Life Sciences 221 (2019) 327–334

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Ameliorative effect of gossypin against acute lung injury in experimental sepsis model of rats

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ARTICLE INFO

Keywords:
CLP
Lung injury
Gossypin
NLRP3
HMGβ1
NF-κB

ABSTRACT

Aims: Sepsis is a complex pathophysiological event involving systemic inflammatory response syndrome, multiple organ dysfunction syndrome and tissue damage such as acute lung injury (ALI). Although many new mechanisms are being investigated to enlighten the pathophysiology of sepsis, there is no effective treatment protocol yet. Antioxidant, antibacterial and antiinflammatory effects of gossypin (GOS)-like flavonoids have been shown and we have hypothesized that GOS have roles in sepsis induced inflammation of lungs.
Main methods: Cecal ligation and puncture (CLP) induced sepsis model was induced in rats. Effects of GOS on oxidative stress, histopathology, nuclear factor kappa B (NF-κB), IL-6 positivity and NLRP3, HMGβ1, TNF-α, NF-κB, IL-1β mRNA expression levels were evaluated in lung tissues of the septic rats.
Key findings: GOS 20 (20 mg/kg) administration to septic rats decreased oxidative stress and supported antioxidant system in lungs. GOS administration also decreased the tissue NF-κB and IL-6 immunopositivity, which is high in septic rats; and decreased the sepsis-induced lung injury. HMGβ1, NLRP3, NF-κB, IL-1β, and TNF-α mRNA expression significantly increased in the CLP group. Both doses of GOS significantly reduced these mRNA expression as compared with the levels in the CLP group demonstrating its anti-inflammatory potential.
Significance: GOS administration, may represent a novel treatment for the prevention of lung damage occurred after sepsis induction. This effect of GOS might be related to its anti-inflammatory potential that result in decreased cytokine response and improved oxidative status.

1. Introduction

Sepsis is a complex pathophysiological event characterized by persistent hypotension, progressive metabolic acidosis, and systemic inflammatory response syndrome. It can lead to septic shock, tissue damage, multiple organ dysfunction syndrome, acute respiratory distress syndrome (ARDS), acute lung injury (ALI), and even death [1,2]. Even though many studies focused to enlighten sepsis physiopathology and develop new treatment strategies [3], current treatment protocol is still limited with routine life-support treatment [4]. Therefore studies are still actively being performed on both clinics and in laboratory animals for determination of potential drug candidates [5–8]. For laboratory examinations among different animal models cecal-ligation and puncture induced sepsis model is mostly preferred one, for it mimics the clinical situation of bowel perforation and bacterial infection in humans [9,10].

ARDS and/or ALI is one of the common lethal complications of endotoxemia therefore research is currently focused on the prevention or amelioration of lung injury during sepsis [5,8]. ALI is characterized by the accumulation of a large number of neutrophils in the lungs, increased generation of reactive oxygen species (ROS) and increased production of proinflammatory cytokines [11]. In sepsis-induced ARDS and/or ALI, the dependent lung regions are the sites of greatest inflammation and injury. There are experimental studies suggesting that oxidative stress is involved in the resulting lung injury [12,13]. Therefore, it is considered that ALI can be prevented or attenuated by pharmacological agents that either inhibit the generation of ROS or scavenge these reactive molecules [14].

During sepsis induced ALI many pathophysiological processes are being activated. One of the most important features of sepsis-induced lung injury is inflammation. Inflammation is characterized by the secretion of various inflammatory cytokines, tissue infiltration, and

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https://doi.org/10.1016/j.lfs.2019.02.039
Received 20 November 2018; Received in revised form 8 February 2019; Accepted 18 February 2019
Available online 20 February 2019

activation of phagocytic cells [15]. Uncontrolled secretion of proinflammatory mediators, such as TNF-α, IL-1β, and IL-6, occurs following the activation of phagocytic cells and tissue infiltration. At the same time, proinflammatory cytokines cause secondary cytokines, lipid mediators, and reactive oxygen species to be released. In particular, the lungs are affected, and ALI develops in about 40% of sepsis cases [16]. Recent studies have also shown that oxidative stress plays an important role in sepsis-related mortality and organ dysfunction [8,17,18]. To shed light on the pathophysiology of sepsis induced ALI, some researchers have focused on anticytokines, drug targets, and antioxidants, whereas others have focused on endothelial receptors that influence hemodynamic parameters and the expression of cytokines responsible for damage [17–20]. Also the high mobility group box (HMGβ1) protein plays an important role in the formation and progression of sepsis induced lung injury [21]. Production of HMGβ1 is induced by immune cells, including mononuclear cells, dendritic cells, macrophages and also stimulated by endotoxins, and inflammatory cytokines [22]. Therefore, inhibition of HMGβ1 secretion attenuates systemic inflammatory response syndrome and sepsis-induced organ injury [23]. Previous research also emphasized the critical role of the nod like receptor protein 3 (NLRP3) inflammasome in the inflammatory response [24]. Upregulation of NLRP3 expression led to the maturation and secretion of potential proinflammatory cytokines [24,25], immune cell accumulation at the site of infection, and enhanced activation of the adaptive immune response. Besides; previously Hou et al. reported that NLRP3 mediated alveolar macrophage pyroptosis enhances HMGβ1 secretion in acute lung injury [26]. Thus, agents targeting both HMGβ1 and NLPR3 inhibition can be potentially beneficial for sepsis and related lung injury treatment.

As mentioned above oxidative stress accompanies inflammatory response during sepsis and many different agents with antioxidant properties have been successfully used to treat septic lung injury in murine models [27–29]. Gossypin (GOS) (3,5,8,3,4-pentahidroxy-7-o-glucosyl flavone 8-glucosyl) (GOS), originally isolated from *Hibiscus vitifolius*, is a flavonoid with antioxidant, anti-inflammatory, and analgesic properties [30]. In addition, GOS provides defense against oxidative stress by activating both aminolevulinate dehydratase and antioxidant defense enzymes [31]. Many studies have shown that flavonoids, such as GOS, exhibit an anti-inflammatory effect in addition to antioxidant properties and that this effect is mediated via NLRP3 [32–34]. Although the antioxidant, antibacterial, and anti-inflammatory effects of GOS-like flavonoids have been demonstrated, the potential protective effects of GOS against sepsis-induced lung injury have not been investigated yet.

Therefore, the aim of this study was to investigate the possible effects of GOS on sepsis induced ALI in rats and evaluate possible contribution of oxidative and inflammatory markers by biochemical, molecular, and histopathological methods.

2. Material and methods

2.1. Animals

In total, 24 female albino Wistar rats (250–300 g) obtained from the Experimental Animal Laboratory of the Medicinal and Experimental Application and Research Center of Ataturk University, Turkey were used. The rats were housed on sawdust bedding in typical plastic cages in a well-ventilated room at 22 °C under specific light conditions (a 14:10 h light:dark cycle). Standard rat food and tap water were given ad libitum. The study was approved by the local animal care committee of Ataturk University (24.05.2017/56).

2.2. Experimental design

Four groups were created, with six rats in each, as below:

Group 1: control
Group 2: cecal ligation and puncture (CLP)
Group 3: CLP + GOS, 10 mg/kg (CLP GOS 10)
Group 4: CLP + GOS, 20 mg/kg (CLP GOS 20)

2.3. CLP-induced polymicrobial sepsis model

CLP was used to induce a polymicrobial sepsis model [35]. The control group underwent a sham operation. Anesthesia was induced through intraperitoneal administration of a combination of 100 mg/kg of ketamine and 15 mg/kg of xylazine. The rats' abdomens were shaved, and a 1 cm midline abdominal incision was then made to open the peritoneum. Once the abdominal organs were exposed, the cecum was isolated and ligated with a 4/0 silk ligature just distal to the ileocecal valve. Using a 21-gauge needle, two punctures were made on the opposite sides of the mesentery (four punctures in total) through the cecum distal to the point of ligation. The cecum was then returned to the peritoneal cavity. Next, the abdominal incision was closed in two layers using a 4/0 sterile synthetic absorbable suture. All the animals were given 0.5 ml of normal saline subcutaneously at the time of the surgery. The drugs were administered 1 h after the sepsis procedure was performed. GOS (Cat. no: 652-78-8, Sigma Aldrich, MO, USA) was administered intraperitoneally at doses of 10 mg/kg and 20 mg/kg (CLP GOS 10 and CLP GOS 20, groups, respectively). GOS is insoluble in water, the drug was dissolved in a mixture with one part DMSO (0.1% DMSO) per one thousand parts of normal saline that is administered to control and CLP groups as vehicle. Twelve hours after the surgery and respective treatments, all the rats were sacrificed via an intraperitoneal administration of a combination of ketamine and xylazine, and blood and lung tissue samples were collected. We selected this time point, for previous literature demonstrated that cytokine storm occurs between 12 and 16 h after CLP induced sepsis model in rats [36,37]. Half of each lung was perfused with saline and kept at −80 °C for biochemical and molecular analyses, and the other half was fixed in a 10% formalin solution for histopathological analysis.

2.4. Biochemical investigation of lung tissues

Following surgery, the tissues were stored at −80 °C. The tissue samples from each rat were ground in liquid nitrogen using a TissueLyser II grinding jar set (Qiagen, Hilden, Germany). Approximately 50 mg of ground tissue was homogenized in 1 ml of phosphate buffered saline homogenate buffer in an Eppendorf tube with TissueLyser II (Qiagen), and the samples were then centrifuged. Superoxide dismutase (SOD) activity [38], glutathione (GSH) levels [39], and malondialdehyde (MDA) levels [40] from each sample supernatant and standards were measured at room temperature in duplicate using modified methods with an enzyme-linked immunosorbent assay reader. A standard curve was plotted, and an equation was obtained from the absorbance of the standards. The linear SOD, GSH, and MDA concentrations were calculated according to this equation and were expressed as U/mg of protein, nmol/mg of protein, and nmol/mg of protein, respectively. The data obtained are presented as mean ± standard deviation (SD) as 1 mg of protein. All measurements were performed three times. All chemicals used were of analytical grade and were obtained from Sigma-Aldrich (Germany).

2.5. Real-time polymerase chain reaction (PCR)

2.5.1. Total RNA extraction and cDNA synthesis

The tissues (20 mg) were stabilized in RNA stabilization reagent (RNAlater, Qiagen), and then disrupted using the TissueLyser II (Qiagen). The total RNA was purified using an RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions in a QIAcube (Qiagen). The RNA samples were then reverse-transcribed into complementary DNA using a High-Capacity cDNA Reverse Transcription Kit

(Applied Biosystems). The cDNA concentration and quality were assessed and quantified using the Epoch Spectrophotometer System and Take3 Plate (BioTek).

2.5.2. Relative quantification of gene expression (real-time reverse transcriptase PCR)

The relative expression analyses of HMGβ1, NLRP3, nuclear factor kappa B (NF-κB), IL-1β, and TNF-α were performed with the StepOnePlus Real Time PCR System (Applied Biosystems) using cDNA synthesized from rat lung RNA. The real-time quantitative reverse transcriptase PCR was run using Primer Perfect Probe mix, TaqMan Probe-based technology (Primer Design Ltd., Southampton, UK), and the results were expressed as the relative-fold change in expression as compared with that in the control animals. Specific primers were used for rat gene transcripts, as follows: HMGβ1 (Rn01442665_m1), NLRP3 (Rn04244620_m1), NF-κB (Rn01475473_m1), IL-1β (Rn00580432_m1), and TNF-α (Rn01525860_g1). The gene expression levels were normalized using β-actin (Rn00667869_m1) as a housekeeping gene. For each tissue, triplicate determinations were performed in a 96-well optical plate for all parameters using 9 μL of cDNA (100 ng), 1 μL of Primer Perfect Probe mix, and 10 μL of QuantiTect Probe PCR Master mix (Qiagen) in each 20 ml reaction. The plates were heated for 2 min at 50 °C and 10 min at 95 °C, followed by 40 cycles of 15 s at 94 °C and 60 s at 60 °C. All data are expressed as the fold-change in expression as compared with the expression in other animal groups, using the $2^{-\Delta\Delta Ct}$ method [41].

2.6. Histopathological analyses

The lung materials obtained from the rats were sampled after being fixed in 10% formaldehyde. These samples were embedded in paraffin after routine follow-up. Sections 4 μm thick were then taken. After deparaffinization and rehydration procedures, the sections were stained with hematoxylin-eosin (H&E) [42]. All the sections were examined by two pathologists using a light microscope (Nikon Eclipse 80i), and photographic images of the slides were then taken using a digital camera. A minimum of five fields for each lung slide at ×40 magnification were evaluated and assigned to determine the severity of the changes using scores on a scale [43] where Grade 0: − (0% negative), Grade 1: + (0–33% mild positive), Grade 2: + + (33–66% moderate positive), Grade 3: + + + (66–100% severe positive).

For immunohistochemistry of IL-6 and NF-κB in the paraffin sections, the sections were mounted on polylysine slides and stained with an IL-6 Ab Leica Bond Polymer Refine Detection Kit and NF-κB in an immunohistochemical staining machine (Leica Bond-Max). Immunohistochemical data was scored in the light of previous literature [44].

2.7. Statistical analysis

IBM SPSS Statistics 25.0 software was used for comparing the molecular results. Differences among the groups were analyzed using Duncan's multiple comparison test and a one-way analysis of variance ($p < 0.05$). All the results are expressed as mean ± SD for the rats in each group.

3. Results

3.1. Biochemical results

SOD, which is a free radical scavenging enzyme, activity was significantly decreased in lungs of CLP group when compared to control rats ($p < 0.05$). GOS administration to septic rats significantly increased lung SOD activity in a dose dependent manner (Fig. 1a). When we evaluated the MDA levels, which is the end-product of membrane lipid peroxidation during oxidative stress, determined that it significantly increased in septic lungs when compared to control (Fig. 1b). Both doses of GOS administration to septic rats significantly decreased MDA levels and so decreased the level of membrane lipid peroxidation in lung tissue ($p < 0.05$). The levels of GSH, an antioxidant molecule, were significantly decreased in the CLP group when compared with that of control group ($p < 0.05$). However, GOS administration significantly increased GSH levels in septic lungs at both doses ($p < 0.05$) (Fig. 1c). Effects of GOS administration on oxidative parameters were dose dependent, namely 20 mg/kg dose of GOS exerted higher anti-oxidative properties than 10 mg/kg for all parameters evaluated (Fig. 1a-b-c).

3.2. Molecular results

To evaluate whether the low and high doses (10 mg/kg and 20 mg/kg, respectively) of GOS attenuated inflammatory response in lungs of septic rats, the expression levels of HMGβ1, NLRP3, NF-κB, IL-1β, and TNF-α mRNA were analyzed using real-time PCR (Fig. 2). HMGβ1, NLRP3, NF-κB, IL-1β, and TNF-α mRNA expression significantly increased in the CLP group as compared with that in the control group ($p < 0.05$). Both doses of GOS significantly reduced HMGβ1, NLRP3, NF-κB, IL-1β, and TNF-α mRNA expression as compared with the levels in the CLP group ($p < 0.05$). Of note, the level of TNF-α mRNA expression in CLP GOS 20 group decreased to a level comparable to that in the control group (Fig. 2e) ($p < 0.05$).

3.3. Histopathological results

Fig. 3a shows the histological appearance of the lungs in the control group (H&E). Sections of whole structures of the lung, such as bronchioles, alveoli (thin black arrows), and pulmonary vessels, showed a normal appearance. The appearance of the interstitial area was also normal. Thus, lung injury was scored as 0 in the control group.

Fig. 3b depicts the histopathological appearance of the lungs in the CLP model of sepsis (H&E). The lungs were characterized by diffuse, nodular, and dense inflammatory cell infiltrations, in addition to thickening of alveolar septa. These inflammatory cells were located in both interstitial and perivascular areas. There were also many neutrophils, lymphocytes, and necrotic cells, which had a hyperchromatic nucleus and eosinophilic cytoplasm, with hemorrhagic areas (Fig. 3b). Lung injury was scored as 3 in the CLP group.

The histopathological changes in the low-dose (CLP GOS 10) and high-dose (CLP GOS 20) groups are shown in Fig. 3c (H&E). The CLP GOS 10 group was characterized by significant neutrophil infiltration and slight bleeding, in addition to a decrease in the thickness of alveolar septa. Lung injury was scored as 2 in the CLP GOS 10 group. In contrast, in the CLP GOS 20 group, only a very small number of neutrophils and little bleeding were seen (Fig. 3d). The histological appearance of the CLP GOS 20 group was similar to that of the control group. Thus, lung injury was scored as 1 in the CLP GOS 20 group (Fig. 4).

3.3.1. Immunohistochemical results

Immunohistochemical staining with the NF-κB antibody showed severe positive staining in the CLP group and moderate staining in the control, CLP + GOS 10, and CLP + GOS 20 groups (Table 1, Fig. 5).

Immunohistochemical staining with the IL-6 antibody revealed moderate staining in the CLP group and a negative immune response and moderate staining in the CLP + GOS 10 and CLP + GOS 20 groups and mild immune positivity (Table 1, Fig. 5).

4. Discussion

This study investigated the protective effects of GOS against inflammatory lung injury in a CLP-induced polymicrobial sepsis model of rats. The effects of GOS on the inflammatory response of lung tissue due to sepsis were investigated by examining HMGß1, NLPR3, NF-κB, IL-1β,

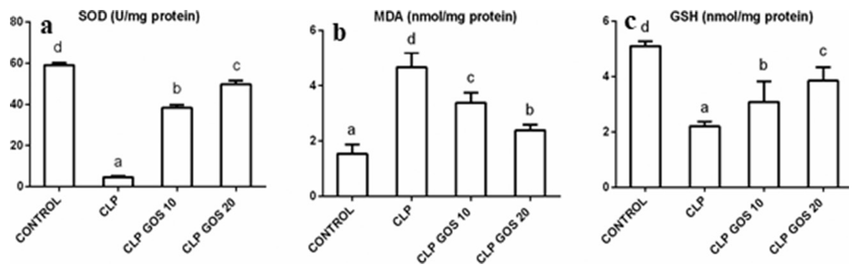

Fig. 1. Effects of gossipin (GOS), on lung SOD activity (a), MDA (b), and GSH (c) levels in septic rats. Each value is mean ± S.D. for six samples in each group. Values not sharing a common superscript differ significantly at P < 0.05 Duncan's Multiple Range Test (DMRT).

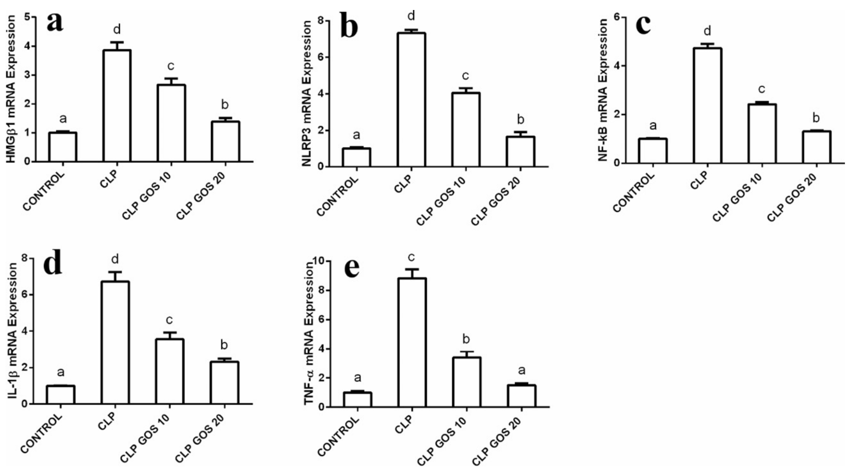

Fig. 2. Effects of gossypin (GOS) on lung HMGβ1 (a), NLRP3 (b), NF-κB (c) IL-1β (d) and TNF-α (e) mRNA expression levels in septic rats. The relative expression levels were calculated by the $2^{-\Delta\Delta}$Ct method. Each value is mean ± S.D. for six samples in each group. Values not sharing a common superscript differ significantly at $P < 0.05$ Duncan's Multiple Range Test (DMRT).

and TNF-α mRNA expression levels, as well as investigating the effects of GOS on oxidative status. In addition, the findings were supported by histopathological and immunohistochemical analyses.

As is well known, among natural antioxidants, flavonoids are the most effective [45,46]. Various studies have demonstrated the powerful anti-inflammatory and immuno-modulatory properties of GOS [31,46,47]. However, the factors that mediate the anti-inflammatory action of GOS remain unclear. Therefore, information on the effects of GOS on sepsis induced inflammatory lung injury and on its underlying anti-inflammatory mechanisms can guide the treatment of other inflammatory diseases.

The early stage of sepsis is dominated by hyperinflammation, mediated by the systemic production of inflammatory cytokines, including IL-1, IL-6, and TNF-α [48]. This cytokine storm can lead to organ damage and death in a subgroup of patients. The proinflammatory factor TNF-α, the first multifunctional cytokine produced from lipopolysaccharide-induced monocytes and macrophages, induces an inflammatory cascade and contributes to the severity of lung injury [49]. Previous studies showed that an increase in TNF-α levels led to an increase in free radicals [20]. In the present study, the levels of MDA,

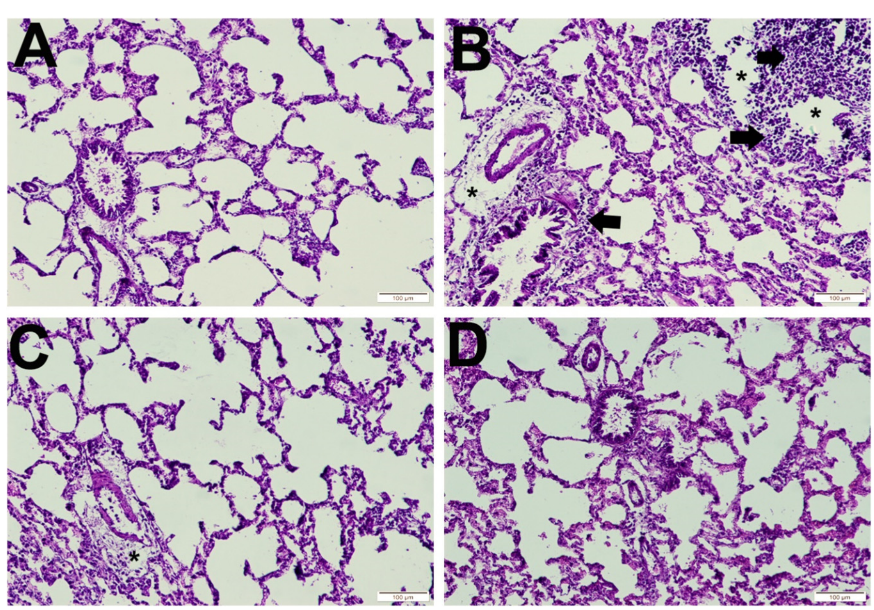

Fig. 3. Sample histopathological results of hematoxylin-eosin staining (*: edema, arrow: inflammation area. A) CONTROL, B) CLP, C) CLP + GOS 10, D) CLP + GOS 20 (each group consist of 6 rats).

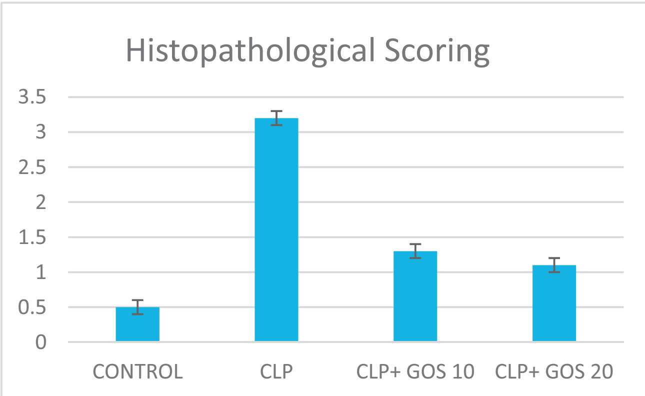

Fig. 4. Histopathological scoring of H&E staining. Inflamaturvar cells, alveolar septal thickness and edema based on the area, 0 (0% negative), 1 (0–33% mild), 2 (33 (66% moderate)), 3 (66 ve100% severe) as histopathological results (each group consist of 6 rats).

Table 1
Immunohistochemistry scoring results.

GROUPS	NFKB	IL-6
CONTROL	0	0
CLP	3	3
CLP + GOS 10	1	1
CLP + GOS 20	1	1

CLP: Sepsis, GOS 10 and GOS 20: Gossypin 10 mg/kg and 20 mg/kg. Grade 0: − (0% negative), Grade 1: + (0–33% mild positive), Grade 2: + + (33–66% moderate positive), Grade 3: + + + (66–100% severe positive).

endproduct of lipid peroxidation due to oxidative stress, increased in the presence of sepsis. Free oxygen radicals are an important defense mechanism against bacterial infections [50], and the production of free oxygen radicals is increased in response to sepsis [51]. In previous research, lipid peroxidation increased in patients with sepsis [52], whereas antioxidant enzymes decreased [53]. Increased oxidant levels in sepsis cause cellular damage by affecting proteins, lipids, and nucleic acids, leading to endothelial dysfunction [3,54]. Therefore, antioxidant treatment protocols are being studied experimentally and clinically in sepsis. In one study, MDA levels increased and GSH and SOD levels decreased in CLP-induced sepsis [55]. In another study, GOS treatment decreased TNF-α and IL-6 levels in rats with gentamicin-induced nephrotoxicity and increased kidney GSH levels and SOD activity [56]. Similar findings were obtained in the present study, with GSH and SOD levels increased in the GOS-treated groups when compared to septic group. Also, GOS administration to septic rats decreased lung MDA levels suggesting that GOS exerts antioxidant capacity in lung tissue damaged by sepsis. These findings can be related to both initial anti-inflammatory effects of GOS that resulted in improved oxidative status and own antioxidant properties of GOS during lung injury.

HMGβ1 acts as an extracellular signaling molecule and has extracellular effects on inflammation, cell differentiation, cell migration, and tumor metastasis [57]. Recent data research showed that it served as a critical transitional mediator in fatal systemic sepsis and that it possessed important extracellular functions [58]. A previous study that exploited in vivo models of sepsis reported that HMGβ1 circulated 8 h after the onset of sepsis and peaked after 16–32 h [59]. Sunden-Cullberg et al. reported that HMGβ1 levels were significantly higher in sepsis patients than in healthy controls [60]. In another study, an increase in HMGβ1 led to a subsequent increase in TNF-α, which resulted in the release of IL-6, reflecting co-activation between early inflammatory cytokines and HMGβ1 [61]. Thus, HMGβ1 acts as a regulator, or modulator, in the sepsis-induced organ response. In the present study, HMGβ1 was significantly increased in the CLP group, whereas the levels of HMGβ1 mRNA were significantly decreased in the GOS-treated groups. This finding suggests that GOS inhibits the inflammatory response of septic lungs by decreasing HMGβ1 and thus protects the lung tissue against ALI. Furthermore, the effect of GOS on HMGβ1 was not investigated before. The effects were shown for the first time in our study.

In vitro studies have shown that advanced glycation end-products [62] and toll-like receptor 4 (TLR4) [63,64] mediate the inflammatory response stimulated by HMGß1. In addition to low cerebral ischemia-reperfusion damage, mice with TLR4 deficiency exhibited down-regulation of inflammatory cytokines, pointing to an important role for TLR4 in the inflammatory response [65]. Recent studies indicated that HMGβ1 interacted with TLR4 and increased TLR-mediated NF-κB activation [66,67]. Studies also suggested that TLR4 receptors and NF-κB signaling pathways played a critical role in the inflammatory response [68–70]. NF-κB is an inducible nuclear transcription factor. It plays a central role in regulating the transcription of several genes, including those encoded by proinflammatory cytokines [71,72]. In previous studies, GOS exhibited anti-inflammatory activity in a carrageenan-induced paw edema model in rats [73] and inhibited the NF-κB activation pathway [47]. In addition to its potential role in the suppression of inflammation, GOS is thought to play a role in the suppression of carcinogenesis and angiogenesis. In the present study, the administration of GOS decreased HMGβ1, as well as NF-κB mRNA expression, in a CLP-induced sepsis models. Previous studies that investigated the role of NF-κB activation and signal transduction pathways in sepsis pathophysiology revealed that NF-κB acted as a transcription factor, which triggered the upregulation of cytokines, such as TNF-α and IL-6, to initiate the inflammatory response and apoptosis [18–20,28,74]. In an osteoclastogenesis study, GOS suppressed NF-κB [47]. In the current study, the expression of HMGβ1 and NF-κB mRNA increased in the lung tissue of rats with CLP-induced model of sepsis, and the administration of GOS decreased both these parameters. These results indicate that HMGβ1 can activate the TLR4 mediated NF-κB signal pathway and that both parameters return to normal as a result of suppression of the inflammatory response with GOS in the lung tissue. Thus, the levels of all pro-inflammatory mediators, including TNF-α and IL-1β, upregulated by the release of NF-κB were significantly reduced in the GOS-treated lungs.

Inflammasomes are multiprotein signaling complexes that trigger the activation of inflammatory caspases and the maturation of IL-1β. Among various inflammasome complexes, the NLRP3 inflammasome is best characterized and has been linked with various human autoinflammatory and autoimmune diseases. The NLRP3 inflammasome is thought to be a promising target for anti-inflammatory therapies [75]. The NLRP3 inflammasome responds to activating signals through a two-step activation model. The first step is typically triggered by ligand binding (e.g., lipopolysaccharide) to TLRs (e.g., TLR4) and related receptors. This results in the activation of NF-κB, which translocates to the nucleus and activates the transcription of inflammasome components, including NLRP3, and pro forms of inflammasome-related cytokines (i.e., pro IL-1β and pro IL-18) [76]. In human studies, the expression levels of inflammasome-related caspase-1, IL-18, and IL-1β messenger RNA transcripts in plasma from critically ill patients with sepsis-induced ARDS were significantly higher than those in patients with systemic inflammatory response syndrome (SIRS) [77] suggesting that sepsis induced lung damage might be related with inflammasome activation. Different from sepsis induced ARDS, SIRS can be related to many other inflammatory situations such as rheumatic diseases and is not always life threating like sepsis induced organ dysfunction [4]. Importantly, circulating IL-18, which is associated with NLRP3 inflammasome activation, was significantly elevated in plasma from critically ill human patients with sepsis and sepsis-induced ARDS and significantly associated with intensive care unit mortality [77,78]. In light of these data, we evaluated lung NLRP3 inflammasome mRNA expression as a marker of inflammation in septic lungs. The results

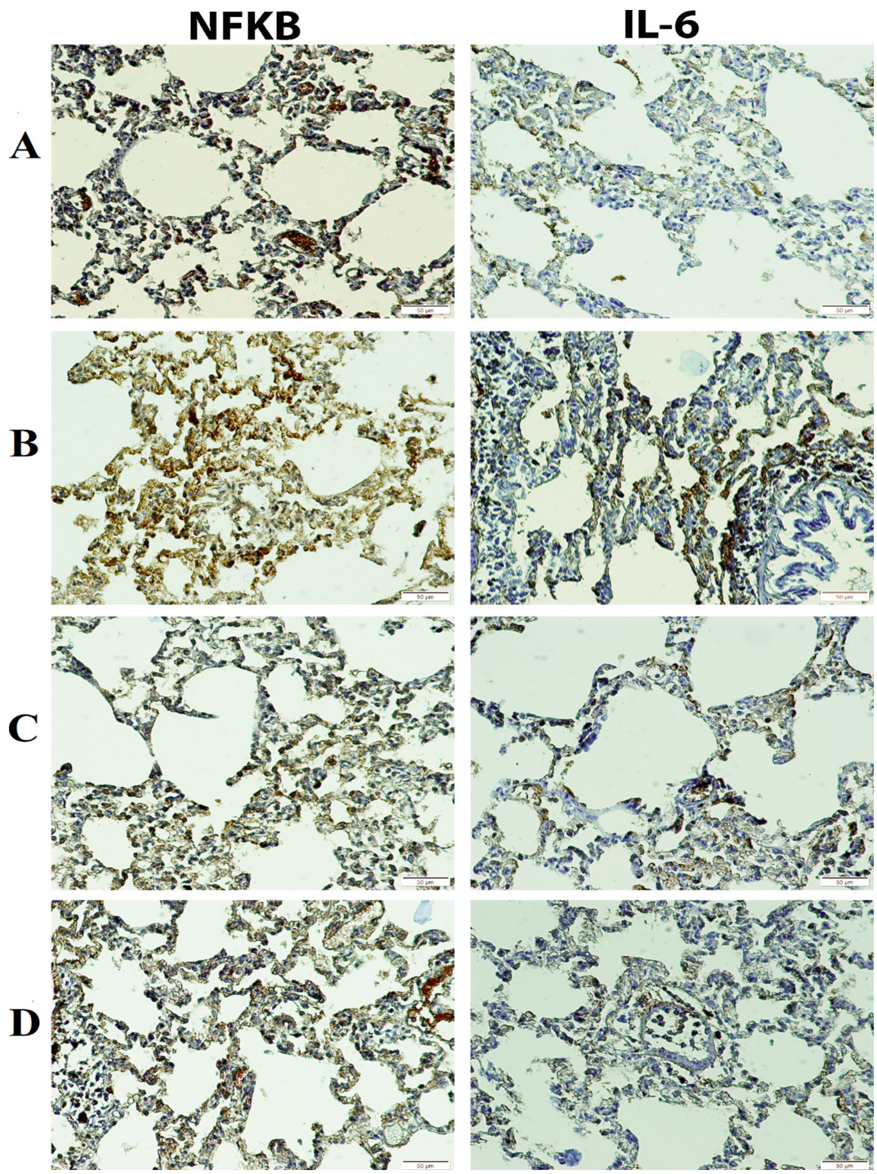

Fig. 5. Immunohistochemistry results of IL-6 and NF-κB Ab dyed lung sections A) CONTROL, B) CLP, C) CLP + GOS 10, D) CLP + GOS 20 (each group consist of 6 rats).

demonstrated dose-related inhibition of NLRP3 activation that led to decreased IL-1β release by GOS, which has not been shown previously. Dose-related inhibition of NLRP3 activation can related to decreased levels of HMGβ1 and NF-κB, which stimulated a TLR4 response in the septic lungs. Previously Yu et al. demonstrated that inhibition of HMGB1 improves necrotizing enterocolitis, which is an inflammatory condition as well as sepsis induced lung injury, by inhibiting NLRP3 via TLR4 and NF-κB signaling pathways [79]. We propose that GOS exerts anti-inflammatory effects in septic lungs by suppressing the NF-κB stimulated TLR4 pathway, thereby resulting in decreased HMGβ1, NLRP3, and proinflammatory cytokine levels. These results show that GOS exert protective effect in lung tissue damaged by sepsis via anti-inflammatory and antioxidant effects. Also, the histopathological and immunohistological results in the present study confirmed the molecular and biochemical findings. Histopathological damage of lung tissues induced by sepsis was decreased in the GOS-treated groups.

5. Conclusion

This study demonstrated for the first time that GOS provides protection against acute lung injury caused by CLP-induced sepsis. Although this effect may be due to the strong antioxidant properties of GOS, it may also be related to the suppression of the aggravated inflammation cascade, which causes severe damage, thus reducing the levels of NF-κB, HMGβ1, and NLRP3 and preventing a severe cytokine storm. The results suggest that the administration of GOS may represent a novel treatment for the prevention of inflammatory lung damage due to septic conditions.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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