NARINGENIN AMELIORATES LIPOPOLYSACCHARIDE-INDUCED KIDNEY AND LUNG INJURIES IN MICE: DECISIVE ROLE OF IL-1β, IL-6, IL8, AND TNF-α

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Abstract. Cytokine storms are a major contributor to acute respiratory failure, causing numerous organ dysfunction, including kidney damage, as a result of inflammation-induced tubular injury, particularly evident in COVID-19 infections. This occurrence poses a global health risk. Experimental evidence suggests that naringenin, a natural flavonoid, has a variety of biological activities. The present study was conducted to examine the protective effects of naringenin in lipopolysaccharide (LPS)-induced lung and kidney injuries in mice and determine its relationship with the suppression of pro-inflammatory mediators. Forty-eight Swiss albino male mice were divided into four groups. Group I received 0.9% normal saline, Group II received 5 mg/kg LPS only, and Groups III and IV received 50 or 100 mg/kg naringenin, respectively, one hour before LPS administration. The effects of naringenin, vehicle, or LPS administration on mortality rate, pro-inflammatory cytokine (IL-6, IL-1, IL-8, and TNF-α) levels, and lung/renal histological changes were evaluated after 48 hours. The results showed that the naringenin-treated groups exhibited a significant ($p \leq 0.01$) reduction in pro-inflammatory cytokine levels compared to Group II. Histological examinations revealed that mice in Group II displayed significant ($p \leq 0.01$) lung and renal tissue injuries, while Groups III and IV exhibited a significant ($p \leq 0.01$) reduction in pulmonary and renal injuries, as demonstrated by improved macroscopic scores and reduced mortality. The findings of this study strongly suggest that naringenin has potent protective effects in mice against LPS-induced lung damage and associated kidney dysfunction. Consequently, naringenin has promise as a human anti-cytokine storm therapeutic agent.

Keywords: cytokine storm, inflammation, lipopolysaccharide, naringenin, lung injury, kidney injury.

List of Abbreviations

ALI – Acute lung injury
ARDS – Acute respiratory distress syndrome
AKI – Acute kidney injury
ANOVA – Analysis of variance
b.w – Body weight
CCl4 – Carbon tetrachloride
COPD – Chronic obstructive pulmonary disease
COX-2 – Cyclooxygenase2
COVID – Coronavirus disease of 2019
CRS – Cytokine release syndrome
CSS – Cytokine Storm Syndrome
DDW – Double distilled water
DMF – Dimethyl formamide
DMSO – Dimethyl sulfoxide
ELISA – Enzyme-linked Immunosorbent assay
ESRD – End-stage renal disease
H&E – Hematoxylin and eosin
IL-1β – Interleukin-1 beta
IL-6 – Interleukin-6
IL-8 – Interleukin-8
TNFα – Tumor necrosis factor α
iNOS – Inducible nitric oxide synthase
i.p – Intraperitoneal
LSD – Least significant difference test
LPS – Lipopolysaccharide
LOX – Lipo-oxygenase
MAPKs – Mitogen-activated protein kinase
NF-κB – Nuclear factor kappa B
pg/mL – Picograms per milliliter
μm – Micrometer
nm – Nanometer
N/S – Normal saline
OD – Optical density
ROS – Reactive oxygen species
SEM – Standard error of the mean
SIRS – Systemic inflammatory response syndrome
TLR4 – Toll-like receptor 4

Introduction

Cytokine storm syndrome (CSS), also referred to as cytokine release syndrome (CRS)

and/or hyper-cytokine syndrome, is a disorder associated with unregulated increased, circulating pro-inflammatory cytokine biomarkers that cause systemic-inflammatory clinical symptoms and severe secondary organ damage (Jarczak & Nierhaus, 2022). It has resurfaced as a result of its association with the coronavirus of the 2020 global pandemic. Such abundant circulating pro-inflammatory cytokine/chemokine is associated with a variety of infectious and immune-mediated conditions rather than the viral load (Mangalmurti & Hunter, 2020; Montazersaheb *et al*., 2022). Lung injury is one of the significant cytokine-release storms that could progress to serious and potentially life-threatening events. Acute lung injury (ALI) or its severe manifestation, acute respiratory distress syndrome (ARDS) is characterized by infiltration of pulmonary cells, hypoxemia, and edema (Goodman *et al*., 2003). On the other hand, kidney damage may cause problems with distant organs such as the lungs. However, ALI/ARDS massive alveolar epithelial damage could trigger systemic inflammation involving renal tissue damage and progress to acute kidney injury (AKI) accompanying higher morbidity and mortality rate by indirect mechanism (Salsabila *et al*., 2022). The major origins of AKI (such as heart failure, surgical intervention, sepsis, and hypovolemia) are linked with hypo-perfusion, shock, and ischemic damage that could cause extensive cell injury (ex. severe tubular-necrosis; Pabla *et al*., 2022). Although hypovolemic circulation may be the most common cause in patients, some robust proofs indicating a cytokine storm due to immune system exaggeration being the main causative factor have proven that the kidneys are also one of the key sites of disease incidence. This could lead to end-stage renal disease (ESRD) and is associated with in-hospital death (Ebrahimi-Dehkordi *et al*., 2021). Consequently, it gained serious clinical attention. Thus, non-classical pathways such as the development of autoantibodies that target both the kidneys and the lungs, excessive cytokine release, and stimulation of systemic inflammation may also confirm kidney-lung interaction (Fajgenbaum & June, 2020).

Lipopolysaccharide (LPS), an essential constituent of the Gram-negative bacteria outer membrane, and still the additional key source of systemic inflammatory response syndrome (SIRS), has been extensively utilized for establishing SIRS-related conditions mimicking the cytokine storm model in rodents as well as for exploration of drug development (Rittirsch *et al.,* 2008; Takahashi *et al*., 2012; Plotnikov *et al*., 2018). Mechanically, LPS acts as a prototypical endotoxin by binding to the CD14/TLR4/MD2-receptor complex in various immune cell types, specifically in macrophages, B cells, dendritic, and monocytes, which stimulates the various pro-inflammatory cytokine secretion, as well as eicosanoids and nitric oxide. As a portion of the cellular stress response, superoxide is reflected as one of the crucial reactive oxygen species (ROS) brought by LPS on various immune cell types that express the toll-like receptor (TLRs) (Farhana & Khan, 2023). Lipopolysaccharide upon its TLR4-recognition, is up-regulated on bronchial epithelial cells and lung macrophages during LPS-inducing ALI. Similarly, it appears to play an important inflammatory role in AKI and is broadly expressed in leukocytes and renal epithelial cells, accordingly. It is therefore regarded as a key regulator of innate and adaptive immune- responses (Saito *et al*., 2005; Zhang *et al*., 2008). The primary effectors of LPS-induced systemic inflammation are pro-inflammatory cytokines, interleukin (IL)-6, tumor necrosis factor (TNF)-α, and IL1β that appear to be associated with lung/kidney injury in a cytokine storm. However, excessive neutrophil infiltration caused by such cytokine/chemokine is responsible for alveolar-capillary barrier injury (Ware & Matthay, 2000), resulting in the non-cardiogenic pulmonary edema as well as oxidative stress modulated by reactive oxygen species (ROS), which is involved in the pathogenesis of ALI (Ward,2010). Furthermore, they could cause renal endothelial cells to secrete chemokines such as IL-8, which increase vascular permeability and cause vascular leakage, resulting in decreased renal blood flow and glomerular filtration rate and numerous organ damage and death (Li *et al.,* 2020; Ahmadian *et*

al., 2021). There is no specific vaccine or medicine for a patient with acute lung damage or kidney failure owing to the cytokine storm. Therefore, additional clinical trials are required to develop new pharmacologic derivatives with diverse biological activities.

Naringenin (4',5,7-trihydroxyflavonone) is one of the main natural flavonoids belonging to the flavone subclass, contained in citrus fruit, grapefruits, and tomatoes, the most widespread and broadly consumed vegetable crops worldwide. It is produced from phenylalanine, an aromatic amino acid. Naringenin also exists in many conjugated forms, principally as neohesperidoside, aglycone, and glycosylated form (Shakeel *et al*., 2017) and it has been shown to have a variety of pharmacological and biological effects, including anti-proliferative, anti-oxidative, anti-tumor, and anti-inflammatory properties (Shizuka *et al*., 2007; Li., 2015). Thus, it is considered a promising source for drug development owing to its prospective large safety margin as being derived from natural dietary sources.

The current study aimed to investigate the protective effects of naringenin at various concentrations on LPS-induced experimental renal and pulmonary damage in mice models. Correlation with the inhibition of pro-inflammatory mediators for developing a novel generation of anti-cytokine storm remedies was also investigated.

Materials and Methods

Source of animals

Forty-eight Swiss adult albino male mice (25 ± 5 grams; age: 8-10 weeks) were used in the current investigation. The animals were obtained from the Animal House Facility, National Center for Drug Quality Control and Research in Baghdad, Iraq. The animals were kept in the animal house of the Biotechnology Research Center, Al Nahrain University; Baghdad, Iraq in standard plastic cages at 22 °C ± 2 °C, relative humidity, and the air of the room was changed continuously by using a ventilating vacuum under a 12 hours light/dark cycle. All the animals were allowed free access to food and tap water (*ad libitum*). The mice were allowed two weeks to acclimate to the animal house environment before the commencement of the experiment.

Ethical approval

All animal care and experiments were carried out in strict accordance with the recommendations of the Animal Ethical Committee at Al-Nahrain University, College of Pharmacy, under issue number PH-Nah 4. The experimental protocol was approved by the Institutional Review Board (IRB) of the College of Medicine, Al-Nahrain University, Baghdad, Iraq.

Preparation of naringenin working solution

Naringenin (Hangzhou Hyperchem Limited, China, CAS No.480-41-1, HPLC ≥ 98.00% purity) working solution (50 and 100 mg/kg) was prepared by dissolving freshly prepared dimethyl formamide (DMF; BDH Chemical Ltd, Poole, England) solvent, then diluted with sterile double distilled water (DDW) and left for 1 hour. Before LPS treatment, a single dose (i.p [intraperitoneally]) was used to determine a dose that demonstrated effective survival in mice after 48 hours.

Induction of lung and kidney injury in a mouse model

A working solution of LPS was first prepared. The LPS stock solution (Hangzhou Hyperchem. Limited, China; B.No.: 0000114326) was prepared by dissolving lyophilized powder of LPS in 0.9% normal saline (Pioneer, Iraq) and was reconstituted to the concentration of 1 mg/mL in a glass tube. It was then vortexed for thirty minutes before each usage. For a 5 mg/kg, i.p) dose, approximately 125-140 µl of the solution was injected intraperitoneally into each mouse based on body weight. To accurately calculate the dosage of LPS, healthy animals were weighed before each injection (Fig. 1).

Monitoring of experimental mice

Mice were observed every 2 hours post LPS injection up to 48 hours for the occurrence and severity of endotoxemia. The principal investigator of the current study and a veterinarian

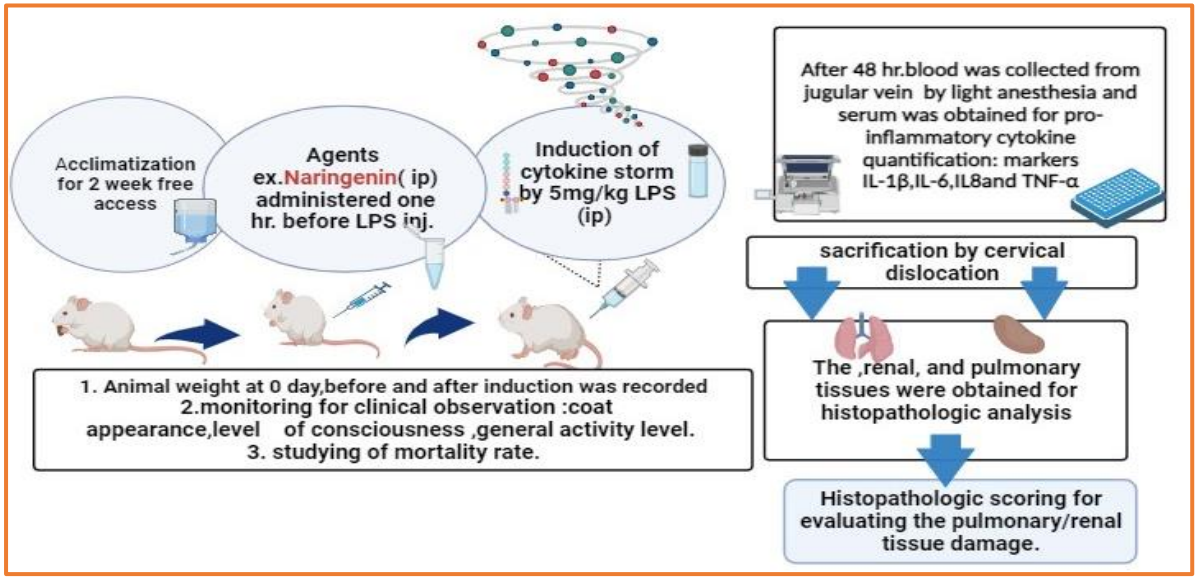

Fig. 1. Overview of the experimental protocol for cytokine storm induction in animal models (Adapted from BioRender.com)

from the Animal Care and Veterinary Services (Biotechnology Research Center, Al-Nahrain University, Baghdad, Iraq) jointly evaluated the animals utilizing variables that have been designated in the previous literature and as follows:

1. Checking the appearance of the fur, which is usually smooth, ruffled, spiky, or puffy fur, and shows, discomfort and/or pain.

2. Checking the activity of the mouse: Mice were generally easily moved across the cage, eating, drinking, and ascending, but restricted activity could be a symptom of pain.

3. Checking the consciousness level: Mice were very snoopy, and they normally moved around the cage inspecting the surroundings. Lack of or reduced movement indicated pain and discomfort.

4. Checking the eye condition: Fully opened eyes were anticipated in apparently healthy mice, but partly or fully closed eyes, probably with secretion, could be an indication of pain.

5. Checking the respiratory quality: Difficulty in breathing with/without gasping is a sign of pain.

6. Checking the respiratory rate: Healthy mice frequently have a regular and rapid respiratory rate, while reduced respiration might be a sign of distress.

Grouping of experimental mice

The forty-eight mice were assigned at random (to avoid experimental bias) into four subgroups (n =12) after being left for two weeks for acclimatization. Group I (control group) contained twelve mice that received 0.9% normal saline vehicle i.p. In Group II, twelve mice received 0.9% normal saline vehicle and LPS lipopolysaccharide (5 mg\kg, i.p, b.w) only (Ramírez *et al*., 2019). Group III was made up of twelve mice that received 50 mg/kg, i.p, b.w of naringenin, then within one hour, 5 mg\kg, i.p, b.w of LPS were administered through injection. In Group IV, twelve mice received 100 mg/kg, i.p, b.w of naringenin, then within one hour, 5 mg\kg, i.p, b.w of LPS were injected.

Sample collection

Forty-eight hours after the LPS administration, all mice were anesthetized with diethyl ether (BDH Chemical Ltd, Poole, England) by light anesthesia (Kaela *et al*., 2021), before being sacrificed by cervical dislocation. Then blood, lung, and renal tissue samples were collected.

Collection and preparation of blood samples

Blood samples were obtained by using the direct cardiac puncture technique (Parasuraman

et al., 2010) from each mouse before death, in which about 0.5-1.5 ml of blood was collected. To collect serum, extracted blood was placed in a gel tube for about 20 minutes until clotting occurred, then centrifuged for about 10 minutes at 10000 rpm. The collected serum was centrifuged at 3000 × g/sec for 1 minute to remove red blood cells (RBCs). The obtained samples with significant hemolysis were discarded. The supernatant was stored at -20 °C for further analysis.

Measurement of inflammatory markers

Following the national security protocols of biological laboratories, the levels of inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and interleukin-8 (IL-8) levels in the collected serum of mice were determined after the experiment. The measurement was conducted using the sandwich-enzyme-linked immunosorbent assay (ELISA) approach with a commercial kit (BioSource, USA) following the manufacturer's instructions. At 450 nm, the absorbance was measured using a microplate reader (Bio-Tek Instruments, Inc., USA). The sample concentrations were calculated with a standard curve and the results were expressed as pg/ml. The experiments were replicated three times.

Histopathological analysis

Forty-eight hours after LPS administration, and immediately following the scarification procedure, histological examinations on mouse-extracted lung and kidney slices were conducted. Five animals from each group were randomly chosen for histopathologic evaluation of their right organ at various times. The 10% buffered formalin (Fluka Co., Switzerland) was used for fixing the lung and kidney tissues, and then dehydrated with alcohol. After overnight fixation, paraffin was embedded, cleared, and infiltrated. As a result, the tissue samples were placed in molds and waxed until they were hardened. Temporarily, tissue sections were partitioned into 4-5 μm thick and the sectioning was performed with the aid of a microtome (Sakura, Japan). Hematoxylin and eosin (H&E; Thermo Shandon, USA) stain was used to examine morphology and inflammatory infiltration under a light microscope (Carl Zeiss Microscope), as previously described. A Zeiss imager M2 microscope (Carl Zeiss Micro-Imaging) fitted with an Axio-CamHRc CCD camera was applied to observe histopathological changes. The slides for lung and renal sections were coded, randomized, and evaluated by professional pathologists who were not part of the study group. The grading was done as follows:

A. The grade of lung tissue injury that was assessed based on the following histological changes: edema, inflammatory cell infiltration, interstitial inflammation, alveolar wall damage, and congestion.

B. The grade of kidney tissue alterations and severity of tubulointerstitial injuries was recorded as renal sections were arbitrarily alienated into 3 sections: the cortex, inner, and outer medulla. In each zone, the level of tubular cast formation, tubular dilatation, and degeneration (vascular changes, injury of brush border, tubular epithelial cells detachment, and condensations of tubular nuclei) was evaluated semiquantitatively.

Assessment of macroscopic tissue scores

Histologic sections for each animal were assigned a semiquantitative average score value as the histological index of lung injury was recorded (Matute-Bello *et al*., 2001). For renal tissue, the injury was scored according to Nomura *et al*. (1995) criteria with some modifications (Table 1). The resulting scores were summed to indicate the rank of organ injury. Finally, statistical analysis and graphs were plotted according to the study scoring outcomes.

Statistical analysis

The data were expressed as the quantitative mean ± standard deviation (SD). Statistical analysis was performed using the statistical analysis system (SAS, 2018) to detect the effect of the different factors in the study parameters. The analysis of variance (ANOVA) and least significant difference (LSD) tests were utilized to compare between means. A Chi-square test was employed to compare the levels of significance (0.05 and 0.01 probability) between the percentages in the current study.

Table 1

Lung and tubular damage scoring system

Tissue findings	Score
0% = no damage	Grade 0
0-30% = mild damage	Grade 1
30% - 70% = moderate damage	Grade 2
70-100% = widespread/severe damage	Grade 3

Table 2

Clinical observations

Clinical criteria	Description
1. General appearance of fur	Piloerection of hair; might or not exist, as the mouse seems "puffy".
2. Consciousness level	Activity was impaired. The mouse only moves when triggered. With possible tremors.
3. General activity	The mouse is inactive with occasional inspective movements.
4. Responses to stimulus	Sluggish or no responding to auditory stimulus; or tactile stimuli. (no locomotion)
5. Quality of respiration	Labored with recurrent gasps
6. Respiratory rate	Considerably reduced respiration (rate not assessable by eye)
7. Eye condition	Eyes were half closed or more, perhaps with secretions

Results

Survival analysis

All animals receiving (0.9% normal saline [N/S]) survived until they were killed. The mortality rate of the 5 mg\kg LPS only-injected mice (group II) was 66.67% (8/12 mice) in the untreated mice after 48 hours of LPS administration. The mortality rate was significantly ($p \leq 0.01$) reduced to 16.67% (2/12) with the 100 mg/kg naringenin treatment compared with untreated animals. The decrease in mortality rate was also achieved in mice administered with 50 mg/kg naringenin to 25% (3/12). However, there was no significant difference between naringenin-pretreated groups (III and IV) as shown in Fig. 2. Although the former had a better chance of survival.

In the naringenin (50 and 100 mg/kg) pretreated groups III and IV, respectively, before LPS injection, the death rate was suppressed significantly to 25% and 16.7%, respectively. All mice in group I (0.9% N/S) survived. The data representing the proportion of dead animals (numbers recorded above bars) ($p \leq 0.01$) compared to group I. A high significant difference ($p \leq 0.01$) was observed compared to group II.

Description of mice at the end of the experiments

According to the results presented in Table 2, all untreated mice in the LPS-induced group demonstrated reduced activity, were lethargic, and clustered together.

Effects of naringenin on inflammatory cytokine levels

Collected serum of Swiss albino male mice that received 5 mg/kg LPS only (group II) after 48 hours revealed significantly elevated IL-6, IL-1β, IL-8, and TNF-α serum levels, which were approximately 6-6.5-fold compared to those of group I, that received 0.9% N/S (Figs. 3-6). To find out the impact of different naringenin doses (50 and 100 mg/kg) on the inflammatory response that occurred 48 hours after LPS administration in mice model, the in flammatory cytokine serum levels including IL-6, IL-1β, IL-8, and TNF-α were measured

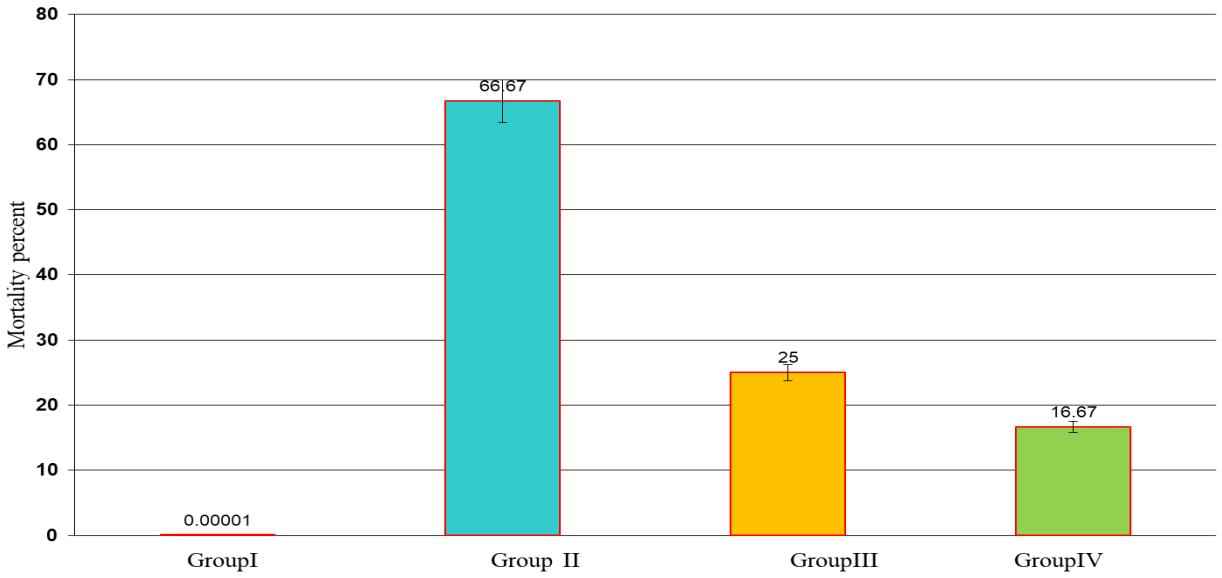

Fig. 2. Effect of lipopolysaccharide only (group II) on mortality rate, 48 hours after single dose (5 mg\kg) injection (n=12)

with ELISA technique. It is worth noting that pre- treated group with naringenin at both doses revealed that serum levels of TNF-α, IL-8, IL-6, and IL-1β were reduced significantly ($p \leq 0.01$) in groups III and IV, respectively, compared to group II. Naringenin-pretreated mice at a dose of 100 mg/kg (group IV) revealed no significant changes in the reduction of IL-8, IL-6, and IL-1β) serum levels, while highly significant ($p \leq 0.01$) reduction was observed in TNF-α serum level compared to group III (pretreated with naringenin at a dose of 50 mg/kg) and in a dose-dependent manner (Figs. 3-6).

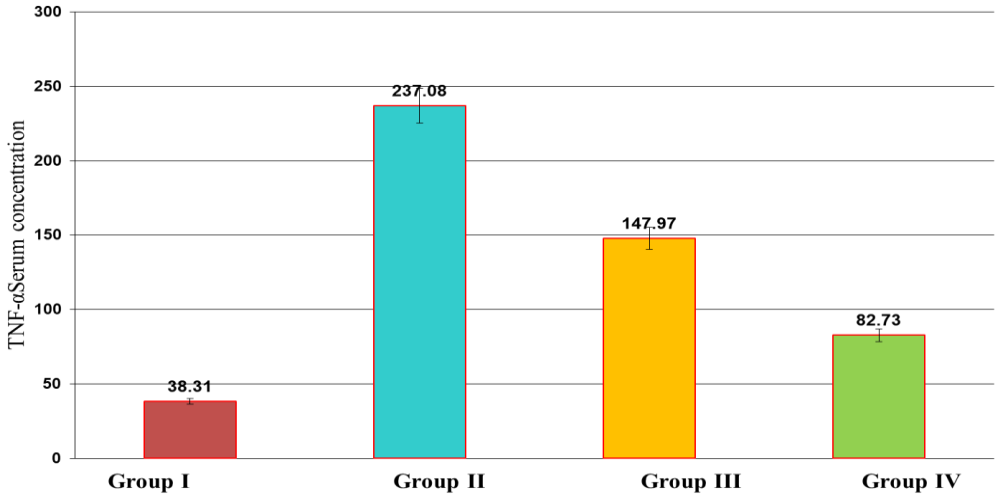

Fig. 3. Mean serum level of TNF-α (pg/mL) in different study groups (n = 12). Group I: healthy control (received 0.9% normal saline); Group II: injected with lipopolysaccharides (LPS) only; Group III: received 50 mg/kg naringenin one hour before being injected with LPS; Group IV: was given 100 mg/kg naringenin one hour before being injected with LPS; The data were expressed as mean ± standard deviation (SD); $P \leq 0.01$: highly significant difference compared with group I (healthy control) and also highly significant difference compared with group II (the LPS only treatment)

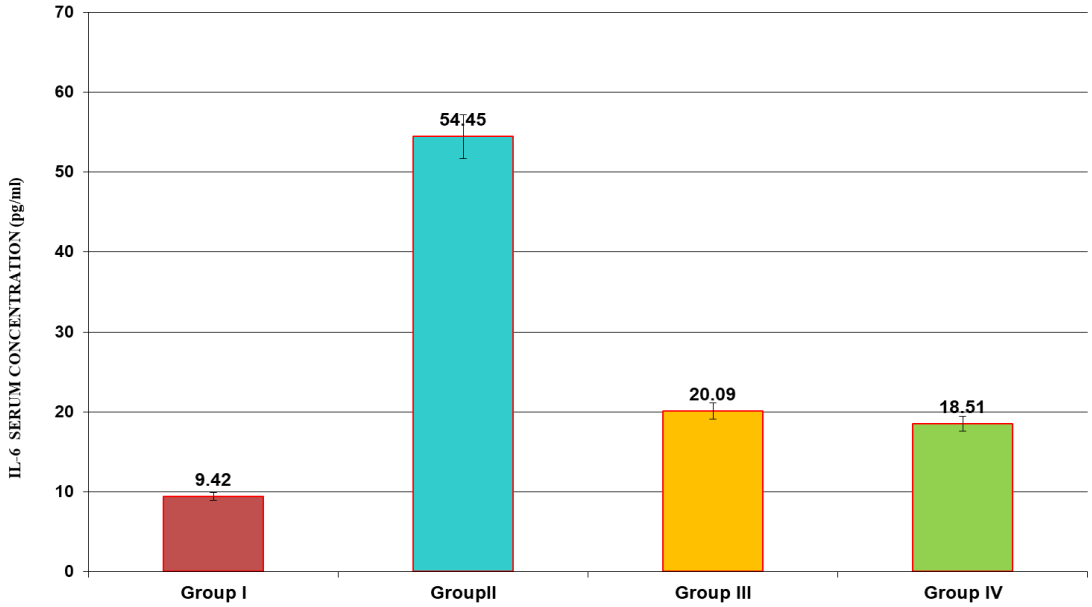

Fig. 4. Mean serum level of IL-6 (pg/mL) in different study groups (n =12). Group I: healthy control (received 0.9% normal saline); Group II: injected with lipopolysaccharides (LPS) only; Group III: received 50 mg/kg naringenin one hour before being injected with LPS; Group IV: was given 100 mg/kg naringenin one hour before being injected with LPS; The data were expressed as mean ± standard deviation (SD); $P \leq 0.01$: highly significant difference compared with group I (healthy control) and also highly significant difference compared with group II (the LPS only treatment)

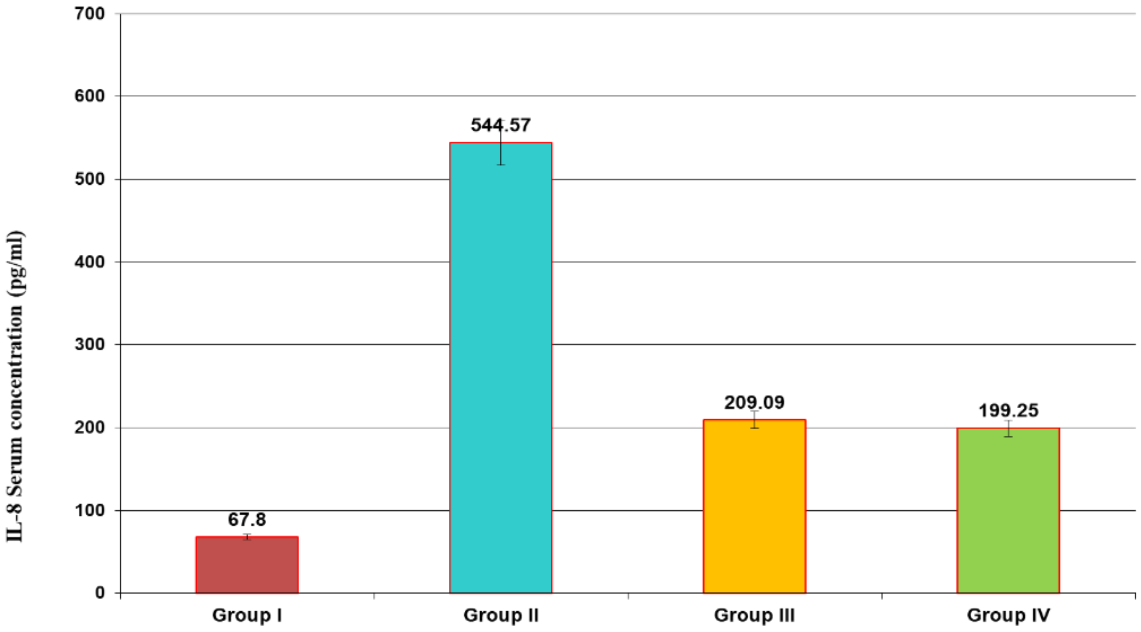

Fig. 5. Mean serum level of IL-8 (pg/mL) in different study groups (n = 12). Group I: healthy control (received 0.9% normal saline); Group II: injected with lipopolysaccharides (LPS) only; Group III: received 50 mg/kg naringenin one hour before being injected with LPS; Group IV: was given 100 mg/kg naringenin one hour before being injected with LPS; The data were expressed as mean ± standard deviation (SD); $P \leq 0.01$: highly significant difference compared with group I (healthy control) and also highly significant difference compared with group II (the LPS only treatment)

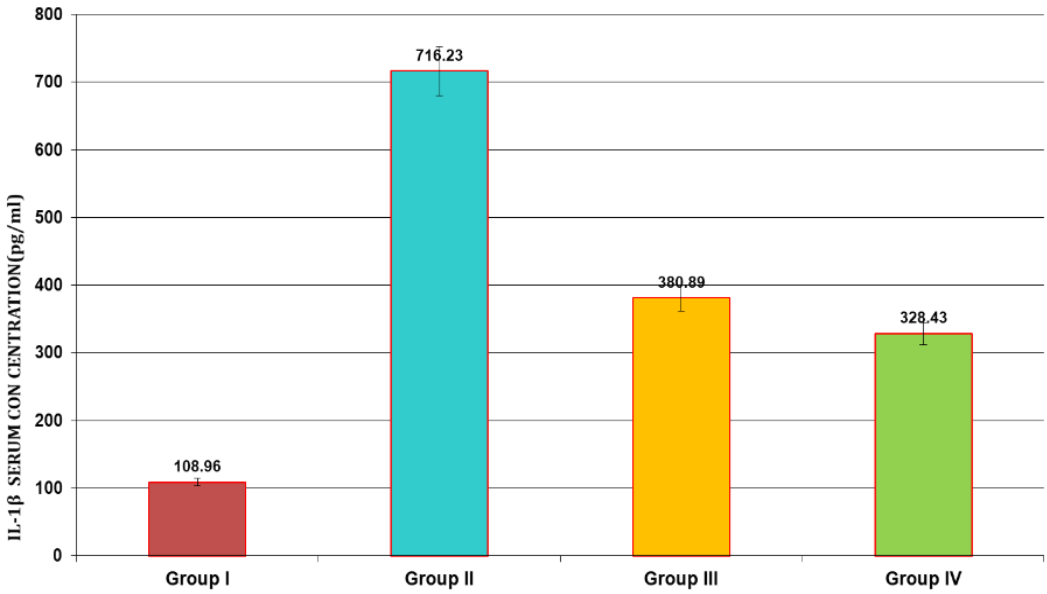

Fig. 6. Mean serum level of IL-1β (pg/mL) in different study groups (n = 12). Group I: healthy control (received 0.9% normal saline); Group II: injected with lipopolysaccharides (LPS) only; Group III: received 50 mg/kg naringenin one hour before being injected with LPS; Group IV: was given 100 mg/kg naringenin one hour before being injected with LPS; The data were expressed as mean ± standard deviation (SD); $P \leq 0.01$: highly significant difference compared with group I (healthy control) and also highly significant difference compared with group II (the LPS only treatment)

Histopathological observations of pulmonary tissues

The histological investigation on pulmonary tissue was directed to provide more evidence about the effect of naringenin on LPS-induced endotoxemia and mediated experimental lung injury. As presented in Fig. 7A, specimens from group I revealed a typical lung tissue histological appearance, and there were no pathological findings detected (grade 0). Intraperitoneal LPS injection at a dose of 5 mg/kg caused diffused damage, alveolar destruction, and marked vascular congestion, concomitant with massive infiltration of inflammatory cells and interstitial edema. Hence, the LPS receiving group II displayed a significantly ($p \leq 0.01$) greater histopathological score of 3 than the control group I (Fig. 7B). However, pre-treatment with naringenin (50 or 100 mg/kg) groups III and IV, respectively, ameliorated these histopathological observations. Moreover, naringenin at a dose of 100 mg/kg resulted in a significantly improved histological pattern and significantly lower microscopic scores (grade 1) in group IV compared to the half dose (50 mg/kg) as displayed in Figs. 7C and D).

Histopathological observations of renal tissues

As shown in Fig. 8A, the histopathological outcome of renal tissues stained with H&E revealed normal histology in the 0.9% N/S treated group I. Meanwhile, in the LPS-treated group II, the proximal convoluted tubules were predominantly influenced by dilation, congested vessels, extensive tubular epithelial desquamation, notable inflammatory cell infiltration, and intraluminal cast formation. In addition to tubular epithelial swelling and vacuolar degeneration (Fig. 8B), the renal score of tubular injury was correspondingly elevated, accordingly. The score of tissue injury reached 3, indicating about 70-100% of the renal tissue being affected. On the contrary, both naringenin doses (100 and 50 mg/kg), respectively, reduced the LPS-inducing renal tissue injury. It is worth noting that pre-treatment with naringenin at a dose of 100 mg/kg exhibited a more reno protective effect with a histological profile almost similar to group I compared to the lower dose (50 mg/kg) as displayed in Figs. 8C and D).

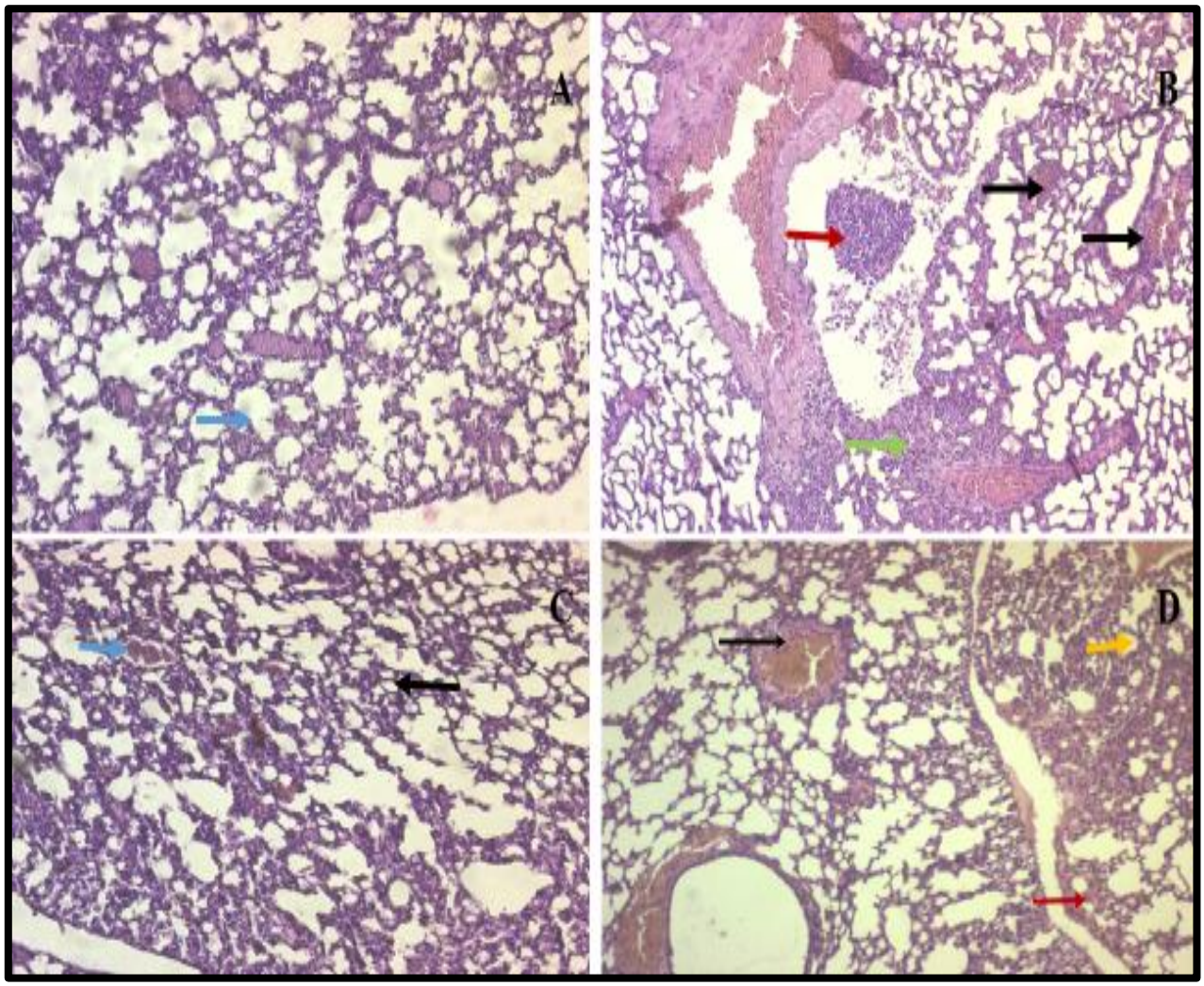

Fig. 7. Histopathological observations of lung tissues stained with hematoxylin and eosin. A. Group I (Control; 10x), which was treated with 0.9% N/S, showed normal histology of the lung consisting of alveoli bordered by normal alveolar septate (blue arrow) with no infiltration of inflammatory cells (no tissue damage; score 0); B. Group II (LPS-injected only; 20x) showing heavy infiltration of inflammatory cells (green arrow), obvious vascular congestion (black arrow), diffused alveolar damage and intra-alveolar suppurative inflammation rich in neutrophils (red arrow) (severe tissue damage; score 3); C. Group III (LPS-injected and pretreated with 50 mg/kg naringenin; 10x) showing some areas of reduced inflammatory cell infiltration and reduced inflammation (black arrow), reduced congestion (blue arrow; moderate tissue damage; score 2); D. Group IV (LPS-injected and pretreated with 100 mg/kg naringenin; 10x) showing reduced inflammatory cell infiltration in some areas, mild inflammation (yellow arrow), mild edema (red arrow), mild congestion (black arrow; mild tissue damage, score 1)

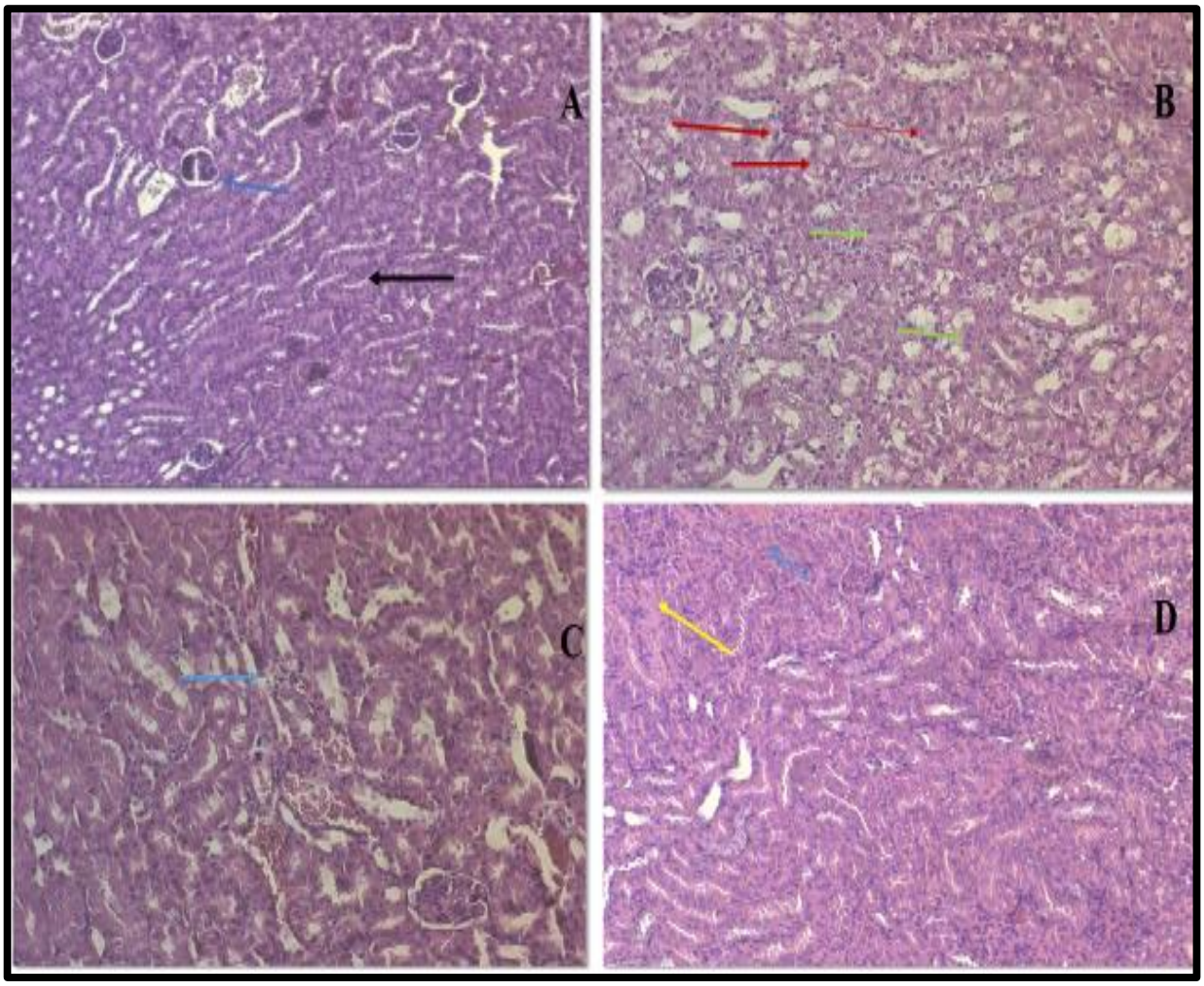

Fig. 8. Histopathological examinations of the kidney tissues stained with hematoxylin and eosin. A. Group I (Control; 10x), which was treated with 0.9% N/S, showed a normal histological appearance comprising of proximal and distal convoluted tubules (black arrow), glomeruli (blue arrow), with no inflammatory cell infiltration (no tissue damage; score 0); B. Group II (LPS-injected; 40x) showing marked distortion of kidney architecture, tubular epithelial swelling of cells and vacuolar degeneration (red arrow), dilated and congested vessels (green arrow), inflammatory cell infiltration and tubular cast formation (sever tissue damage, score 3); C. Group III (LPS-injected and pretreated with 50 mg/kg naringenin; 20x) showing moderate degenerative changes of renal tubules (blue arrow), moderate congested and dilated glomerular capillaries, reduced inflammatory cell infiltration in some areas (moderate tissue damage, score 2); D. Group IV (LPS-injected and 100 mg/kg naringenin; 20x) showing reduced infiltration of inflammatory cells (yellow arrow), and mild congestion (blue arrow; mild tissue damage; score 1)

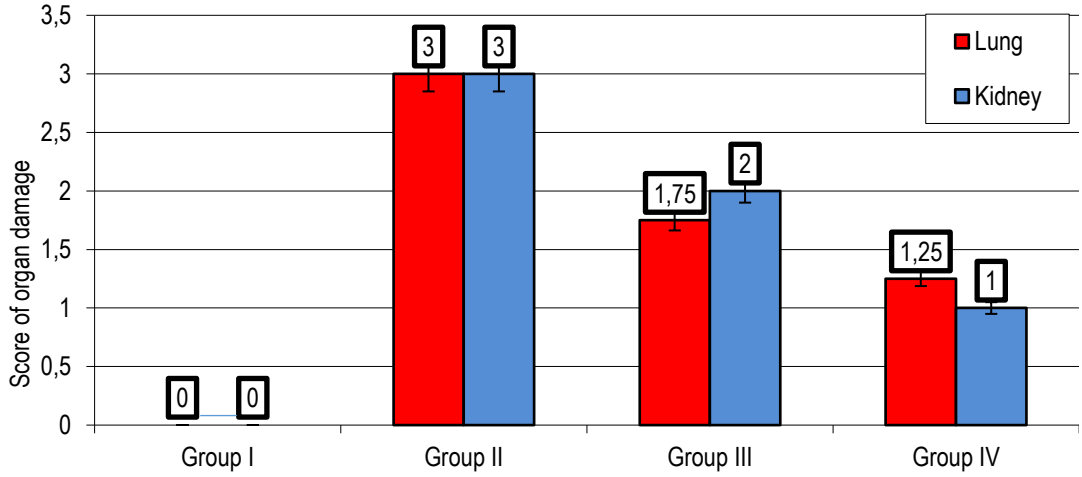

Fig. 9. The pathological scores of representative lung and kidney samples in each group. Data were expressed as mean ± SD, n = 5; The graph revealed that group II (LPS-injected) produced a highly significant ($p \leq 0.01$) degree score of injury compared with group I (healthy control). Meanwhile, the naringenin pre-treated groups III and IV at doses 50 and 100 mg/kg, respectively, one hour before receiving LPS showed highly significant ($p \leq 0.01$) enhancement of histopathological variations compared with group II (LPS-injected)

Discussion

Although lung and kidney injuries are distinct entities, they frequently coexist and frequently interact in severely ill patients. With a mortality rate of over 40%, most patients in the intensive care unit will die from numerous rather than discrete organ failures (Vincent, 2011). Cytokine storm syndrome is the detrimental prominent core of multi-organ failure, predominantly the lungs and kidneys, as it is characterized by systemic hyper-inflammatory conditions, as well as endothelial damage, oxidative stress, mitochondrial dysfunction, and apoptosis. All these are complications in the injury mechanism(s) of such organs (Gomez *et al*., 2014; Moreira *et al*., 2014). The current study focused on evaluating the prophylactic effects of naringenin in the lung, and associated dysfunctioning kidney injury after 48 hours of LPS administration on male mice by targeting the exaggerated inflammatory response while assessing the anti-cytokine storm activity via modulating the inflammatory biomarker outcomes, and their decisive role in addition to evidence of histopathological findings.

In terms of animal welfare, current study findings successfully reflected changes during endotoxemia, as well as assess its severity on a mouse model based on symptoms identified in humans. In addition, it is considered reliable in an i.p. injection with the LPS administration) as reported by Huet *et al.* (2013). Researchers have utilized an endotoxin-treated mouse approach as an efficient strategy for inducing lung and kidney injuries in animal models by triggering inflammatory response pathways (Liu *et al*., 2019; Sahib *et al*., 2022). In human beings, a clinical pattern of outcomes termed the systemic inflammatory response syndrome (SIRS) describes a resident in danger of lung and kidney injuries (Pietrantoni *et al*., 2003; Zheng *et al*., 2019). The term SIRS refers to the systemic release of a variety of proinflammatory cytokines/chemokines that have been implicated in the pathophysiology of ALI/AKI in humans (Takala *et al*., 2002). Although intraperitoneal LPS injection significantly increased pro-inflammatory cytokines (IL-1β, IL-8, IL-6, and TNF-α) in the current investigation to approximately 6-6.5-fold compared to apparently healthy control. Several lines of evidence suggested that cecal ligation and puncture could up-regulate such cytokines upon inducing an experimental ALI/ AKI animal model in a similar pattern (Bhargava *et al*., 2013; Zhou *et al*., 2019; Raheem *et al*., 2022; Qiongying *et al*.,

2023). The results of the remarkable elevation of TNF-α, IL-1β, IL-6, and IL-8 serum levels in group II may be attributed to the capability of LPS linked to its unique LPS-binding protein (LBP), forming a complex of LPS/LBP. At that point, it triggers the stimulation of the CD14/TLR4 receptor on the macrophages and monocytes (a TLR4 dimer binding to the MyD88 proteins), thereby stimulating the downstream of the nuclear factor kappa light chain kappa factor (NF-κB) and mitogen-activated protein kinase (MAP kinases) signaling pathways (Kolb *et al*., 2001; Vellaisamy *et al.*, 2015). Subsequently, there is an over-expression of various pro-inflammatory cytokines such as TNF-α, IL-8, IL-6, and IL-1 β (Mansoor & Raghif, 2022; Farhana & Khan, 2023).

Pro-inflammatory cytokines/chemokines, such as TNF-α, IL-8, IL-1β, and IL-6, could aid the host defense and/or paradoxically induce inflammatory tissue injury. Among various pro-inflammatory mediators accountable for the amplification and transduction of the inflammatory responses is TNF-α. It has been strongly linked to the development of lung injury (Lu *et al*., 2012), as it directly ameliorates lung damage characterized by increased edema, permeability, and neutrophil entrapment in the microvasculatures. Meanwhile, IL-1β initiates the deterioration of the lung's epithelial and endothelial cellular layers (Negri *et al*., 2002). Thus, TNF-α and IL-1β together could induce bronchial epithelial cells to secrete IL-8, which is further increased by LPS as well. Hence, IL-8 plays an essential role in modifiable leukocyte trafficking in pulmonary illnesses, such as asthma, COPD, and ARDS (Okusawa *et al*., 1988; Ortiz *et al.*, 2001; Pease & Sabroe, 2002). IL-8 is regarded as a critical chemoattractant for monocytes/macrophages, neutrophils, and T-lymphocytes during renal inflammation. Moreover, the chemokine initiates local infiltration of monocytes/macrophages and subsequently its activation (Yu *et al*., 2015), which further increases lung and kidney injuries induced by LPS administration, as presented in the current study's observations.

On the other hand, IL-6 plays a crucial role in the pathogenesis and progression of ALI. The elevated level of IL-6 in the lung tissues is due to its vital role in the phosphorylation of activator and signal transducer of transcription 3 (STAT3), resulting in the enhancement of neutrophils recruitment and lowering endotoxin stress (Zhao *et al.*, 2016). Moreover, other signal transduction pathways that comprise JAK/STAT, NF-κB, as well as mitogen-activated protein kinases (MAPK), also contributed to mediating lung/kidney hyperactive inflammatory disorder (Fang *et al*., 2007). Thus, IL-6 is reported to be intensely involved in an inflammatory cascade of LPS-induced lung and kidney injuries as evidenced in the results of the current study. Conversely, the significant reduction in TNF-α, IL-1β, IL-8, and IL-6 serum levels in groups III and IV (pretreated groups with the naringenin at different doses) may be due to multiple pharmacological and biological effects, such as anti-oxidative, anti-inflammatory, anti-proliferative, and anti-tumor, as well as immune-modulating activity that the phytochemical has (Alberca *et al.*, 2020). Furthermore, naringenin treatment inhibits inflammation-producing mediators such as inducible nitric oxide synthase (iNOS), lipo-oxygenase (LOX), and cyclooxygenase 2 (COX-2), which produce thromboxane A2 and prostaglandin E2 that could worsen cytokine storm (Park *et al*., 2012). Scientific evidence shows that naringenin treatment prevents the STAT-phosphorylation, thus blocking the JAK/STAT inflammatory signaling pathway that is responsible for most cytokines signaling and growth factors affecting many essential cellular functions and promoting growth, differentiation, cell proliferation, migration, apoptosis, immune response, and inflammation (Lingtao *et al*., 2017).

In the survival experiment, the animals in group II that were exposed to the inflammatory stimulation had a significantly higher mortality rate than the animals in group I that were given saline. This increased mortality may be connected to a strong SIRS induced by LPS, resulting in various organ dysfunctions. However, pretreatment with naringenin at doses of 50 and 100 mg/kg for one hour before LPS administration resulted in the greatest reduction in mortal-

ity rate. Built on this outcome, the current study team predicted that naringenin at a dose of 100 mg/kg might act as an immune-modulatory agent, inhibiting the LPS biological toxicity, suggestive of providing an innovative therapeutic approach for pulmonary/renoprotective activity. Thus, naringenin specifically promotes cytokine trafficking and degradation and improves the survival capability of naringenin-pretreated mice, hence, providing a modality for drug discovery in this field.

According to the pathophysiological aspect, cytokine storms can result in oxidative stress injury, deregulated inflammatory response, and severe histological changes. Hence, histopathological assessment reinforced the outcomes obtained in the current study. According to earlier reports, the histological sign of the LPS-inducing group of animals had a high score value of 3 (serious tissue damage) on the previously indicated lung/kidney scoring system. The typical symptom in response to systemic as well as local inflammation is edema. Pretreatment with naringenin improved the pulmonary histopathological changes and attenuated the severity of lung edema significantly. Moreover, neutrophil infiltration, cell structure damage, and thickening of the alveolar wall in the pre-treatment group with naringenin significantly improved the renal histopathological alteration to mild changes only (rank 1) compared with those in the LPS-induced lung injury groups.

LPS stimulation of the renal tubules considerably increased the formation of reactive oxygen species (ROS) and thus the stimulation of MAPKs. However, ROSs are produced by infiltrating cells in injured kidneys or endogenous sources and producing AKI and/or increasing renal damage (Kang *et al*., 2005; Aal-Aaboda *et al*., 2021). Hence, ROS endogenous scavengers could ameliorate vascular or renal tubular injury (Schrier & Wang, 2004; Shi *et al*., 2021). However, oxidative stress promotes the activation and recruitment of residential neutrophils and macrophages, prominent to hyper-inflammatory responses and thus cellular injury. The results of the current study and several evidence specified that extracts of citrus fruit and naringenin have the prospective for suppressing oxidative stress, metal chelating, and anti-free radical scavenging activity and improving the kidney's antioxidant defense system (Jasemi *et al*., 2022; Zhang *et al*., 2022; Alaqeel & Hariri, 2023). However, in comparison to the current study findings, only a few reports have revealed citrus naringenin's renoprotective activity. It effectively protects against CCl_4-inducing renal injury at doses of 20 and 40 mg/kg in rats by reducing creatinine, urea, and uric acid serum levels in a dose-dependent manner (Ammar *et al*., 2022). Hence, alleviating inflammation and oxidative stress pathways could preserve normal lung and kidney functions and prevent histopathological changes (Abu-Raghif *et al*., 2015; Al-Saedi *et al* 2015; Ahmad *et al*., 2022).

Conclusion and recommendations

The findings of the current research show that the kidney plays an important role in the elimination and synthesis of mediators of lung injury and that prolonged exposure to mediators contributes to pulmonary injury. Furthermore, the study highlights the importance of the kidney in the regulation of systemic inflammatory cytokines/chemokines and pulmonary homeostasis. The naringenin's pulmonary/renoprotective effects were observed at a dose of 100 mg/kg, which was more significant compared to 50 mg/kg. This observation could be due possibly to its anti-inflammatory effect and significantly improving the survival rate. Furthermore, these phytochemicals have a pulmonary/nephroprotective effect by decreasing histological alterations caused by LPS administration and improving microscopic scores. Additional research with a more comprehensive study is required to fully understand the molecular mechanism(s) underlying naringenin's protective action against LPS-inducing in an experimental pulmonary/renal injury model or, ideally, in clinical investigations.

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References

AAL-AABODA M., ABU RAGHIF A.R., ALMUDHAFER R.H. & HADI N.R. (2022): Lipopolysaccharides from Rhodobacter Spheroids Modulate Toll-Like Receptor Expression and Tissue Damage in an Animal Model of Bilateral Renal Ischemic Reperfusion Injury. *J Med Life* **15**(5), 685–697.

ABU-RAGHIF A.R., QASIM B.J., ABADY A.H. & SAHIB H.B. (2015): Effects of Aqueous Thyme Extract Against Cisplatin-Induced Nephrotoxicity in Rabbits. *Int J Pharm Sci Rev Res* **30**(1), 190–194.

AHMAD A., PRAKASH P., KHAN M.S., ALTWAIJRY N., ASGHAR M.N., RAZA S.S., *et al.* (2022): Enhanced Antioxidant Effects of Naringenin Nanoparticles Synthesized Using the High-Energy Ball Milling Method. *ACS Omega* **7**(38), 34476–84.

AHMADIAN E., HOSSEINIYAN KHATIBI S.M., RAZI SOOFIYANI S., ABEDIAZAR S., SHOJA M.M., ARDALAN M., *et al.* (2021): COVID-19 and Kidney Injury: Pathophysiology and Molecular Mechanisms. *Rev Med Virol.* **31**(3), e2176.

AL-SAEDI H.F., AL-ZUBAIDY A.A., KHATTAB Y.I. & SAHIB H.B. (2015): Effect of Pentoxifylline Against Doxorubicin-Induced Nephrotoxicity in Rabbits. *International Journal of Pharmaceutical Sciences Review and Research* **30**(1), 195-9.

ALAQEEL N.K. & AL-HARIRI M.T. (2023): Naringenin Ameliorates Cyclophosphamide-Induced Nephrotoxicity in an Experimental Model. *Saudi J Biol Sci.* **30**(6), 103674.

ALBERCA R.W., TEIXEIRA F.M.E., BESERRA D.R., DE OLIVEIRA E.A., ANDRADE M.M.S., PIETROBON A.J. & SATO M.N. (2020): Perspective: The Potential Effects of Naringenin in COVID-19. *Front Immunol*. **25**(11), 570919.

AMMAR N.M., HASSAN H.A., ABDALLAH H.M.I., AFIFI S.M., ELGAMAL A.M., FARRAG A.R.H., EL-GENDY A.E.G., FARAG M.A. & ELSHAMY A.I. (2022): Protective Effects of Naringenin from Citrus Sinensis (Var. Valencia) Peels Against CCl4-Induced Hepatic and Renal Injuries in Rats Assessed by Metabolomics, Histological and Biochemical Analyses. *Nutrients* **14**(4), 841.

BHARGAVA R., ALTMANN C.J., ANDRES-HERNANDO A., WEBB R.G., OKAMURA K., YANG Y., *et al.* (2013): Acute Lung Injury and Acute Kidney Injury Are Established by Four Hours in Experimental Sepsis and Are Improved with Pre, but Not Post, Sepsis Administration of TNF-α Antibodies. *PLoS ONE* **8**(11), e79037.

CARDIFF R.D., MILLER C.H. & MUNN R.J. (2014): Manual Hematoxylin and Eosin Staining of Mouse Tissue Sections. *Cold Spring HarbProtoc.* **6**, 655–8.

EBRAHIMI-DEHKORDI S., BANITALEBI H. & HASANPOUR-DEHKORDI A. (2021): Acute Kidney Injury Due to Cytokine Storm in Patients with COVID-19 Infection. *J Nephropathol.* **10**(4), e37.

FAJGENBAUM D.C. & JUNE C.H. (2020): Cytokine Storm. *N Engl J Med.* **383**(23), 2255–73.

FANG W., CAI S.X., WANG C.L., SUN X.X., LI K., YAN X.W., SUN Y.B., SUN X.Z., GU C.K., DAI M.Y., *et al.* (2017): Modulation of Mitogen-Activated Protein Kinase Attenuates Sepsis-Induced Acute Lung Injury in Acute Respiratory Distress Syndrome Rats. *Mol. Med. Rep*. **16**, 9652–9658.

FARHANA A. & KHAN Y.S. (2023): Biochemistry, Lipopolysaccharide. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing.

GOMEZ H., *et al.* (2014): A Unified Theory of Sepsis-Induced Acute Kidney Injury: Inflammation, Microcirculatory Dysfunction, Bioenergetics, and the Tubular Cell Adaptation to Injury. *Shock* **41**, 3–11.

GOODMAN R.B., PUGIN J., LEE J.S. & MATTHAY M.A. (2003): Cytokine-mediated inflammation in acute lung injury. *Cytokine Growth Factor Rev.* **14**, 523–535.

HAYDER B.S., KATHUM O.A., ALANEE R.S., JAWAD R.A.M. & AL-SHAMMARI A.M. (2022): The Anti-Cytokine Storm Activity of Quercetin Zinc and Vitamin C Complex. *Advances in Virology*, vol.article ID 1575605, 6 pages.

HIRAI S., KIM Y., GOTO T., KANG M., YOSHIMURA M., OBATA A., YU R. & KAWADA T. (2007): Inhibitory Effect of Naringenin Chalcone on Inflammatory Changes in the Interaction Between Adipocytes and Macrophages. *Life Sciences* **81**(16), 1272–1279.

HUET O., RAMSEY D., MILJAVEC S., JENNEY A., AUBRON C., APRICO A., STEFANOVIC N., BALKAU B., HEAD G.A., DE HAAN J.B. & CHIN-DUSTING J.P.F. (2013): Ensuring Animal Welfare While Meeting Scientific Aims Using a Murine Pneumonia Model of Septic Shock. *Shock* **39**(6), 488–494.

JARCZAK D. & NIERHAUS A. (2022): Cytokine storm-definition, causes, and implications. *Int J Mol Sci.* **23**(19), 11740.

JASEMI S.V., KHAZAEI H., FAKHRI S., MOHAMMADI-NOORI E. & FARZAEI, M.H. (2022): Naringenin Improves Ovalbumin-Induced Allergic Asthma in Rats Through Antioxidant and Anti-Inflammatory Effects. *Evid Based Complement Alternat Med.*, 9110798.

KANG D.G., SOHN E.J., MOON M.K., LEE Y.M. & LEE H.S. (2005): Rehmannia Glutinose Ameliorates Renal Function in the Ischemia/Reperfusion-Induced Acute Renal Failure Rats. *Biol. Pharm. Bull*. **28**, 1662–1667.

KOLB M., MARGETTS P.J., ANTHONY D.C., PITOSSI F. & GAULDIE J. (2001): Transient Expression of IL-1β Induces Acute Lung Injury and Chronic Repair Leading to Pulmonary Fibrosis. *J Clin Invest*. **107**, 1529–36.

LI R.F., FENG Y.Q., CHEN J.H., GE L.T., XIAO S.Y. & ZUO X.L. (2015): Naringenin Suppresses K562 Human Leukemia Cell Proliferation and Ameliorates Adriamycin-Induced Oxidative Damage in Polymorphonuclear Leukocytes. *Exp Ther Med.* **9**, 697–706.

LI X., GENG M., PENG Y., MENG L. & LU S. (2020): Molecular Immune Pathogenesis and Diagnosis of COVID-19. *J Pharm Anal*. **10**(2), 102–108.

LINGTAO J., ZENG W., ZHANG F., ZHANG C. & LIANG W. (2017): Naringenin Ameliorates Acute Inflammation by Regulating Intracellular Cytokine Degradation. *J Immunol* **199**(10), 3466–3477.

LIU H., HAO J., WU C., *et al.* (2019): Eupatilin Alleviates Lipopolysaccharide-Induced Acute Lung Injury by Inhibiting Inflammation and Oxidative Stress. *Med Sci Monit*. **25**, 8289–96.

LU C.N., YUAN Z.G., ZHANG X.L., YAN R., ZHAO Y.Q., LIAO M. & CHEN J.X. (2012): Saikosaponin a and its epimer saikosaponin d exhibit anti-inflammatory activity by suppressing activation of NF-κB signaling pathway. *International immunopharmacology* **14**(1), 121-126.

MANGALMURTI N. & HUNTER C.A. (2020): Cytokine storms understanding COVID-19. *Immunity* **53**, 19–25.

MANSOOR A.F.A. & RAGHIF A.R.A. (2022): Attenuated Effects of Rivastigmine in Induced Cytokine Storm in Mice, Journal of Emergency Medicine, Trauma & Acute Care. (3):12.

MATUTE-BELLO G., *et al.* (2001): Fas (CD95) Induces Alveolar Epithelial Cell Apoptosis in Vivo: Implications for Acute Pulmonary Inflammation. *The American Journal of Pathology* **158**(1), 153–161.

MONTAZERSAHEB S., HOSSEINIYAN KHATIBI S.M., HEJAZI M.S., *et al.* (2022): COVID-19 infection: An overview on cytokine storm and related interventions. *Virol J* **19,** 92.

MOREIRA R.S., *et al.* (2014): Apolipoprotein A-I Mimetic Peptide 4F Attenuates Kidney Injury, Heart Injury, and Endothelial Dysfunction in Sepsis. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **307**, R514–R524. Epub.11.

NAVARRO K.L., HUSS M., SMITH J.C., SHARP P., MARX J.O. & PACHARINSAK C. (2021): Mouse Anesthesia: The Art and Science. *ILAR Journal* **62**(1-2), 238–273.

NEGRI E.M, MONTES G.S., SALDIVA P.H. & CAPELOZZI V.L. (2000): Architectural Remodeling in Acute and Chronic Interstitial Lung Disease: Fibrosis or Fibroelastosis? *Histopathology* **37**, 393–401.

NOMURA A., NISHIKAWA K., YUZAWA Y., OKADA H., OKADA N., MORGAN, B.P., PIDDLESDEN S.J., NADAI M., HASEGAWA T. & MATSUO S. (1995): Tubulointerstitial Injury Induced in Rats by a Monoclonal Antibody That Inhibits the Function of a Membrane Inhibitor of Complement. *J Clin Invest* **96**(5), 2348–56.

ORTIZ L.A., FRIEDMAN M. & BANKS W.A. (2001): Role of LPS and Receptor Subtypes in the Uptake of TNF by the Murine Lung. *Life Sci **69***, 791–802.

OKUSAWA S., GELFAND J.A., IKEJIMA T., CONNOLLY R.J. & DINARELLO C.A. (1988): Interleukin 1 Induces a Shock-Like State in Rabbits. Synergism with Tumor Necrosis Factor and the Effect of Cyclooxygenase Inhibition. *J Clin Invest* **81**, 1162–1172.

PABLA N., SCINDIA Y., GIGLIOTTI J. & BAJWA A. (2022): Mouse Models of Acute Kidney Injury. *Preclinical Animal Modeling in Medicine*. IntechOpen.

PARASURAMAN S., RAVEENDRAN R. & KESAVAN R. (2010): Blood Sample Collection in Small Laboratory Animals. *J Pharmacol Pharmacother*. **1**(2), 87–93.

PARK H., KIM G. & CHOI Y. (2012): Naringenin Attenuates the Release of Pro-Inflammatory Mediators from Lipopolysaccharide-Stimulated BV2 Microglia by Inactivating Nuclear Factor-κB and Inhibiting Mitogen-Activated Protein Kinases. *International Journal of Molecular Medicine* **30**(1).

PEASE J.E. & SABROE I. (2002): The Role of Interleukin-8 and Its Receptors in Inflammatory Lung Disease. *Am J Respir Med* **1**, 19–25.

PIETRANTONI C., MINAI O.A., YU N.C., MAURER J.R., HAUG M.T. III, MEHTA A.C. & ARROLIGA A.C. (2003): Respiratory Failure and Sepsis are the Major Causes of ICU Admissions and Mortality in Survivors of Lung Transplants. *Chest* **123**, 504–509.

PLOTNIKOV E.Y., BREZGUNOVA A.A., PEVZNER I.B., ZOROVA L.D., MANSKIKH V.N., POPKOV V.A., SILACHEV D.N. & ZOROV D.B. (2018): Mechanisms of LPS-Induced Acute Kidney Injury in Neonatal and Adult Rats. *Antioxidants (Basel)* **7**(8), 105.

QIONGYING W., LIN J. & LI Y. (2023): Albiflorin Attenuates Sepsis-Induced Acute Lung Injury (ALI) via the TLR-4/NF-κB Pathway. *Journal of Functional Foods* **107**, 105633.

RAHEEM A.K., ABU-RAGHIF A.R. & ABD-ALAKHWA S.Z. (2022): Irbesartan Attenuates Sepsis-Induced Renal Injury In Mice Models. *Journal of Pharmaceutical Negative Results* 662–669.

RAMÍREZ K., QUESADA-YAMASAKI D. & FORNAGUERA-TRÍAS J.A. (2019): Protocol to Perform Systemic Lipopolysaccharide (LPS) Challenge in Rats. *Odovtos International Journal of Dental Sciences* **21**(1), 53–66.

RITTIRSCH D., FLIERL M.A., DAY D.E., NADEAU B.A., MCGUIRE S.R., HOESEL L.M., IPAKTCHI K., ZETOUNE F.S., SARMA J.V., LENG L., HUBER-LANG M.S., NEFF T.A., BUCALA R. & WARD P.A. (2008): Acute Lung Injury Induced by Lipopolysaccharide is Independent of Complement Activation. *J Immunol*. **180**(11), 7664–72.

SAITO T., YAMAMOTO T., KAZAWA T., GEJYO H. & NAITO M. (2005): Expression of Toll-like Receptor 2 and 4 in Lipopolysaccharide-Induced Lung Injury in Mouse. *Cell Tissue Res* **321**, 75–88.

SALSABILA S.A., ROSYID A.N., EMPITU M.A., KADARISWANTININGSIH I.N., SURYANTORO S.D., HARYATI M.R., THAHA M. & SUZUKI Y. (2022): Kidney-pulmonary crosstalk from pathophysiological perspective. *JR* **8**(1), 44–51.

SAS (2018): Statistical Analysis System, User's Guide. Statistical. Version 9.6th ed. SAS Inst. Inc. Cary. N.C. The USA.

SCHRIER R.W. & WANG W. (2004): Acute Renal Failure and Sepsis. *N. Engl. J. Med.* **351**, 159–169.

SHAKEEL S., REHMAN M. U., TABASSUM N., AMIN U. & MIR M.U.R. (2017): Effect of Naringenin (a Naturally Occurring Flavanone) Against Pilocarpine-Induced Status Epilepticus and Oxidative Stress in Mice. *Pharmacognosy Magazine* **13**(49), 154.

SHI Q., LANG W., WANG S., LI G., BAI X., YAN X. & ZHANG H. (2021): Echinacea Polysaccharide Attenuates Lipopolysaccharide-Induced Acute Kidney Injury via Inhibiting Inflammation, Oxidative Stress, and the MAPK Signaling Pathway. *Int. J. Mol. Med.* **47**, 243–255.

TAKAHASHI K., MIZUKAMI H., KAMATA K., INABA W., KATO N., *et al.* (2012): Amelioration of Acute Kidney Injury in Lipopolysaccharide-Induced Systemic Inflammatory Response Syndrome by an Aldose Reductase Inhibitor, Fidarestat. *PLOS ONE* **7**(1), e30134.

TAKALA A., NUPPONEN I., KYLANPAA-BACK M.L. & REPO H. (2002): Markers of Inflammation in Sepsis. *Ann Med* **34**, 614–623.

VELLAISAMY S., NEPAL N., ROGERS S., MANNE N.D.P.K., ARVAPALLI R., RICE K.M., ASANO S., FANKENHANEL E., MA J.Y., SHOKUHFAR T., MAHESHWARI M. & BLOUGH E.R. (2015): Lipopolysaccharide-Induced MAP Kinase Activation in RAW 264.7 *Cells Attenuated by Cerium Oxide Nanoparticles* **4**, 96–99.

VINCENT J. L. (2011): Acute Kidney Injury, Acute Lung Injury, and Sepsis: How Does Mortality Compare? *Controversies in Acute Kidney Injury* **174**, 71-77.

WARE L.B. & MATTHAY M.A. (2000): Acute Respiratory Distress Syndrome. *N Engl J Med.* **342**, 1334–1349.

YU C., QI D., SUN J.F., *et al.* (2015): Rhein Prevents Endotoxin-Induced Acute Kidney Injury by Inhibiting NF-κB Activities. *Sci Rep* **5**, 11822.

ZHANG B., RAMESH G., UEMATSU S., AKIRA S. & REEVES W.B. (2008): TLR4 Signaling Mediates Inflammation and Tissue Injury in Nephrotoxicity. *J Am Soc Nephrol.* **19**(5), 923-32.

ZHANG B., WAN S., LIU H., QIU Q., CHEN H., CHEN Z., WANG L. & LIU, X. (2022): Naringenin Alleviates Renal Ischemia-Reperfusion Injury by Suppressing ER Stress-Induced Pyroptosis and Apoptosis Through Activating Nrf2/HO-1 Signaling Pathway. *Oxid Med Cell Longev.*, 5992436.

ZHAO J., YU H., LIU Y., GIBSON S.A., YAN Z., XU X., GAGGAR A., LI P.K., LI C., WEI S., *et al.* (2016): Protective Effect of Suppressing STAT3 Activity in LPS-Induced Acute Lung Injury. *Am. J. Physiol. Lung Cell. Mol. Physiol*. **311**, L868–L880.

ZHENG C., ZHOU Y., HUANG Y., CHEN B., WU M., XIE Y. ... PAN J. (2019): Effect of ATM on Inflammatory Response and Autophagy in Renal Tubular Epithelial Cells in LPS-Induced Septic AKI. *Experimental and Therapeutic Medicine* **18**, 4707-4717.

ZHOU Y., XU W. & ZHU H. (2019): CXCL8(3-72) K11R/G31P Protects Against Sepsis-Induced Acute Kidney Injury Via NF-κB and JAK2/STAT3 Pathway. *Biol Res.* **52**(1), 29.