal Experimentation Ethics Committee (Meeting date, 25-06-2021; Meeting no, 5; Decision no: 172).

Preparation of bacterial isolate and inoculum: The standard *S. aureus* ATCC 29213 strain was obtained from the Medical Microbiology Laboratory of Erzincan Mengücek Gazi Training and Research Hospital. The standard strain stored at -80°C was inoculated into 5% sheep blood medium (BioMérieux, France) and incubated at 37°C for 16-18 hrs. The next day, the same procedure was repeated a second time and pure colonies grown in sheep blood medium (BioMérieux, France) were suspended in fresh Mueller Hinton liquid medium and the turbidity was adjusted to 0.5 MacFarland (1.5×10^8 CFU mL^{-1}) on a DensiCHEK Plus (BioMérieux, France) densitometer.

Chemicals: The thiopental sodium used in the study was obtained from IE. Ulagay (Turkey) and taxifolin was obtained from Evalar (Russia).

Experimental groups: The rats used in the experiment were divided into the following three groups: Healthy control (HG), *S. aureus* inoculated (SaG) and *S. aureus* inoculated+taxifolin treated (SaT).

Experimental procedure: Animals were anesthetized with thiopental sodium. The area around the nostrils of animals in all groups was sterilized with povidone-iodine. To perform the experiment, 0.1 mL^{-1} of bacterial mixture (*S. aureus* strain ATCC 29213, suspended at a concentration of 900×10^6 CFU colony forming units per mL) was administered into the left nostrils of the rats in the SaG (n = 6) and SaT (n = 6) groups (excluding HG) and the animals were held upright for one minute after inoculation. Taxifolin (50 mg kg^{-1}) was administered orally to the SaT group 24 hrs after *S. aureus* administration. The HG and SaG groups were given the same volume of solvent. This procedure was performed once a day for 7 days. At the end of this period, the animals were euthanized with high dose anesthesia (thiopental sodium 50 mg kg^{-1}) and lung tissues were removed. The MDA, total Glutathione (tGSH), NF-κB, TNF-α and IL-1β levels were measured in the excised tissues. Lung tissues were examined histopathologically. All biochemical and histopathological results obtained from the HG and SaT groups were compared with those obtained from the SaG group.

Biochemical analyzes

Preparation of samples: All tissues were rinsed with phosphate buffered saline prior to dissection. Tissues were homogenized in ice-cold phosphate buffer (50 mM, pH 7.4) appropriate for the variable that needed to be measured. Tissue homogenates were subjected to centrifugation at 5000 rpm for 20 min at 4°C. For analysis of MDA and tGSH, the supernatants were extracted. The results for all the tissues were stated by dividing them by G protein. A microplate reader was handled for all spectrophotometric measurements. (Bio-Tek, USA).

Determination of tissue MDA and tGSH: Tissue MDA was evaluated handling the method as described by Ohkawa *et al.*[20] which involves spectrophotometric measurement of the absorbance of the pink complex created by thiobarbituric acid (TBA) and MDA. The measurement of tGSH was carried out according to the method described by Sedlak and Lindsay[21].

NF-κB, TNF-α and IL-1β analysis: The following rat-specific Enzyme-Linked Immunosorbent Assay (ELISA) kits were used to evaluate tissue-homogenized NF-κB, TNF-α and IL-1β concentrations rat: NF-κB ELISA immunoassay kits (Catalog Number 201-11-0288, Shanghai SunRed Biological Technology Co. Ltd., Shanghai, China), rat TNF-α ELISA kits (Catalog Number YHB1098Ra, Shanghai LZ Biotech Co. Ltd., Shanghai, China), rat IL-1β ELISA kits (Catalog number YHB0616Ra, China), rat IL-1β ELISA kits (Catalog number YHB0616Ra, Shanghai LZ Biotech Co. Ltd., Shanghai, China). The analyses were carried out in accordance with the manufacturer's instructions.

Histopathological examination: After necropsy, the lung tissues were placed in 10% buffered formalin solution. The specimens were then subjected to routine follow-up procedures and embedded in paraffin blocks. The 5 µm-thick sections taken from the blocks on slides were stained with hematoxylin-eosin and examined under light microscopy for histopathological findings. The evaluation was semi-quantitatively graded as absent (0), mild (1), moderate (2) and severe (3) for mononuclear cell infiltration in interstitial areas, lymphoid hyperplasia in bronchial-associated lymphoid tissue (BALT) and desquamation in bronchial epithelium.

Statistical analysis: Results are stated as Mean±Standard Deviation (mean±SD). The IBM SPSS Statistics program for Windows (IBM Corp., v. 22.0, 2013 release, Armonk, New York, USA) was handled for statistical analysis. The normality of the distribution for continuous variables in the biochemical test results was checked via the Shapiro-Wilk's Test. The significance of the intergroup differences in the biochemical test results was determined using one-way ANOVA test considering that the results were normally distributed except for TNF-α. Levene's Test was performed to determine whether the homogeneity of variances was achieved. Tukey's honestly significant difference (HSD) for MDA, NF-κB and IL-1β and Games-Howell's Test for tGSH were subsequently performed as *post-hoc* Tests. Considering that TNF-α levels were not normally distributed, Kruskal–Wallis Test, a non-parametric test, was used and Mann-Whitney's U-test was used as a *post-hoc* Test to determine the group that created the difference. The $p<0.05$ indicated statistical significance in all analyses. Histopathological data were analyzed with IBM SPSS Statistics for Windows program (IBM Corp., version 20.00, released in 2011, Armonk, New York, USA). The difference between the groups was determined by Kruskal Wallis, one of the non-parametric tests. Mann-Whitney's U-Test was used as a *post-hoc* Test to determine the group that created the difference ($p<0.05$).

RESULTS

Biochemical results

MDA and tGSH analysis results: Figure 1a shows that *S. aureus* inoculation significantly increased MDA levels in lung tissue ($p<0.001$). Treatment with taxifolin significantly suppressed the increase in MDA levels induced by *S. aureus* inoculation in lung tissue ($p<0.001$). The difference between

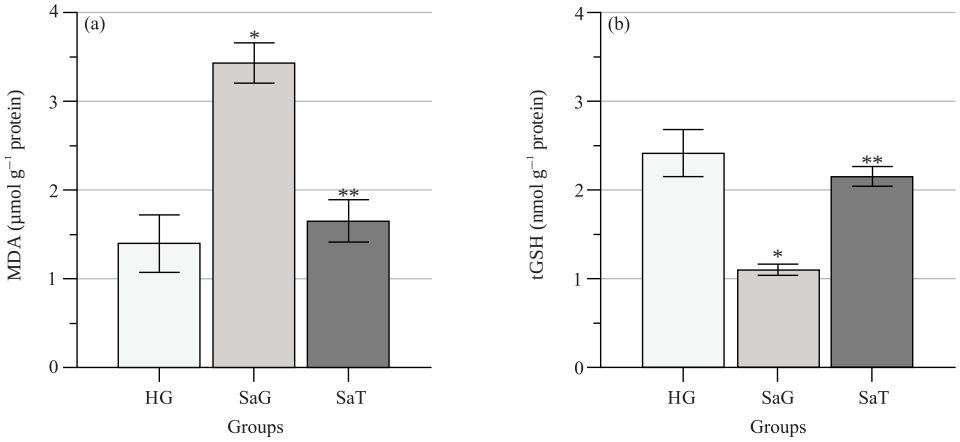

Fig. 1(a-b): Lung tissue of experimental groups, (a) MDA and (b) tGSH levels

Bars are mean±SD (standard deviation),*means p<0.001 when SaG group was compared with the HG control group,**means p<0.001 when the SaT group was compared with the SaG group and for each group n = 6, MDA: Malondialdehyde, tGSH: Total Glutathione, HG: Healthy control group, SaG: *Staphylococcus aureus* inoculated group and SaT: *Staphylococcus aureus* inoculated+taxifolin treated group

Table 1: Mean and standard deviation values of oxidant, antioxidant and proinflammatory cytokine levels in lung tissue

Group	MDA	tGSH	NF-κB	TNF-α	IL-1β
HG	1.40±0.32	2.41±0.26	2.19±0.14	1.86±0.33	2.45±0.19
SaG	3.43±0.23	1.10±0.06	4.53±0.34	3.89±0.10	5.27±0.20
SaT	1.66±0.24	2.15±0.11	2.55±0.23	2.01±0.33	2.61±0.30

HG: Healthy control group, SaG: *Staphylococcus aureus* inoculated group, SaT: *Staphylococcus aureus* inoculated+taxifolin treated group, MDA: Malondialdehyde, tGSH: Total Glutathione, NF-κB: Nuclear Factor kappa B, TNF-α: Tumor Necrosis Factor-alpha and IL-1β: Interleukin one beta

Table 2: Effect of taxifolin on oxidant, antioxidant and proinflammatory cytokine levels on *Staphylococcus aureus*-induced lung injury in rats

Variable	HG vs SaG	HG vs SaT	SaG vs SaT
MDA*	<0.001	0.257	<0.001
tGSH**	<0.001	0.133	<0.001
NF-κB*	<0.001	0.065	<0.001
TNF-α***	0.004	1.000	0.028
IL-1β*	<0.001	0.476	<0.001

*Statistical evaluation was performed using one-way ANOVA followed by Tukey HSD *post-hoc* Test. **Statistical evaluation was performed using one-way ANOVA followed by Games-Howell *post-hoc* Test. ***Statistical evaluation was performed using Kruskal-Wallis Test followed by Mann-Whitney U *post-hoc* Test, p values comparison of the experimental groups' biochemical test results. Significance level was considered as p<0.05, HG: Healthy control group, SaG: *Staphylococcus aureus* inoculated group, SaT: *Staphylococcus aureus* inoculated+taxifolin treated group, MDA: Malondialdehyde, tGSH: Total glutathione, NF-κB: Nuclear Factor kappa B, TNF-α: Tumor Necrosis Factor-alpha and IL-1β: Interleukin one beta

the MDA levels of the taxifolin-treated group and the healthy group was no significant (p = 0.257, Table 1 and 2).

Staphylococcus aureus inoculation significantly decreased tGSH levels in lung tissue (p<0.001). Treatment with taxifolin significantly prevented the decrease in tGSH levels induced by *S. aureus* inoculation in lung tissue (p<0.001). The difference between the tGSH levels of the taxifolin-treated group and the healthy group was not significant (p = 0.133, Table 1 and 2, Fig. 1b).

NF-κB, TNF-α and IL-1β analysis results: *Staphylococcus aureus* inoculation significantly increased NF-κB levels in lung tissue (p<0.001, Fig. 2a). Treatment with taxifolin significantly suppressed the increase in NF-κB levels induced by *S. aureus* inoculation in lung tissue (p<0.001). The difference between the NF-κB levels of the taxifolin-treated group and the healthy group was not significant (p = 0.065, Table 1 and 2).

Staphylococcus aureus inoculation also significantly increased TNF-α levels in lung tissue (p = 0.004). Taxifolin significantly suppressed the *S. aureus*-induced increase in TNF-α levels in lung tissue (p = 0.028). The difference between the TNF-α levels of the taxifolin-treated group and the healthy group was not significant (p = 1.000, Table 1 and 2, Fig. 2b).

Figure 2c shows that *S. aureus* inoculation significantly increased IL-1β levels in lung tissue (p<0.001). Taxifolin significantly inhibited the *S. aureus*-induced increase in IL-1β levels in lung tissue (p<0.001). The difference between the IL-1β levels of the taxifolin-treated group and the healthy group was nonsignificant (p = 0.476, Table 1 and 2).

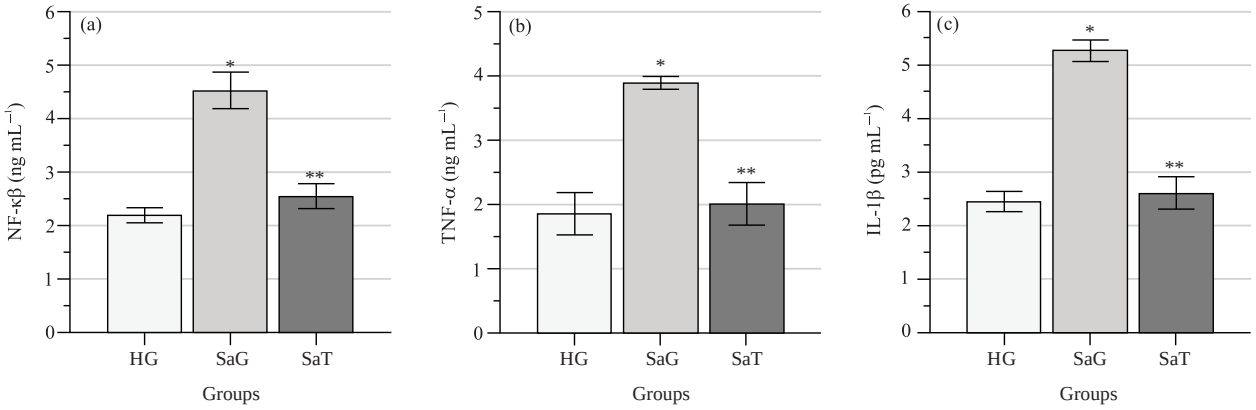

Fig. 2(a-c): Levels in the lung tissue of experimental groups (a) NF-κB, (b) TNF-α and (c) IL-1β,
Bars are mean±SD (standard deviation). *means p<0.05 when SaG group was compared with the HG control group.**means p<0.05 when the SaT group was compared with the SaG group. For each group n = 6. NF-κB: Nuclear Factor kappa B, TNF-α: Tumor Necrosis Factor-alpha, IL-1β: Interleukin one beta, HG: Healthy control group, SaG: *Staphylococcus aureus* inoculated group and SaT: *Staphylococcus aureus* inoculated+taxifolin treated group

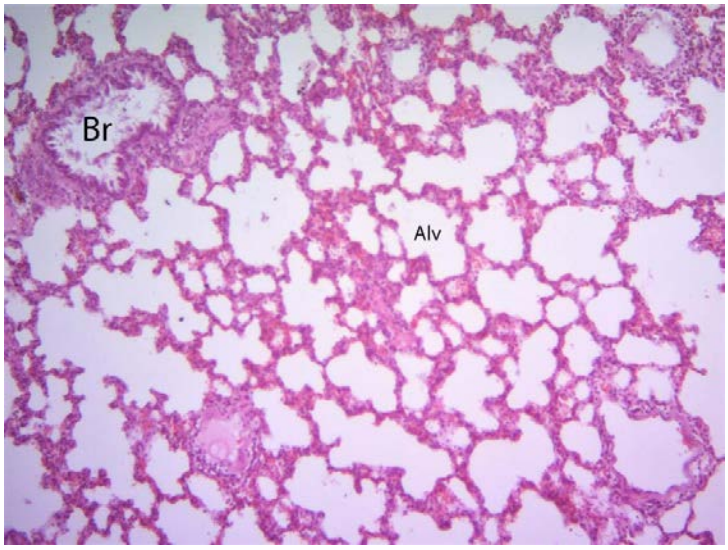

Fig. 3: HG control group, normal histologic appearance, Br: Bronchioles and Alv: Alveoli

Table 3: Histopathological comparison of the experimental groups in terms of MNC infiltration in interstitial areas, hyperplasia in BALT and desquamation in bronchial epithelium

Group	MNC infiltration in interstitial areas	Hyperplasia in BALT	Desquamation in bronchial epithelium
HG (mean±SD)	0.16±0.51[a]	0.16± 0.51[a]	0.33±0.40[a]
SaG (mean±SD)	2.66±0.51[b]	2.66± 0.51[b]	2.83±0.40[b]
SaT (mean±SD)	2.16±0.40[c]	1.16± 0.40[c]	2.16±0.40[c]

There is a significant difference between groups including different letters in the same column (significance level was considered as p<0.05), MNC: Mononuclear cell, BALT: Bronchial-associated lymphoid tissue, HG: Healthy control group, SaG: *Staphylococcus aureus* inoculated group, SaT: *Staphylococcus aureus* inoculated+taxifolin treated group

Histopathological results: A significant intergroup difference was observed in terms of the histopathological results (p<0.05, Table 3). Lung tissues in the healthy group had normal histological appearance (Fig. 3). The *S. aureus* inoculated group showed severe mononuclear cell infiltration (MNC) in interstitial areas, severe lymphoid hyperplasia in bronchial-associated lymphoid tissue (BALT) and severe desquamation in bronchial epithelium (Fig. 4a-c). These histopathological findings were attenuated in the taxifolin-treated group (Fig. 4d-f).

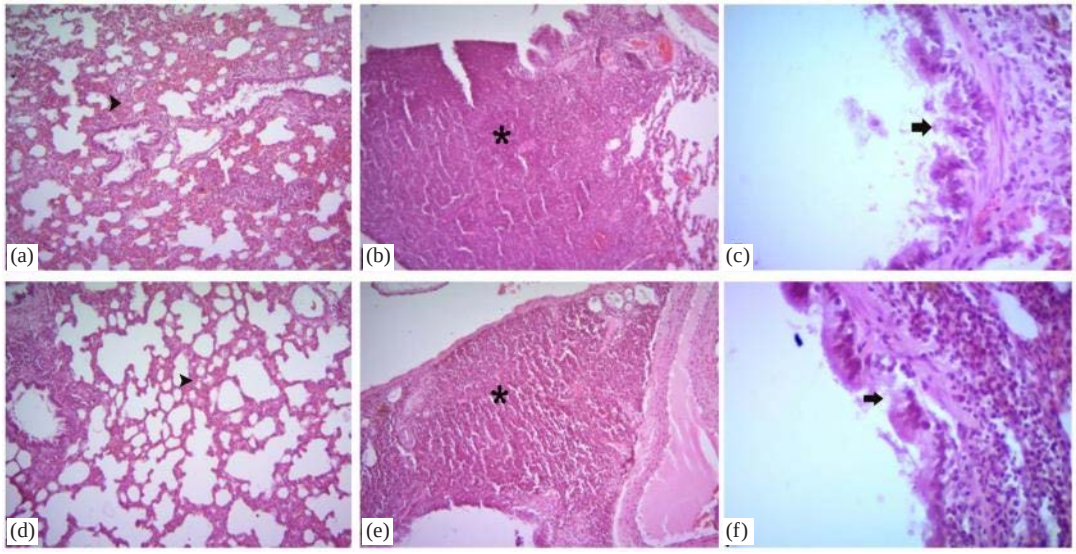

Fig. 4(a-f): SaG group, (a) Severe mononuclear cell (MNC) infiltration in interstitial areas (arrowhead), (b) Severe hyperplasia in bronchial-associated lymphoid tissue (BALT) (*), (c) Severe desquamation in bronchial epithelium (arrow), SaT group, (d) Mild MNC infiltration in interstitial areas (arrowhead), (e) Mild hyperplasia in BALT (*) and (f) Mild desquamation in bronchial epithelium (arrow)

DISCUSSION

The present study examined the protective effect of taxifolin against oxidative and inflammatory lung injury induced by intranasal inoculation of *S. aureus* in rats by biochemical and histopathological methods. As, *S. aureus* is one of the leading reasons of morbidity and mortality around the world[22]. The results of the current biochemical experiments showed that intranasally inoculated *S. aureus* caused an increase in oxidant MDA levels and a decrease in antioxidant tGSH levels in lung tissue. In a recent study, intranasal *S. aureus* inoculation caused an increase in oxidant MDA levels and a decrease in antioxidant levels in Kunming mice[23]. Dilber *et al.*[24] reported that intranasal inoculation of *S. aureus* in albino Wistar rats increased MDA levels in the sinonasal tissue. Indeed, MDA is one of the end products of the LPO reaction[25]. In addition, MDA-induced damage can significantly affect the structure and functions of the membrane[26]. *Staphylococcus aureus* reportedly has the ability to induce ROS production[27]. Experimental results of the current study and the literature evidence revealed that MDA is an important factor in the pathogenesis of oxidative damage caused by *S. aureus* in lung tissue.

The antioxidant tGSH was another parameter used in the present study to determine oxidative damage in tissues. The tGSH levels appeared to decrease in the lung tissue of the *Staphylococcus aureus* inoculated group. The GSH is a tripeptide chemically composed of glutaminic acid, cysteine and glycine[28]. The GSH is the most important reducing agent responsible for scavenging overproduced ROS to maintain cellular redox balance[29]. Decreased GSH levels further increase ROS production[29]. Therefore, any factor that causes GSH depletion can lead to ROS/GSH imbalance[29]. This imbalance in the ROS/GSH ratio results in oxidative stress and cell death[29]. In parallel with the results of the present study, Wang *et al.*[30] also reported that *S. aureus* increased oxidant parameters and decreased GSH levels in lung tissue. These findings indicated that *S. aureus* alters the oxidant/antioxidant balance in lung tissue in favor of oxidants. In the literature, oxidative stress is defined as the disruption of cell redox balance[31].

In the present study, the lung tissues of animals inoculated with *S. aureus* showed high levels of proinflammatory cytokines including NF-κB, TNF-α and IL-1β. These findings were in agreement with the data showing the role of the increase in cytokines such as NF-κB, TNF-α and IL-1β in the pathogenesis of inflammatory lung injury induced by *S. aureus*[23-32]. The transcription factor NF-κB was recognized as a key regulator of inducible gene expression in the immune system[33]. The NF-κB is a dimeric transcription factor involved in inflammation, host immune response and numerous physiological and pathological events[34]. Oxidative stress activates the transcription factor NF-κB, which regulates the expression of proinflammatory cytokines[35]. The ROSs was reported to induce NF-κB activation[36]. As previously reported,

NF-κB increases the production of proinflammatory cytokines through proinflammatory gene induction during inflammation onset. The TNF-α, one of the most important proinflammatory cytokines, causes a wide range of biological effects including vasodilatation and edema formation, adhesion of leukocytes to epithelium, inflammatory response, oxidative stress[37] and cell death[38]. The TNF-α reportedly induced oxidative stress and caused cell dysfunction[39]. The IL-1β, another proinflammatory cytokine, is recognized as the main mediator of the inflammatory response[40]. The IL-1β is essential for host response and resistance to pathogens, however, it exacerbates damage during chronic disease and acute tissue injury[40]. The IL-1β has potent proinflammatory activity, inducing various proinflammatory mediators including cytokines and chemokines[41]. Liu *et al.*[42] observed that IL-1β significantly increased intracellular ROS levels. Literature evidence suggests that *S. aureus* causes oxidative and inflammatory damage in lung tissue, furthermore, the evidence points to a link between oxidative stress and inflammation.

The biochemical results obtained in the present study were consistent with the histopathological findings. The lung tissues of the group inoculated with *S. aureus* showed high levels of MDA, NF-κB, TNF-α and IL-1β, low levels of tGSH, severe MNC infiltration in interstitial areas, severe lymphoid hyperplasia in BALT and severe desquamation in bronchial epithelium. These histopathological findings indicated the presence of inflammation in the lungs. Previous studies also showed that intranasal inoculation of *S. aureus* induced acute lung inflammation[43]. Similarly, inflammatory cell infiltration and edema were observed in studies conducted by Wu *et al.*[44]. During pulmonary infection, neutrophils are the first immune cells recruited to the site of injury, which has been reported to lead to alveolar basement membrane breakdown, edema formation and proinflammatory cytokine production, which eventually leads to lung injury[44]. Although neutrophil activation is vital for host defense, over activation leads to tissue damage through the release of cytotoxic and immune cell activating agents including proteinases, cationic polypeptides, cytokines and ROS[45].

In the present study, taxifolin significantly inhibited the increase in MDA, NF-κB, TNF-α and IL-1β and the decrease in tGSH caused by *S. aureus* inoculation in the lung tissues of animals. Taxifolin, which significantly inhibited the increase in oxidant and proinflammatory cytokines and the decrease in antioxidant tGSH, also alleviated the abovementioned histopathological damage. Indeed, taxifolin is effective against many pathogenic microorganisms including *Staphylococcus epidermidis*, *Streptococcus sobrinus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Micrococcus luteus* and *Mycobacterium tuberculosis* as well as *S. aureus*[16]. To the best of our knowledge, no study has investigated the antioxidant effect of taxifolin against *S. aureus*-induced oxidative lung injury. However, taxifolin was shown to prevent oxidative neuronal damage by inhibiting LPO and scavenging ROS in rat cortical cell cultures[46]. In another study, Teselkin *et al.*[47] demonstrated the antioxidant activity of taxifolin in albino Wistar rats *in vivo*. Taxifolin was reported to show broad antioxidant effects, including reducing the formation of ROS, ROS scavenging and metal binding activities[48]. A correlation exists between oxidant and antioxidant parameters and an increase in proinflammatory cytokines in lung injury induced by *S. aureus* inoculation of rats[23]. Reportedly, taxifolin had an anti-inflammatory effect by inhibiting leukocyte infiltration, COX-2 and iNOS expression and NF-κB activity[49]. Zhang *et al.*[50] reported that taxifolin suppressed the NF-κB signaling pathway in macrophages obtained from bone marrow. Additionally, Cai *et al.*[51] found that taxifolin had anti-inflammatory effects in mice by decreasing proinflammatory cytokine levels such as TNF-α, IL-1β and IL-6 in serum.

Experimental results obtained in the present study suggest that taxifolin may be beneficial in the treatment of oxidative and inflammatory lung injury associated with *S. aureus* infection. Further in-depth studies are needed to clarify the mechanism of the pulmonary protective effect of taxifolin against *Staphylococcus aureus* infection.

CONCLUSION

Intranasal inoculation of *S. aureus* induced oxidative and inflammatory damage in rat lung tissue by stimulating oxidant and proinflammatory cytokine production and depleting antioxidant stores. Oxidative and inflammatory lung injury associated with *S. aureus* was demonstrated both biochemically and histopathologically. Taxifolin significantly reduced the lung injury caused by *S. aureus* by preventing the increase in oxidant MDA and proinflammatory NF-κB, TNF-α and IL-1β levels and the decrease in antioxidant tGSH levels.

SIGNIFICANCE STATEMENT

Staphylococcus aureus is known to lead to pneumonia and as a consequence, acute lung injury. The present study discussed whether taxifolin may have a protective effect against acute lung oxidative and inflammatory damage due to *S. aureus* infection in rats. Experimental results of the present study revealed that intranasal inoculation of *S. aureus* induced oxidative and inflammatory damage in rat lung tissue by stimulating oxidant and proinflammatory cytokine production and depleting antioxidant stores. Taxifolin significantly

reduced the lung injury caused by *S. aureus* by preventing the increase in oxidants and proinflammatory cytokines and the decrease in antioxidants. In this respect, taxifolin may be beneficial in the treatment of oxidative and inflammatory lung injury associated with *S. aureus* infection.

ACKNOWLEDGMENT

The authors thank Enago (https://www.enago.com.tr/ceviri/) for their assistance in manuscript translation and editing.

REFERENCES

1. Clark, S.B. and M.A. Hicks, 2023. Staphylococcal Pneumonia. StatPearls Publishing, Treasure Island.
2. Esposito, S., F. Blasi, N. Curtis, S. Kaplan and T. Lazzarotto *et al*., 2023. New antibiotics for *Staphylococcus aureus* infection: An update from the world association of infectious diseases and immunological disorders (WAidid) and the Italian society of anti-infective therapy (SITA). Antibiotics, Vol. 12. 10.3390/antibiotics12040742.
3. Lister, J.L. and A.R. Horswill, 2014. *Staphylococcus aureus* biofilms: Recent developments in biofilm dispersal. Front. Cell. Infec. Microbiol., Vol. 4. 10.3389/fcimb.2014.00178.
4. Guo, Y., G. Song, M. Sun, J. Wang and Y. Wang, 2020. Prevalence and therapies of antibiotic-resistance in *Staphylococcus aureus*. Front. Cell. Infect. Microbiol., Vol. 10. 10.3389/fcimb.2020.00107.
5. Schreiber, M.P., C.M. Chan and A.F. Shorr, 2011. Bacteremia in *Staphylococcus aureus* pneumonia: Outcomes and epidemiology. J. Crit. Care, 26: 395-401.
6. Long, M.E., R.K. Mallampalli and J.C. Horowitz, 2022. Pathogenesis of pneumonia and acute lung injury. Clin. Sci., 136: 747-769.
7. Butt, Y., A. Kurdowska and T.C. Allen, 2016. Acute lung injury: A clinical and molecular review. Arch. Pathol. Lab. Med., 140: 345-350.
8. Mohamed, W., E. Domann, T. Chakraborty, G. Mannala and K.S. Lips *et al*., 2016. TLR9 mediates *S. aureus* killing inside osteoblasts via induction of oxidative stress. BMC Microbiol., Vol. 16. 10.1186/s12866-016-0855-8.
9. Pizzino, G., N. Irrera, M. Cucinotta, G. Pallio and F. Mannino *et al*., 2017. Oxidative stress: Harms and benefits for human health. Oxid. Med. Cell. Longevity, Vol. 2017. 10.1155/2017/8416763.
10. Koç, A., U. Kuyrukluyildiz, M. Gazi, A.C. Sayar and D. Altuner *et al*., 2023. The effects of sugammadex on gastric ischemia-reperfusion injury in rats: Biochemical and histopathological evaluation. Gen. Physiol. Biophys., and histopathological evaluation. Gen. Physiol. Biophys., 42: 67-75.
11. Jiang, K.F., G. Zhao, G.Z. Deng, H.C. Wu and N.N. Yin *et al*., 2017. Polydatin ameliorates *Staphylococcus aureus*-induced mastitis in mice via inhibiting TLR2-mediated activation of the p38 MAPK/NF-κB pathway. Acta Pharmacol. Sin., 38: 211-222.
12. Tsai, M.H., C.H. Wu, W.N. Lin, C.Y. Cheng and C.C. Chuang *et al*., 2018. Infection with *Staphylococcus aureus* elicits COX-2/PGE_2/IL-6/MMP-9-dependent aorta inflammation via the inhibition of intracellular ROS production. Biomed. Pharmacother., 107: 889-900.
13. Yang, P., F. Xu, H.F. Li, Y. Wang and F.C. Li *et al*., 2016. Detection of 191 taxifolin metabolites and their distribution in rats using HPLC-ESI-IT-TOF-MS^n. Molecules, Vol. 21. 10.3390/molecules21091209.
14. Liu, Y., X. Shi, Y. Tian, S. Zhai, Y. Liu, Z. Xiong and S. Chu, 2023. An insight into novel therapeutic potentials of taxifolin. Front. Pharmacol., Vol. 14. 10.3389/fphar. 2023.1173855.
15. Saito, S., M. Tanaka, N. Satoh-Asahara, R.O. Carare and M. Ihara, 2021. Taxifolin: A potential therapeutic agent for cerebral amyloid angiopathy. Front. Pharmacol., Vol. 12. 10.3389/fphar.2021.643357.
16. Das, A., R. Baidya, T. Chakraborty, A.K. Samanta and S. Roy, 2021. Pharmacological basis and new insights of taxifolin: A comprehensive review. Biomed. Pharmacother., Vol. 142. 10.1016/j.biopha.2021.112004.
17. Topal, F., M. Nar, H. Gocer, P. Kalin, U.M. Kocyigit, İ. Gülçin and S.H. Alwasel, 2016. Antioxidant activity of taxifolin: An activity-structure relationship. J. Enzyme Inhib. Med. Chem., 31: 674-683.
18. Shubina, V.S., V.I. Kozina and Y.V. Shatalin, 2021. Comparison of antioxidant properties of a conjugate of taxifolin with glyoxylic acid and selected flavonoids. Antioxidants, Vol. 10. 10.3390/antiox10081262.
19. Sunil, C. and B. Xu, 2019. An insight into the health-promoting effects of taxifolin (dihydroquercetin). Phytochemistry, Vol. 166. 10.1016/j.phytochem. 2019.112066.
20. Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.
21. Sedlak, J. and R.H. Lindsay, 1968. Estimation of total, protein-bound and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal. Biochem., 25: 192-205.
22. Cheung, G.Y.C., J.S. Bae and M. Otto, 2021. Pathogenicity and virulence of *Staphylococcus aureus*. Virulence, 12: 547-569.
23. Shaukat, A., C. Yang, Y. Yang, Y.F. Guo and K. Jiang *et al*., 2020. Ginsenoside Rb 1: A novel therapeutic agent in *Staphylococcus aureus*-induced acute lung injury with special reference to oxidative stress and apoptosis. Microb. Pathogen., Vol. 143. 10.1016/j.micpath.2020.104109.
24. Dilber, M., E. Erhan, S. Bulut, M. Kuzucu, S. Akyuz and H. Suleyman, 2022. Effect of *Usnea longisima* ethyl acetate extract on *Staphylococcus aureus*-induced experimental rhinosinusitis in rats. Lat. Am. J. Pharm., 41: 58-66.

25. Gawel, S., M. Wardas, E. Niedworok and P. Wardas, 2004. Malondialdehyde (MDA) as a lipid peroxidation marker. Wiad. Lek., 57: 453-455, (In Polish).
26. Süleyman, H. and A. Özçiçek, 2020. Molecular mechanism of ischemia reperfusion injury. Arch. Basic Clin. Res., 2: 25-27.
27. Clements, M.O. and S.J. Foster, 1999. Stress resistance in *Staphylococcus aureus*. Trends Microbiol., 7: 458-462.
28. Kwiecien, S., K. Jasnos, M. Magierowski, Z. Sliwowski and R. Pajdo *et al*., 2014. Lipid peroxidation, reactive oxygen species and antioxidative factors in the pathogenesis of gastric mucosal lesions and mechanism of protection against oxidative stress-induced gastric injury. J. Physiol. Pharmacol., 65: 613-622.
29. Liu, T., L. Sun, Y. Zhang, Y. Wang and J. Zheng, 2022. Imbalanced GSH/ROS and sequential cell death. J. Biochem. Mol. Toxicol., Vol. 36. 10.1002/jbt.22942.
30. Wang, F., F. Liu and H. Liu, 2016. Effect of exposure to *Staphylococcus aureus*, particulate matter, and their combination on the neurobehavioral function of mice. Environ. Toxicol. Pharmacol., 47: 175-181.
31. Pisoschi, A.M. and A. Pop, 2015. The role of antioxidants in the chemistry of oxidative stress: A review. Eur. J. Med. Chem., 97: 55-74.
32. Yao, L. and T. Sun, 2019. Glycyrrhizin administration ameliorates *Streptococcus aureus*-induced acute lung injury. Int. Immunopharmacol., 70: 504-511.
33. Mitchell, J.P. and R.J. Carmody, 2018. NF-κB and the Transcriptional Control of Inflammation. In: International Review of Cell and Molecular Biology, Loos, F. (Ed.), Academic Press, Cambridge, Massachusetts, ISBN: 9780128123393, pp: 41-84.
34. Alharbi, K.S., N.K. Fuloria, S. Fuloria, S.B. Rahman and W.H. Al-Malki *et al*., 2021. Nuclear factor-kappa B and its role in inflammatory lung disease. Chem. Biol. Interact., Vol. 345. 10.1016/j.cbi.2021.109568.
35. Kumari, N., B.S. Dwarakanath, A. Das and A.N. Bhatt, 2016. Role of interleukin-6 in cancer progression and therapeutic resistance. Tumor Biol., 37: 11553-11572.
36. Zhang, J., X. Wang, V. Vikash, Q. Ye, D. Wu, Y. Liu and W. Dong, 2016. ROS and ROS-mediated cellular signaling. Oxid. Med. Cell. Longevity, Vol. 2016. 10.1155/2016/ 4350965.
37. Zelová, H. and J. Hošek, 2013. TNF-α signalling and inflammation: Interactions between old acquaintances. Inflammation Res., 62: 641-651.
38. Brenner, C., L. Galluzzi, O. Kepp and G. Kroemer, 2013. Decoding cell death signals in liver inflammation. J. Hepatol., 59: 583-594.
39. Wang, S., B. Sarriá, R. Mateos, L. Goya and L. Bravo-Clemente, 2019. TNF-α-induced oxidative stress and endothelial dysfunction in EA.hy926 cells is prevented by mate and green coffee extracts, 5-caffeoylquinic acid and its microbial metabolite, dihydrocaffeic acid. Int. J. Food Sci. Nutr., 70: 267-284.
40. Lopez-Castejon, G. and D. Brough, 2011. Understanding the mechanism of IL-1β secretion. Cytokine Growth Factor Rev., 22: 189-195.
41. Wang, Y., M. Che, J. Xin, Z. Zheng, J. Li and S. Zhang, 2020. The role of IL-1β and TNF-α in intervertebral disc degeneration. Biomed. Pharmacother., Vol. 131. 10.1016/j.biopha. 2020.110660.
42. Liu, Q., L. Jin, F.H. Shen, G. Balian and X.J. Li, 2013. Fullerol nanoparticles suppress inflammatory response and adipogenesis of vertebral bone marrow stromal cells-A potential novel treatment for intervertebral disc degeneration. Spine J., 13: 1571-1580.
43. Kapetanovic, R., G. Jouvion, C. Fitting, M. Parlato and C. Blanchet *et al*., 2010. Contribution of NOD2 to lung inflammation during *Staphylococcus aureus*-induced pneumonia. Microb. Infect., 12: 759-767.
44. Wu, Y.X., F.J. Jiang, G. Liu, Y.Y. Wang and Z.Q. Gao *et al*., 2021. Dehydrocostus lactone attenuates methicillin-resistant *Staphylococcus aureus*-induced inflammation and acute lung injury via modulating macrophage polarization. Int. J. Mol. Sci., Vol. 22. 10.3390/ijms22189754.
45. Grommes, J. and O. Soehnlein, 2011. Contribution of neutrophils to acute lung injury. Mol. Med., 17: 293-307.
46. Dok-Go, H., K.H. Lee, H.J. Kim, E.H. Lee and J. Lee *et al*., 2003. Neuroprotective effects of antioxidative flavonoids, quercetin, (+)-dihydroquercetin and quercetin 3-methyl ether, isolated from *Opuntia ficus-indica* var. *saboten*. Brain Res., 965: 130-136.
47. Teselkin, Y.O., I.V. Babenkova, V.K. Kolhir, A.I. Baginskaya and N.A. Tjukavkina *et al*., 2000. Dihydroquercetin as a means of antioxidative defence in rats with tetrachloromethane hepatitis. Phytother. Res., 14: 160-162.
48. Jain, S. and A. Vaidya, 2023. Comprehensive review on pharmacological effects and mechanism of actions of taxifolin: A bioactive flavonoid. Pharmacol. Res. Mod. Chin. Med., Vol. 7. 10.1016/j.prmcm.2023.100240.
49. Wang, Y.H., W.Y. Wang, C.C. Chang, K.T. Liou and Y.J. Sung *et al*., 2006. Taxifolin ameliorates cerebral ischemia-reperfusion injury in rats through its anti-oxidative effect and modulation of NF-kappa B activation. J. Biomed. Sci., 13: 127-141.
50. Zhang, H.Q., Y.J. Wang, G.T. Yang, Q.L. Gao and M.X. Tang, 2019. Taxifolin inhibits receptor activator of NF-κB ligand-induced osteoclastogenesis of human bone marrow-derived macrophages *in vitro* and prevents lipopolysaccharide-induced bone loss *in vivo*. Pharmacology, 103: 101-109.
51. Cai, C., C. Liu, L. Zhao, H. Liu, W. Li, H. Guan, L. Zhao and J. Xiao, 2018. Effects of taxifolin on osteoclastogenesis *in vitro* and *in vivo*. Front. Pharmacol., Vol. 9. 10.3389/fphar.2018.01286.