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Original Article

Protective effect of taurine on lipopolysaccharide induced acute lung injury in mice

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Abstract: Acute lung injury (ALI), a serious respiratory distress has been associated with considerable human morbidity and mortality. Taurine, a physiological semi-essential amino acid has been shown to be tissue-protective in several models of oxidant-induced injury. Therefore, the current study was designed to explore protective effects of the taurine in ALI mice models. The possible tissue protective effects of taurine were evaluated in ALI mice models by investigating the lung water content, cell counts, pro-inflammatory and anti-inflammatory cytokine and levels antioxidant defence system in the broncho-alveolar fluid (BALF). Lung tissue histopathology has also been examined to investigate the degree of protection or tissue damage in ALI mice models. Treatment taurine to ALI mice models significantly reduced lung water content, lung inflammation as evidenced by decrease in neutrophil migration into BALF. Additionally, treatment with taurine significantly ($P < 0.05$) inverted the LPS-induced inhibition of IL-10 in the lung tissues LPS treated animals compared to saline treated animals. Treatment with taurine to LPS challenged mice reversed LPS induced oxidative stress, as demonstrated by a rise in the anti-oxidant biomarker ratio of reduced/oxidized glutathione (GSH/GSSG ratio) and T-AOC, CAT and SOD activities. Moreover, taurine treatment significantly prevented the LPS induced lung tissue injury induced by LPS challenge in ALI mice. Taurine treatment to ALI mice models conferred protection against acute LPS induced lung injury, which may be attributed to its potential role in the anti-oxidant and anti-inflammatory properties. Therefore, the present study merits further exploration of the clinical applicability of taurine in the prevention and treatment of ALI.

Keywords: Taurine, acute lung injury, inflammatory, antioxidant

Introduction

Acute lung injury (ALI) is a clinical manifestation of acute respiratory failure with substantial leads significant mortality and morbidity in humans. Subjects who survive ALI, their long-term quality of life is badly affected [1, 2]. This acute respiratory distress is more common across the globe. Both ALI and its more serious form, acute respiratory distress syndrome (ARDS), are severe and acute respiratory dysfunctions with both the diseases causing the large irresistible lung inflammation.

Migration of neutrophils in lung of patients with ALI, intravascular coagulation, loss in capillary integrity results in pulmonary edema, and elevated shunt function are main characteristics of this disease. In the recent past, several aspects including understanding of the epidemiology, pathogenesis, and treatment of this disease have been made. However, significant problems need to be challenged to further reduce mortality and morbidity from ALI. Number of therapeutic lines which are directed for the control of inflammatory responses, such as inflammatory cytokines [3], adhesion molecules [4], the compliment system, 6 and oxygen radicals [5] have been widely explored. Additionally, oxidative stress has been evidenced to plays an major role in regulating lung injury. Antioxidant such as N-acetyl cysteine mitigates pulmonary function in ARDS patients [5, 6]. Bacterial lipopolysaccharide (LPS), present in the outer membranes of Gram-negative bacteria, is an endotoxin leads to generation of reactive oxygen species (ROS) and inflammatory mediators and believed to induce pharmacological research models of ALI [7, 8]. LPS is one of the major factors that induce acute lung injury.

Oxidative stress in ALI leads to an increase in the production of ROS, myeloperoxidase (MPO) and reduces the creation of anti-oxidative enzymes, including reduced glutathione (GSH) and superoxide dismutase (SOD), which together protect lung tissues against oxidative damage in vivo [9]. Taurine, a semi-essential sulphur-containing β-amino acid that is not incorporated into proteins. This amino acid is present at high concentrations (e.g., mM range) in excitable cells and tissues where oxidants are generated [10]. Additionally, taurine has been demonstrated to prevent cells damage in a variety of model systems involved in inflammation by mechanisms not completely understood [11]. For example, taurine protected against endotoxin-induced hepatocyte damage by modulating the production of nitric oxide, oxygen radicals, as well as peroxynitrite formation [12].

ALI is reasonably an unmet medical need with treatment limited only to supportive care [13]. Therefore new therapies need to be explored to further improve clinical outcomes of this disease. In the present study we have explored the possible protective effects of taurine in ALI mice models.

Materials and methods

Experimental animals

Female BalB/C mice about seven week old weighing 22 ± 3 g were used in the study. The mice were given free access to pellet diet and water. Animals were kept in well ventilated rooms with controlled setting of light/dark cycle, temperature of 24 ± 2 and humidity of 40-60%. The animal protocols for the study were approved by animal ethical committee of the institute.

Animal grouping and acute lung injury model

Animals were randomly divided into 5 groups with 10 mice/group: group I consisted of normal control mice that were administered normal saline, group II animals were treated with taurine only (at the dosage of 100 mg/kg body weight) dissolved in phosphate buffer saline (PBS, 0.1 M, pH 7.4), group III consisted of animals that were administered LPS only (0.5 mg/kg, dissolved in saline), group IV consisted of animals that were administered LPS (0.5 mg/kg body weight) + Taurine (50 mg/kg body weight) and group V consisted of animals that were administered LPS (0.5 mg/kg body weight) + dexamethasone (Dex, 5 mg/kg body weight dissolved in saline). Both taurine (100 mg/kg body weight) and Dex (5 mg/kg body weight) were administered *via* intrapertoneal (i.p) route. One hour after exposure to taurine or Dex, animals were dosed via i.p injection with pentobarbital sodium (90 mg/kg body weight) to induce anaesthesia. Successively, LPS was administered intranasally (i.n.) to induce lung injury. After 2 administration of LPS, animals were euthanized.

Lung tissue and BALF extraction

Immediately after the animal euthanization, extractions of BALF (Broncho-alveolar lavage fluid) and lung tissue were performed as reported earlier [14]. Lungs tissues were either snap frozen in liquid N_2 for preparation of tissue homogenates or inflation fixed in situ at 25 cmH_2O with 4% paraformaldehyde for histological examination. The BALF and lung tissue samples were used to estimate/determine different biochemical parameters.

Measurement of lung water content and cell count of BALF

To monitor the lung water content, the extracted lungs tissues were first weighed and then dried by the procedure as reported previously [15]. Weight of wet lungs - weight of dry lung/weight of animal was calculated (n=4 in all group). The cell count of the pooled lung lavage fluid were determined by hemocytometer (n=4 in all group). Cells in the BALF were then harvested by centrifugation at 1,000×g for 20 minutes to pellet down the cells and the protein quantification of the BALF freed of cells was determined by the bicinchoninic acid method (Pierce Biotechnology, Rockford, IL). The supernatant samples were used immediately or stored at -80°C for later use.

Cytokine and chemokine analysis

The BALF supernatant was used for quantifying the levels of cytokines such IL-6, IL-8 and anti-inflammatory cytokine IL-10. Cytokine levels were measured using commercially available kits (Jiancheng Bioengineering Institute, China) carefully according to the manufacturer's written instructions.

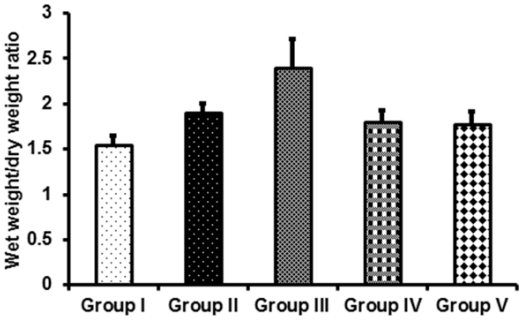

Figure 1. Water content in the lungs of normal or ALI models of mice 24 hours following the intranasal administration of vehicle or LPS (n=5). Lung tissues were dried by keeping the tissues at 60°C for 48 hours.

Myeloperoxidase assay

MPO activity in the BALF was measured to assess infiltration of neutrophils in the lung tissues of LPS-induced ALI mouse [16]. To assay MPO activity, mice lungs were chopped and then homogenized in PBS containing 0.5% hexadecyltrimenthyl ammonium bromide. Cell homogenate was harvested by centrifugation. To the supernatant, phosphate buffer (pH=6.0) containing 0.167 mg/Ml *o*-dianisidine hydrochloride and 0.0005% hydrogen peroxide were added. The MPO activity of the supernatant was measured spectrophotometrically by taking the absorbance at 460 nm.

Antioxidant assays

Catalase (CAT), superoxide dismutase (SOD), Total anti-oxidant capacity (T-AOC) glutathione (GSH) and were determined by the slight modifications of the well-established protocols as reported by Aebi, 1984, Gunzler and Flohe 1986, Rubio et al., 2016, Buege and Aust 1978 respectively.

Histopathological analysis

For histopathological analysis, lungs were washed twice with PBS and fixed in 4% paraformaldehyde at 4°C for 24 h. Afterwards, lung tissues were embedded in paraffin and then sectioned. Subsequently, sectioned tissues were stained with haematoxylin and eosin (HE staining). Pathological changes, such as inflammatory cell infiltration and edema, were observed under Ti-S bright field microscope (Nikon, Melville, NY, USA) [17].

Statistical analysis

All of the above discussed data were expressed as mean ± standard error of the mean (S.E.M.) at least for n=4, and data was analysed using SPSS19.0 (IBM). Comparisons between experimental groups were conducted using one-way ANOVA and Newman-Keuls multiple- range test while multiple comparisons were made using the LSD method. *$P < 0.05$ or **$P < 0.01$ were taken as an indication of a statistically significant difference.

Results

Effect of taurine on lung water content

To determine the role of taurine in lung oedema induced by LPS, we measured the lung water content 24 hours after the intranasal administration of LPS (**Figure 1**). A noticeable increase in lung water content was observed in the group III animals (LPS treated) compared to group I (saline treated) and group II animals (treated with taurine only) with $P < 0.01$. Water content in the lungs of group IV (LPS+ tourine treated) and Group V (LPS+ Dexamathasone) were more or less similar but highly significant from group II animals ($P < 0.05$).

Effect of taurine on lung inflammation and cell count

To define lung inflammation related to neutrophil induced by LPS, cell profile of BALF was determined 24 h succeeding intranasal instillation. LPS treatment to group III animals prompted a great escalation in the total cells (**Figure 2A**) and neutrophil counts (**Figure 2B**) compared to group I animals ($P < 0.05$). However, total cells (**Figure 2A**) and neutrophil counts (**Figure 2B**) in the taurine treated animals were observed to be similar to that of group I animals. In group IV and group V animals, the above cell counts were more or less similar but statistically significant from group IV animals (**Figure 2A** and **2B**) ($P < 0.05$).

Effect of taurine on pro-inflammatory and anti-inflammatory cytokines

Taurine treatment to ALI mice models decreased the elevated IL-6 and IL-8 levels induced by LPS in lung (**Figure 3**). Furthermore, treatment with LPS significantly ($P < 0.05$) inverted the LPS-induced inhibition of IL-10 in the lung

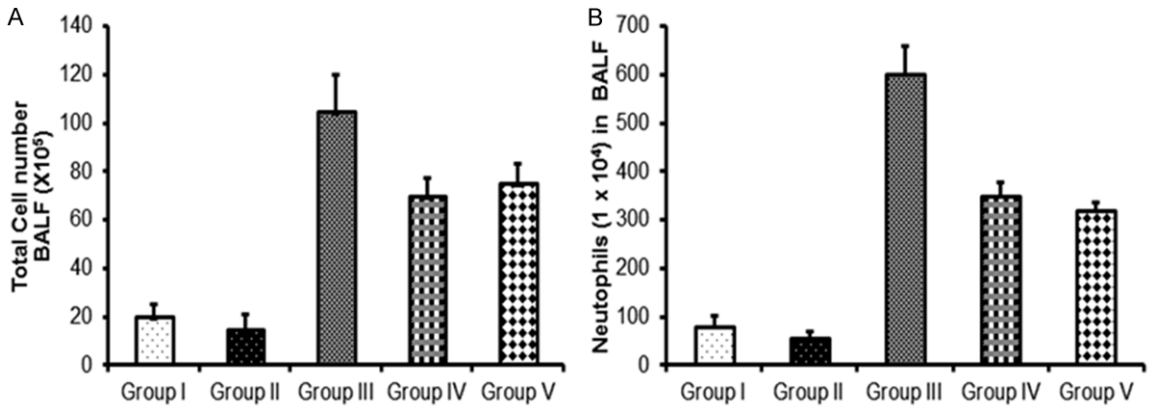

Figure 2. Water content in the lungs of normal or ALI models of mice 24 h after intranasal instillation intranasal instillation with LPS (A) total cell count of the BALF in normal and ALI mice. Total cell count was determined using the haemocytometer (Invitrogen, Waltham, MA, USA) and (B) neutrophil count in the BALF (n=5 for each group).

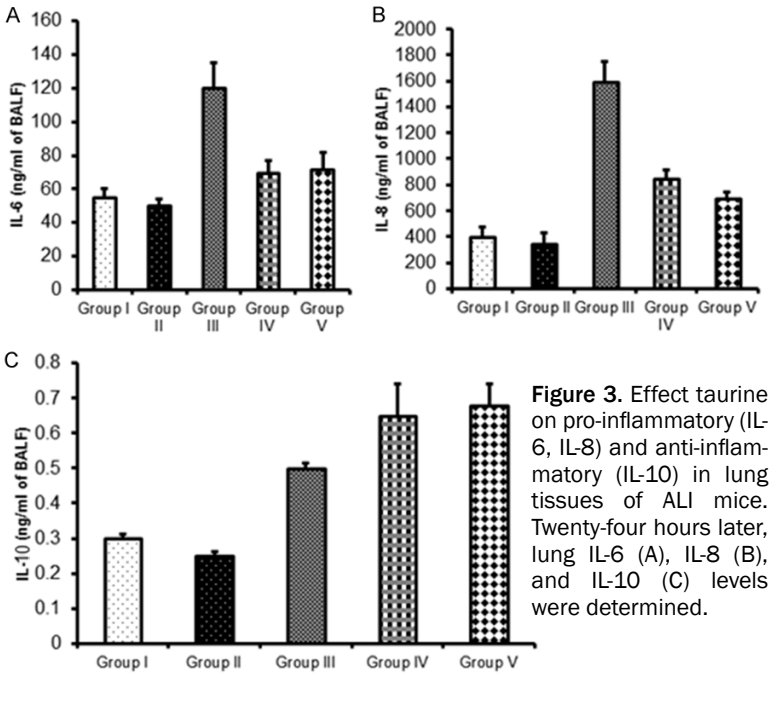

Figure 3. Effect taurine on pro-inflammatory (IL-6, IL-8) and anti-inflammatory (IL-10) in lung tissues of ALI mice. Twenty-four hours later, lung IL-6 (A), IL-8 (B), and IL-10 (C) levels were determined.

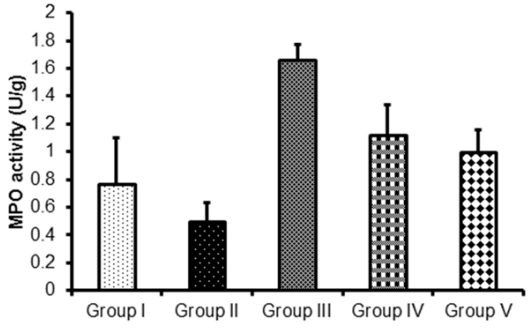

Figure 4. MPO activity monitored in the lung tissues of normal or ALI mice models treated (n=5).

tissues in group III animals compared to group 1 animals. Additionally, the BALF levels of pro-inflammatory cytokines (IL-6 and IL-8) and anti-inflammatory cytokine (IL-10) in group IV and Group V were almost similar.

Effect of taurine on myeloperoxidase activity

Myeloperoxidase (MPO) activity is an important marker of neutrophil infiltration. Neutrophil infiltration was then determined by measuring the MPO activity in lung homogenates of ALI mice (**Figure 4**). It was observed that the LPS increased the MPO activity by at least 2 folds in group III mice compared to group I (saline treated). Taurine significantly reduced the MPO activity in a group IV mice compared to group III (LPS treated) animals ($P < 0.01$). These results suggest that tourine has a favourable effect to prevent of neutrophil migration into the alveolar space.

Oxidative stress

The effect of taurine on several anti-oxidant biomarkers, including the GSH/GSSG ratio, T-AOC activity and the activities of two important anti-oxidant enzymes CAT and SOD were

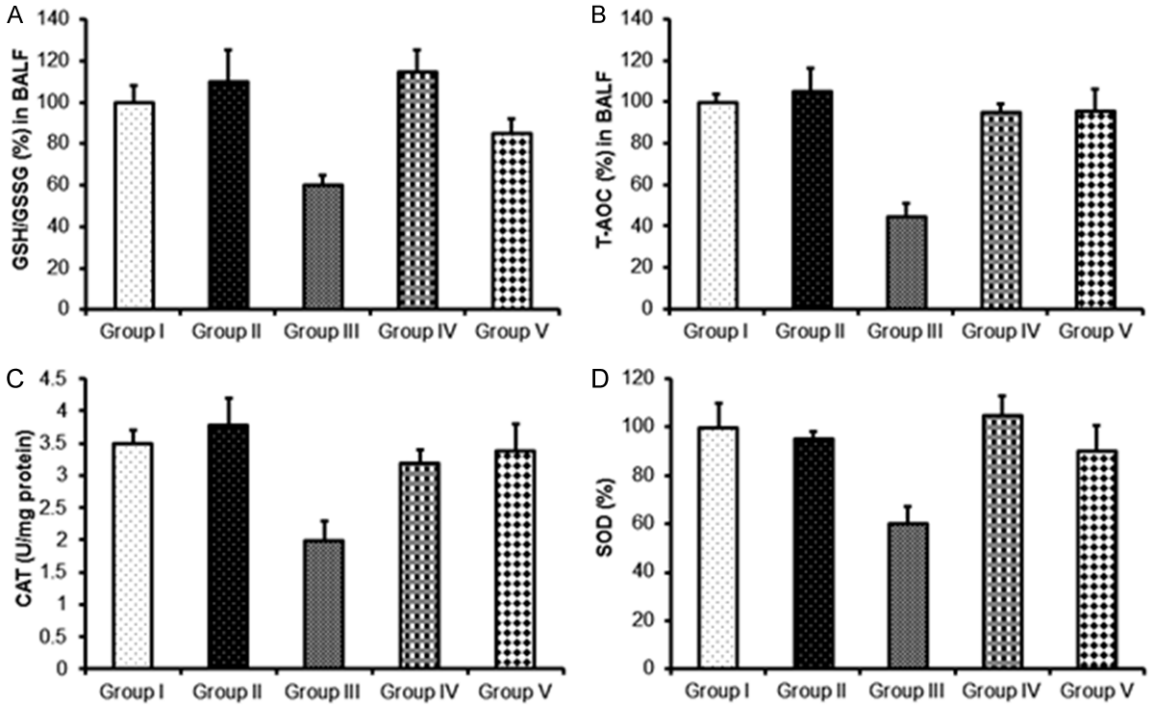

Figure 5. Effect of taurine on antioxidant defence systems in normal and ALI mice models. 24 h after LPS challenge lung GSH/GSSG ratio (A), T-AOC level (B), CAT activity (C) and SOD activity (D) were determined (n=4).

evaluated. We have observed a considerable reduction in the GSH/GSSG ratio and the activity of T-AOC, CAT and SOD were reflected in the animals challenged with the LPS compared with group I (saline treated) animals (**Figure 4A-D**). Additionally, touring treatment significantly attenuated the LPS-induced reduction of all of these anti-oxidative biomarkers. The LPS administration significantly reduced the activity of T-AOC (**Figure 4B**), CAT (**Figure 4C**) and SOD (**Figure 4D**) in lung ALI animal models but GSH/GSSG ratio remained unaffected (**Figure 4A**).

Histological changes in lung tissue sections

For histology examination, sections of lung tissue were H&E stained. **Figure 6A** shows photomicrographs of the normal histology of lungs. The administration of LPS resulted in diffuse interstitial edema, alveolar thickening, extensive leukocyte infiltration into the interstitium and alveoli, and a marked decrease in alveolar air space in group III animals (**Figure 6C**). Treatment with taurine to group IV ALI mice models significantly attenuated these pathological changes in ALI mice compared to group III.

Discussion

LPS in ALI mice models often leads to pulmonary oedema therefore, we estimated the lung water content 24 hours after the intranasal instillation of LPS or saline to mice (**Figure 1**). Taurine treatment to LPS challenged mice significantly ($P < 0.01$) water content of group IV mice compared to group I (saline) mice indicating the role of taurine in relieving the lung oedema. No significant difference in lung water content was observed between group IV and group V animals that were treated with dexamethasone suggesting that the taurine and dexamethasone exert similar protective effects in ALI mice models that were challenged with LPS.

Lung oedema, endothelial and epithelial injury is associated with an influx of neutrophils into the interstitium and broncheo-alveolar space. Neutrophils play an important role in the progression of ALI and ARDS [18], as activation and transmigration of neutrophils is a hallmark event in the progression of ALI and ARDS. Therefore, we have evaluated the total cell count and number of neutrophils in the BALF of normal and control mice (**Figure 2**). Intranasal instillation of LPS to group III mice significantly

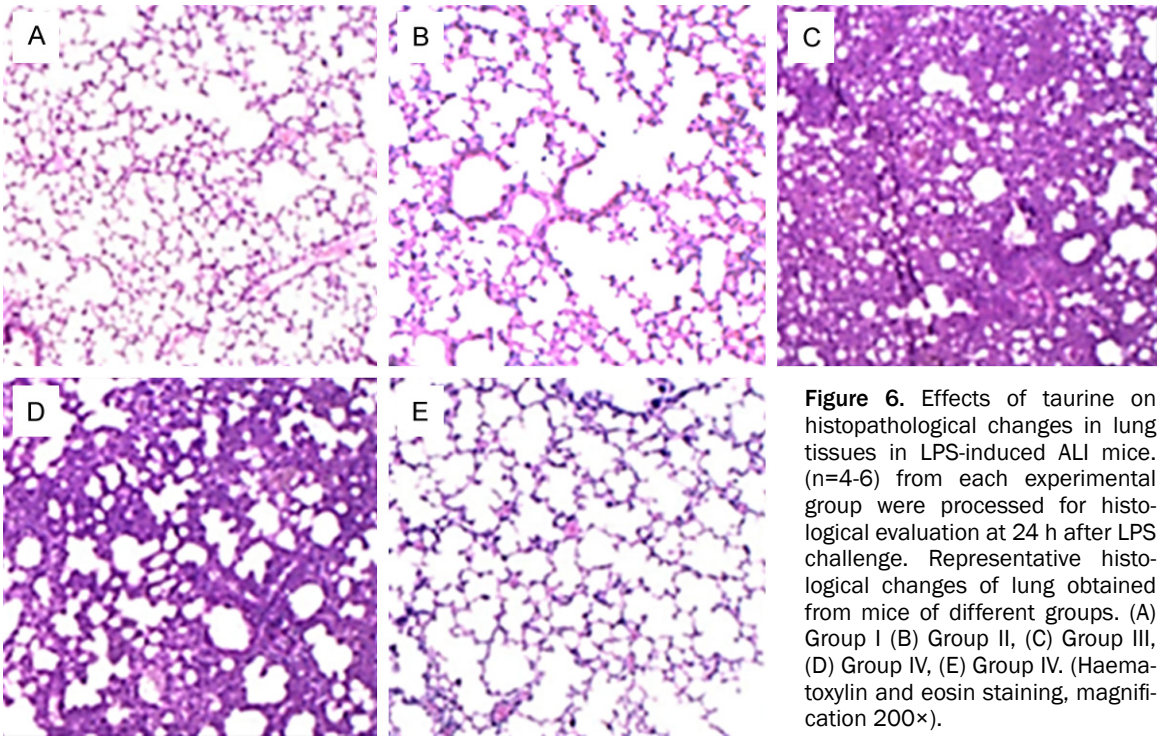

Figure 6. Effects of taurine on histopathological changes in lung tissues in LPS-induced ALI mice. (n=4-6) from each experimental group were processed for histological evaluation at 24 h after LPS challenge. Representative histological changes of lung obtained from mice of different groups. (A) Group I (B) Group II, (C) Group III, (D) Group IV, (E) Group IV. (Haematoxylin and eosin staining, magnification 200×).

($P < 0.05$) increased the total cell count (**Figure 2A**) and neutrophil count (**Figure 2B**) compared to group 1 animals. Taurine treatment to LPS challenged group IV mice significantly ($P < 0.01$) prevented the rise in total cell count and neutrophil cell number in the BALF of ALI mice models. These results indicate that taurine prevented the migration of neutrophil into the lung alveolar space, which could be due to the decrease in chemoattractants in the lung tissues in response to acute lung injury induced by LPS.

In a number of experimental and clinical investigations, it has been indicated that a complex network of inflammatory cytokines and chemokine's have a major role in the onset of inflammatory-induced lung injury from aspiration, sepsis, pneumonia, and shock [19]. In the current study we have shown via quantitative analysis that pro-inflammatory cytokines (IL-6 and IL-8) were increased in the LPS groups compared with control groups; however, the level of IL-10, an anti-inflammatory cytokine was reduced in the LPS treated mice (**Figure 3A-C**). Thus, suggesting the negative modulation of pro-inflammatory factors, including IL-6 and IL-8, and a positive modulation of the anti-inflammatory factor IL-10. Furthermore, we measured the MPO activity, a widely used marker for neutrophil activity. Our results indicated that treatment with LPS increased the MPO levels (**Figure 4**). Thus suggesting that the inflammation induced by LPS administration has a role in the pathogenesis of lung injury mice.

Oxidative stress also induces inflammatory responses which in turn induce the generation of ROS. Both, oxidative stress and inflammation are recognized as interconnected events and both are involved in the pathogenesis of ALI [20]. In earlier studies, it has been reported that LPS intranasal instillation triggers the release of excessive cytokines, chemokines and ROS, thus inducing an ALI model with similar pathological features to ALI in humans [21]. Loss in homeostasis between pro-oxidants and anti-oxidants is often attributed to have role in oxidative stress [22]. Therefore, the current study was designed to further explore the effect of taurine on several anti-oxidant biomarkers, including the GSH/GSSG ratio, CAT and SOD. Considerable reduction in the GSH/GSSG ratio and the activity of catalase (CAT) and superoxide dismutase (SOD) were observed in LPS challenged mice compared with control mice (**Figure 5A-D**). In addition, treatment with tau-

rine significantly diminished the LPS-induced reduction of all of these anti-oxidative biomarkers. The LPS challenge significantly reduced the activity of T-AOC (**Figure 5B**), CAT (**Figure 5C**) and SOD (**Figure 5D**) in lung tissues of mice but not the GSH/GSSG ratio (**Figure 5A**).

The administration of LPS resulted in diffuse interstitial edema, alveolar thickening, extensive leukocyte infiltration into the interstitium and alveoli, and a marked decrease in alveolar air space in LPS challenged mice (**Figure 6**). Treatment with tourine to group ALI mice models significantly attenuated these pathological changes in ALI mice.

Conclusion

Taurine treatment to ALI mice models conferred protection against acute LPS induced lung injury, which may be attributed to its potential role in the anti-oxidant and anti-inflammatory properties. Therefore, the present study merits further exploration of the clinical applicability of tourine in the prevention and treatment of ALI.

Disclosure of conflict of interest

None.

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