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Quercetin Exerted Protective Effects in a Rat Model of Sepsis via Inhibition of Reactive Oxygen Species (ROS) and Downregulation of High Mobility Group Box 1 (HMGB1) Protein Expression

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Sepsis is a severe medical condition. Approximately 0.75 million people are diagnosed with sepsis in the USA annually. Several of anti-inflammatory drugs are used to manage sepsis, but with a very low success rate. This study examined the possible protective effects of a naturally occurring flavanone, quercetin, in a rat model of sepsis.

Material/Methods: The study was carried out using Wistar albino rats. Sepsis was induced by cecal ligation and puncture methods. Histological analysis was performed by hematoxylin and eosin (HE) staining. Reactive oxygen species (ROS) levels were determined by flow cytometery. Superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) activities were determined by standard assays. Protein expression was determined by Western blot analysis.

Results: The results showed that quercetin reduced the tissue edema, congestion, and hemorrhage, increased the alveolar volume, and helped to maintain the lung anatomy of septic rats. Admistration of quercetin at the dosage of 15 and 20 mg/kg to septic rats caused significant reduction in the ROS levels. The activities and the expression of SOD, CAT, and APX were significantly decreased upon administration of quercetin in the septic rats at the dosage of 15 and 20 mg/kg. The effects of quercetin were also examined on the expression of the High mobility group box 1 (HMGB1) protein in septic rats. The results showed that quercetin caused a significant decrease in HMGB1 protein levels.

Conclusions: The findings of this study suggest that quercetin has therapeutic potential in the treatment of sepsis.

MeSH Keywords: Reactive Oxygen Species • Sepsis • Superoxide Dismutase

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Background

Sepsis is a severe medical condition that is the result of intense systemic inflammatory response to an infection, loss of blood, trauma, or neoplasm [1]. Owing to its clinical presentations, it is often challenging to manage [2]. Sepsis may result in multi-organ dysfunction, shock, and death. In critically ill patients, sepsis is a leading cause of death in the USA [3], where approximately 0.75 million people are annually diagnosed with sepsis, and of these, about 0.21 million die [4]. Several anti-inflammatory drugs are used to manage sepsis, but with a very low success rate. It is believed that defining the molecular mechanisms underlying this disease may enable more effective treatment [5]. Over the years, after facing the prolonged impact of industrialization, utilizing extracted edibles and consuming highly active/chemically synthesized/highly specific drugs, there is a growing focus on simplicity and people started opting for natural or close to natural foods and drugs. Moreover, many studies have shown the pharmacological correlation with ayurvedic/alternative medicines. It is important to note that chemically synthesized drugs are generally improved derivatives of prototypes of herbal isolated drugs [6]. Moreover, it has been observed that medicinal plants or extracts, used as co-adjuvant or as alternate therapy to allopathic medicine, are effective and have few adverse effects, as well as being low cost and locally available [7]. Flavonoids are ubiquitous plant secondary metabolites found in plants. They have been shown to exhibit huge pharmacological potential [8]. Due to the structural features of flavonoids, they have shown the potential to interact with different cell types and as such may prove beneficial in the treatment of human diseases [9]. In this study, the effects of a naturally occurring flavonoid, quercetin [10], were examined in a sepsis rat model. The results showed that quercetin may prove beneficial in the treatment of sepsis and warrants further investigation.

Material and Methods

Treatment groups

Twenty-eight 15-week-old Wistar albino rats were obtained from the Animal House of the Shengli Oilfield Central Hospital. The rats were maintained at standard laboratory conditions with a light/dark cycle of 12 h. Food and water were available ad libitum. The rats were randomly divided in 4 groups. Group I (control) consisted of normal rats administered normal saline. Group II consisted of rats in which sepsis was induced by cecal ligation and puncture methods, as described previously [11]. Group III and Group IV consisted of septic rats administered 15 and 25 mg/kg of quercetin, respectively. The Animal Ethics Committee of Shengli Oilfield Central Hospital approved the study (approval number SOCH/IV/992, 2018). At the end of the study, the animals were sacrificed by ketamine/xylazine anesthesia. The lung tissues were extracted and stored at –80°C for further experimentation.

Determination of SOD, APX, and CAT activities

SOD, APX, and CAT activities were determined in the lung tissues, as described previously [12–14]. The enzyme activities were then expressed as units/mg protein.

Hematoxylin and eosin (HE) staining

Lung tissue specimens were washed in ice-cold saline and then fixed in 10% formalin solution for 24 h. Following the embedding of the tissues in paraffin, they were cut into 5-mm sections, followed by HE staining. Finally, the tissues were analyzed by a pathologist using a light microscope.

Estimation of reactive oxygen species (ROS)

Cells were collected from the lung tissues of the 4 rat groups and treated with dihydrofluorescein diacetate (10 μM) at 37°C in the dark for 35 min. Samples were then assessed using a flow cytometer to determine ROS levels.

Western blot analysis

The lung tissues were then lysed in lysis buffer containing the protease inhibitor. Around 45 μg of proteins from each sample were subjected to 10% separation, followed by transfer to a polyvinylidene difluoride (PVDF) membrane. Next, fat-free milk was used to block the membrane at room temperature for 1 h. Thereafter, the membranes were treated with primary antibodies (SOD, CAT, and APX, all obtained from Santa Cruz Biotechnology, Dallas, TX) at 4°C overnight. Subsequently, the membranes were incubated with secondary antibodies. Finally, the signal was detected using the Odyssey Infrared Imaging System. Actin was used as control for normalization.

Statistical analysis

All experimental procedures were performed in 3 biological replicates. The values obtained are presented as mean of these 3 replicates ±SD. * $P<0.05$, ** $P<0.01$, and *** $P<0.001$ were considered statistically significant. Results were assessed using the t test, and statistical analysis was performed using GraphPad prism 7 software.

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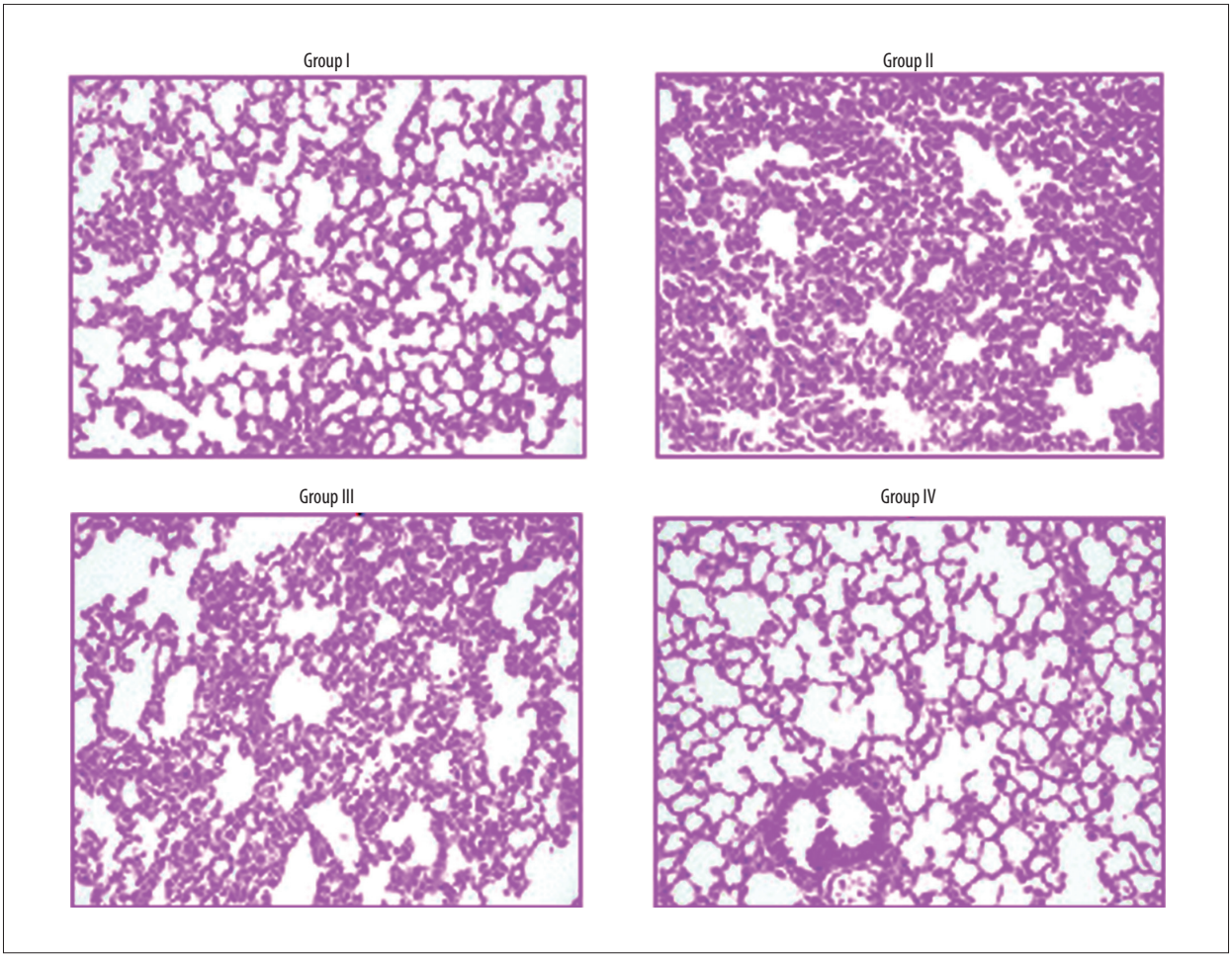

Figure 1. Histological analysis of the lung tissues of different treatment groups by HE staining. Group I (control) consisted of normal rats administered normal saline and Group II consisted of septic rats. Group III and Group IV consisted of septic rats administered 15 and 25 mg/kg of quercetin, respectively. The experiments were performed in triplicate.

Results

Quercetin prevents septic lung injury

The effects of quercetin were examined on the lung injury induced by sepsis, showing that septic rats had severely injured lungs. The lung tissues showed tissue edema, decreased alveolar volume, congestion, and hemorrhage. Treatment of the septic rats with 15 and 25 mg/kg of quercetin significantly prevented or reversed the lethal effects of sepsis on lung histology (Figure 1). Quercetin administration also markedly decreased damage to lung parenchymal tissue (Figure 1).

Quercetin caused decreases ROS levels in lung tissues

As ROS has been implicated by in sepsis-induced lung injury, flow cytometery was used to examine the ROS levels in all the rat groups. The results showed that septic rats showed markedly higher levels of ROS levels (Group II) in comparison to the normal rats (Group I) (Figure 2). However, administration of quercetin at 15 and 25 mg/kg resulted in significant decreases in the ROS levels in lung tissues of septic rats (Group III and IV) (Figure 2). Taken together, these results suggest that quercetin causes inhibition of the sepsis-induced oxidative stress.

Quercetin caused decrease in the ROS scavenging enzyme expression

The effects of quercetin were also examined on the expression of the ROS scavenging enzymes (SOD, CAT, and APX) by Western blot analysis. The results showed that administration of quercetin at the dosage of 15 and 5 mg/kg to septic rats caused a significant increase in SOD, CAT, and APX expression levels (Figure 3).

Quercetin enhanced the SOD, APX, and CAT activity

Standard assays were used to assess the enzyme activities of SOD, APX, and CAT activity. The results showed the activity of

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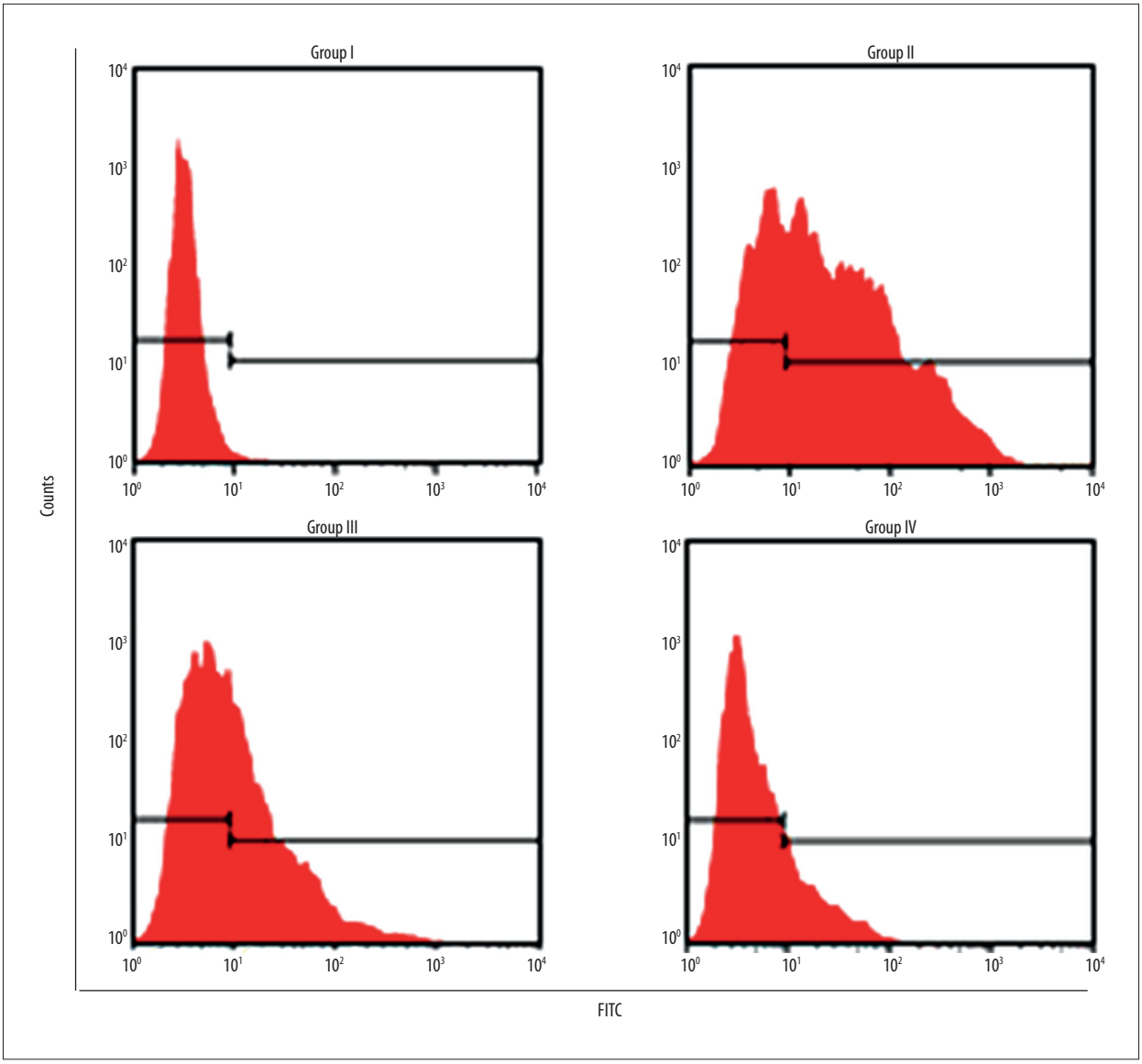

Figure 2. ROS levels in the lung tissues of different treatment groups as determined by flowcytometric analysis. Group I (control) consisted of normal rats administered normal saline and Group II consisted of septic rats. Group III and Group IV consisted of septic rats administered 15 and 25 mg/kg of quercetin, respectively. The experiments were performed in triplicate.

SOD, APX, and CAT were significantly decreased in the septic rats (Group II) as compared to the normal rats (Group I). Administration 15 and 25 mg/kg of quercetin to the septic rats (Group III and IV) caused a significant enhancement in the activity of all 3 antioxidant enzymes (Figure 4A–4C).

Quercetin decreased high mobility group box 1 (HMGB1) protein expression

The expression of the HMGB1 was assessed in all treatment groups by Western blot analysis. The results showed that the expression of HMGB1 was significantly increased in the septic rats (Group II) in comparison to the normal rats (Group I). Moreover, administration of 15 mg/kg (Group III) and 25 mg/kg (group IV) of quercetin resulted in significant decreases in the expression of HMGB1 (Figure 5).

Discussion

Sepsis and septic shock are considered common causes of mortality in intensive care units [15]. Sepsis can cause severe medical conditions such as multi-organ dysfunction and death [16]. Sepsis is associated with lung injury, and ROS has been linked to sepsis-induced damage [17]. Owing to the many deaths due to sepsis, there is pressing need to develop treatment

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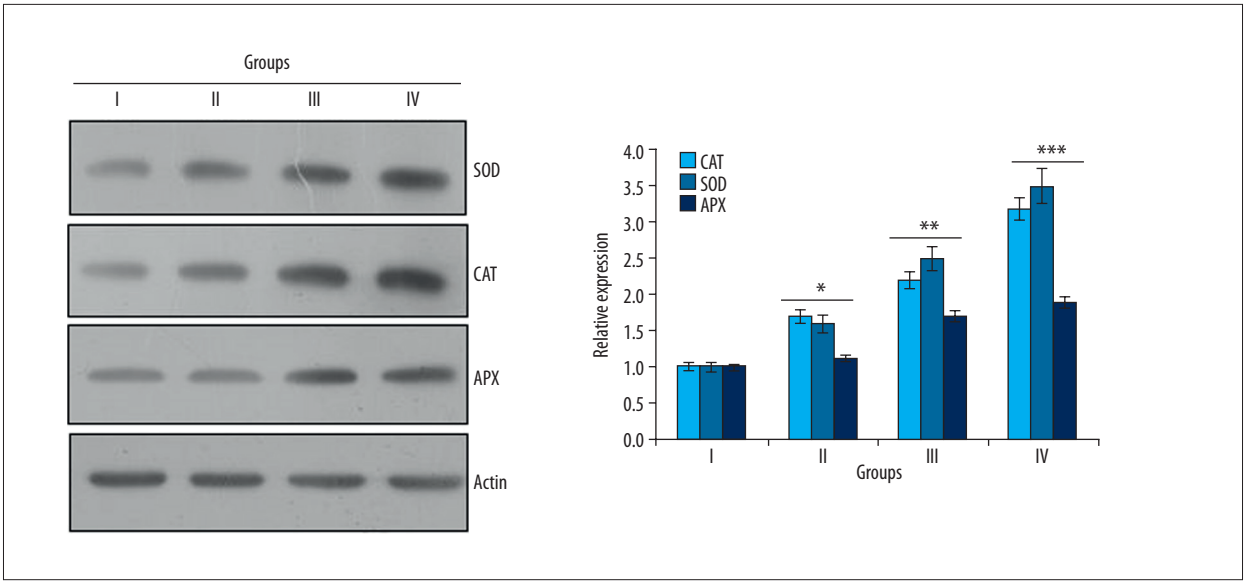

Figure 3. Western blot analysis showing the expression of SOD, CAT, and APX proteins in different treatment groups. Group I (control) consisted of normal rats administered normal saline and Group II consisted septic rats. Group III and Group IV consisted of septic rats administered 15 and 25 mg/kg of quercetin, respectively. The experiments were performed in triplicate and results are expressed as mean ±SD (*P<0.05 for Group I *vs.* II, ** P<0.01 for group II *vs.* Group III and *** P<0.001 for Group II *vs.* Group IV).

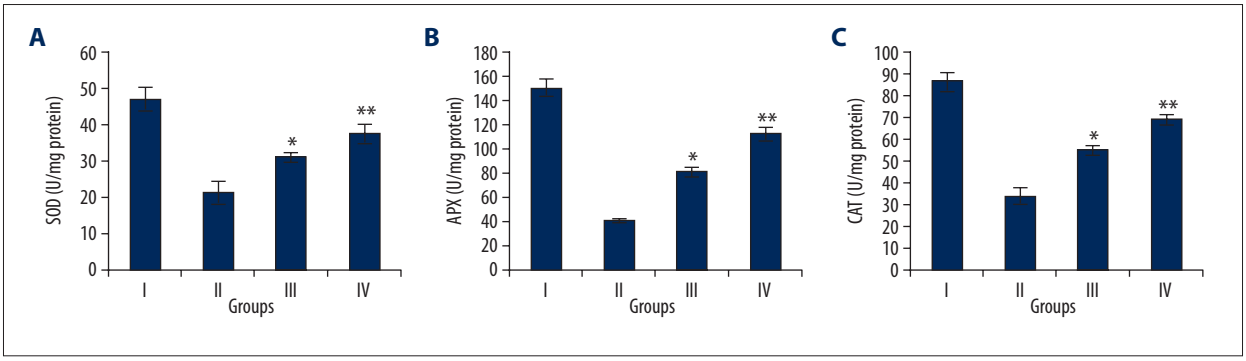

Figure 4. Enzyme activities of (**A**) SOD, (**B**) APX, and (**C**) CAT in different treatment groups. Group I (control) consisted of normal rats administered normal saline and Group II consisted of septic rats. Group III and Group IV consisted of septic rats administered 15 and 25 mg/kg of quercetin, respectively. The experiments were performed in triplicate and results are expressed as mean ±SD (* P<0.05 for Group II *vs.* Group III and ** P<0.01 for Group II *vs.* Group IV).

strategies for management of sepsis [15]. Plants can be rich sources of secondery metabolites that could be screened and ultimately developed into efficient drugs for the treatment of sepsis [18]. One of the advantages of using plant-derived molecules as drugs is their less severe adverse effects [19]. In this study, a common plant flavonoid, quercetin, was evaluated in a rat model of sepsis for its protective effects. Since sepsis is often associated with lung injury, the effects of quercetin were first examined on the lung histology of the different rat groups. We found that sepsis disturbed the histology of the lungs, as evident from the lower alveolar volume and thickness. Nonetheless, quercetin treatment could restore the normal lung anatomy, suggesting the protective effects of quercetin against lung injury induced by sepsis. A previous study has also shown protective effects of quercetin in rat models of sepsis, wherein quercetin was found to reduce the inflammation associated with sepsis [20]. Moreover, studies have shown that the development of sepsis is concomitant with generation of ROS and development of oxidative stress. This oxidative stress damages the cellular membranes and other cellular macromolecules [21]. Quercetin is belived to be a strong antioxidant [22] and in the present study we found that quercetin caused substantial decreases in the ROS levels as assessed by flow cytometry. Western blot analysis also showed a significant increase in the expression of antioxidant enzymes. The activities of the ROS scavenging enzymes were

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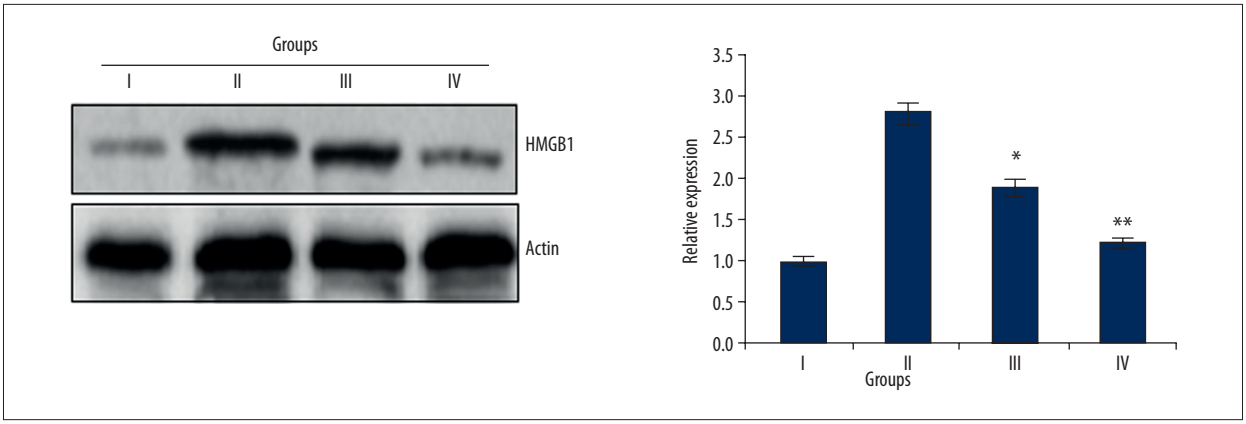

Figure 5. Western blot analysis showing the expression of HMGB1 in lung tissues of different treatment groups. Group III and Group IV consisted of septic rats administered 15 and 25 mg/kg of quercetin, respectively. The experiments were performed in triplicate and results are expressed as mean ±SD (* P<0.05 for Group II *vs.* Group III and ** P<0.01 for Group II *vs.* Group IV).

also significantly increased. These results unequivocally indicate that quercetin quenches the ROS and activates the enzymic antioxidant system of the lung cells. Studies have shown that HMGB1 protein plays an important role in the development of inflammation during sepsis [23]. In the present study, we also examined the effects of quercetin on the expression of HMGB1, showing that the expression of HMGB1 was increased in septic rats. However, quercetin administration caused significant declines in HMGB1 expression, again indicating the protective effects of quercetin.

Conclusions

The findings of the present study suggest that quercetin exerts protective effects in a sepsis rat model by quenching ROS, activation of the enzymic antioxidant system, and reduction of HMGB1 expression. Hence, quercetin may prove beneficial in the treatment of sepsis and warrants further evaluation.

References:

1. Sands KE, Bates DW, Lanken PN et al: Epidemiology of sepsis syndrome in 8 academic medical centers. JAMA, 1997; 278(3): 234–40
2. Drusano GL, Johnson DE, Rosen M, Standiford HC: Pharmacodynamics of a fluoroquinolone antimicrobial agent in a neutropenic rat model of *Pseudomonas sepsis*. Antimicrob Agents Chemother, 1993; 37(3): 483–90
3. Laudes IJ, Chu JC, Sikranth S et al: Anti-c5a ameliorates coagulation/fibrinolytic protein changes in a rat model of sepsis. Am J Pathol, 2002; 160(5): 1867–75
4. NeviÈre R, Fauvel H, Chopin C et al: Caspase inhibition prevents cardiac dysfunction and heart apoptosis in a rat model of sepsis. Am J Respir Crit Care Med, 2001; 163(1): 218–25
5. Şener G, Toklu H, Ercan F, Erkanlı G: Protective effect of β-glucan against oxidative organ injury in a rat model of sepsis. Int Immunopharmacol, 2005; 5(9): 1387–96
6. Dhamija I, Parle M, Kumar S: Antidepressant and anxiolytic effects of *Garcinia indica* fruit rind via monoaminergic pathway. Biotech, 2017; 7: 131
7. Villas Boas GR, Stefanello da Silveira AP, Feitosa Farinelli BC et al: The ethanolic extract obtained from Campomanesia pubescens (DC) O. BERG fruits exerts anxiolytic and antidepressant effects on chronic mild stress model and on anxiety models in Wistar rats: Behavioral evidences. Nutr Neurosci, 2018 [Epub ahead of print]
8. Romano B, Pagano E, Montanaro V et al: Novel insights into the pharmacology of flavonoids. Phytother Res, 2013; 27(11): 1588–96
9. Yao LH, Jiang YM, Shi J et al: Flavonoids in food and their health benefits. Plant Food Hum Nutr, 2004; 59(3): 113–22
10. Formica JV, Regelson W: Review of the biology of quercetin and related bioflavonoids. Food Chemical Toxicol, 1995; 33(12): 1061–80
11. Dejager L, Pinheiro I, Dejonckheere E, Libert C et al: Cecal ligation and puncture: The gold standard model for polymicrobial sepsis? Trends Microbiol, 2011; 19(4): 198–208
12. Sun Y, Oberley LW, Li Y: A simple method for clinical assay of superoxide dismutase. Clin Chem, 1988; 34: 497–500
13. Aebi H: Catalase. Methods enzym. Anal. New York and London, Academic Press, 1974; 673–77
14. Nakano Y, Asada K: Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol, 1981; 22: 867–80
15. Baker CC, Chaudry IH, Gaines HO, Baue AE: Evaluation of factors affecting mortality rate after sepsis in a murine cecal ligation and puncture model. Surgery, 1983; 94(2): 331–35
16. Breuillé D, Arnal M, Rambourdin F et al: Sustained modifications of protein metabolism in various tissues in a rat model of long-lasting sepsis. Clin Sci, 1998; 94(4): 413–23
17. Breuillé D, Arnal M, Rambourdin F et al: Sustained modifications of protein metabolism in various tissues in a rat model of long-lasting sepsis. Clin Sci, 1998; 94(4): 413–23
18. Dartora N, de Souza LM, Paiva SM et al: Rhamnogalacturonan from Ilex paraguariensis: A potential adjuvant in sepsis treatment. Carbohydr Polym, 2013; 92(2): 1776–82
19. Di Carlo G, Mascolo N, Izzo AA, Capasso F: Flavonoids: Old and new aspects of a class of natural therapeutic drugs. Life Sci, 1999; 65(4): 337–53
20. Gerin F, Sener U, Erman H et al: The effects of quercetin on acute lung injury and biomarkers of inflammation and oxidative stress in the rat model of sepsis. Inflammation, 2016; 39(2): 700–5
21. Macdonald J, Galley HF, Webster NR: Oxidative stress and gene expression in sepsis. Br J Anaesth, 2003; 90(2): 221–32
22. Boots AW, Haenen GR, Bast A: Health effects of quercetin: From antioxidant to nutraceutical. Eur J Pharmacol, 2008; 585(2–3): 325–37
23. Huang W, Tang Y, Li L: HMGB1, a potent proinflammatory cytokine in sepsis. Cytokine, 2010; 51(2): 119–26

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