Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.010523

Extraction of echinacoside from *Cistanche tubulosa* (Schenk) R. Wight and investigation of its protective effect on liver injury in sepsis rats

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Abstract

In this study, echinacoside was extracted from *Cistanche tubulosa* (Schenk) R. Wight, and its protective effect on liver injury in sepsis rats was investigated. Forty-five rats were randomly divided into control, sepsis and echinacoside groups, 15 rats in each group. The sepsis model was established in sepsis and echinacoside groups. In echinacoside group, the rats were treated with echinacoside at 1 h before modeling. At 24 h after modeling, compared with sepsis group, in echinacoside group the serum aspartate aminotransferase and alanine aminotransferase levels were decreased, the liver injury score and hepatocyte apoptosis rate were decreased, the serum monocyte chemoattractant protein-1, tumor necrosis factor α, interleukin 6 and interleukin 1β levels were decreased, the liver tissue catalase, superoxide dismutase and glutathione peroxidase levels were increased, the liver tissue malondialdehyde level was decreased, and the liver tissue nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase 1 (HO-1) protein expression levels were increased. The difference of all above comparisons was significant ($P < 0.05$). In conclusion, echinacoside can inhibit the inflammatory response, activate the Nrf2/HO-1 signal pathway, and reduce the oxidative stress, thus alleviating the liver injury in sepsis rats.

Keywords: echinacoside; sepsis; liver injury; Nrf2/HO-1.

Practical Application: This study has provided an experimental basis for preparation of echinacoside and its application to prevention of liver injury in sepsis.

1 Introduction

Sepsis is a fatal disease caused by the host's maladjusted response to infection. It can lead to the systemic inflammation and multiple organ dysfunction syndromes, with high morbidity and mortality (Purcarea & Sovaila, 2020). Liver is one of the most frequently involved organs in the early stage of sepsis, which is related to pathological mechanisms such as liver microcirculation disorder, oxidation and anti-oxidation imbalance, intestinal bacterial translocation and uncontrolled inflammatory response (Nesseler et al., 2012; Woźnica et al., 2018; Kim & Choi, 2020). In clinical practice, the liver injury of sepsis patients is mainly alleviated by controlling infection, restoring hemodynamic stability, anti-oxidation, anti-inflammation and other means (Wu et al., 2018; Fang et al., 2021; Xiao et al., 2022), but the treatment effect is not ideal in some patients. Echinacoside is a phenylethanolic glycoside compound extracted from *Cistanche tubulosa* (Schenk) R. Wight, a medical herb (Wu et al., 2019). In recent years, studies have shown that echinacoside has a variety of biological activities including the anti-inflammatory (Li et al., 2018), antioxidant (Wei et al., 2019), anti-aging (Chen et al., 2018) and anti-tumor (Lin, 2021) effects. It is found that echinacoside can alleviate the acetaminophen-induced liver injury (Thida et al., 2021) and D-galactosamine plus lipopolysaccharide-induced acute liver injury (Li et al., 2014) in mice. It is assumed that echinacoside has the protective effect on sepsis-induced liver injury. In this study, echinacoside was extracted from *Cistanche tubulosa* (Schenk) R. Wight, and its protective effect on liver injury in sepsis rats was investigated.

2 Materials and methods

2.1 Extraction of echinacoside from Cistanche tubulosa (Schenk) R. Wight

About 1 kg dried fleshy stems of *Cistanche tubulosa* (Schenk) R. Wight was taken, and was crushed to obtain the powder. The powder was extracted with 10 times (volume to mass) of 70% ethanol by refluxing for two times, 2 h of each time. After filtering, the filtrate was obtained. The filtrates of two extractions were combined, followed by concentrating under reduced pressure. The concentrated solution was loaded to HP-20 adsorption resin column. The resin column was eluted with water until there is no sugar reaction, and then eluted with 30% ethanol. The target elution solution was obtained. After concentrating under reduced pressure and drying, the crude echinacoside product was taken. After purifying by silica-gel column chromatography, the final echinacoside product was obtained. The high-performance liquid chromatograph showed that the purity of echinacoside was 95.49%.

Received 23 Jan., 2023
Accepted 13 Feb., 2023
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2.2 Animal grouping and treatment

Forty-five specific pathogen-free grade healthy male Sprague Dawley rats with body mass of (180-220 g) were randomly divided into control, sepsis and echinacoside groups, with 15 rats in each group. The echinacoside group was injected with 80 mg/kg echinacoside through the tail vein at 1 h before establishment of sepsis model. The other two groups were synchronously injected with the same volume of normal saline through the tail vein.

2.3 Establishment of sepsis model

Sepsis model was established in sepsis and echinacoside groups using cecal ligation and perforation (CLP) method. The rats were anesthetized using 20 g/L sodium pentobarbital by intraperitoneal injection. After removing the abdominal hair, a 3 cm incision was made along the middle of abdomen. The cecum was exposed, and the end of cecum was ligated. Then, the ligated cecum was punctured twice using an 18-gauge needle, and a small amount of feces was squeezed out. Then, the muscle and skin tissue were sutured layer by layer. If the rats had symptom such as bristling hair, lethargy, diarrhea and pyuria, the model establishment was judged as successful. In control group, excepting cecum ligating and puncturing, the other operations were the same as other two groups.

2.4 Detection of liver function indexes and inflammatory indexes

At 24 h after CLP, the rats were anesthetized using 20 g/L pentobarbital sodium. The blood was taken from abdominal aorta. After centrifuging at 3000 r/min for 15 min, the serum was collected. The liver function indexes including aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were detected by automatic biochemical analyzer. The inflammatory indexes including monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor α (TNF-α), interleukin 6 (IL-6) and interleukin 1β (IL-1β) were detected by enzyme-linked immunosorbent assay. The detection procedures were according to the instruction of kits.

2.5 Determination on liver injury score and hepatocyte apoptosis rate

Rats were sacrificed by cervical dislocation. The liver tissues were isolated, and were fixed with 40 g/L paraformaldehyde, followed routine sectioning (about 4 μm thickness). One section sample was treated with hematoxylin-eosin staining. The pathological changes of liver tissue were observed under light microscope. The liver injury score was as determined follows: normal: 0 point; light injury: 1 point; medium injury: 2 points; severe injury: 3 points. Another section sample was stained with terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling. The total number of hepatocytes and number of positive cells were counted under light microscope. The hepatocyte apoptosis rate was calculated.

2.6 Determination of oxidative stress indexes

Liver tissues of rats were homogenized with normal saline. After centrifuging at 3000 r/min for 10 min, the supernatant was taken. The oxidative stress indexes including catalase, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) were determined using the corresponding kits. The detection procedures were according to the instruction of kits.

2.7 Western blotting

Expressions of nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase 1 (HO-1) proteins in liver tissues were determined by western blotting. The liver tissues were homogenized. The total protein was extracted using cell lysate. The protein concentration was determined by Coomassie brilliant blue method. A 10 μg protein sample was taken, and the sodium dodecyl sulfonate-polyacrylamide gel electrophoresis was conducted. The separated protein was transferred to the polyvinylidene difluoride membranes, followed by blocking using 5% skimmed milk powder for 2 h. After washing with tris-buffered saline Tween-20 (TBST), the membranes were incubated with rabbit anti-rat Nrf2, HO-1 and β-actin (internal reference) monoclonal antibody at 4 °C overnight, respectively. After washing with TBST, the alkaline phosphatase labeled second antibody IgG was added, and the incubation was performed at room temperature for 2 h. The electrochemiluminescence agent was added for routine development and visualization. The gray value of band was measured by Image J software. The relative expression level of target protein was presented by the ratio of protein band gray value to β-actin band gray value.

2.8 Statistical analysis

SPSS 18.0 statistical software was used for statistical analysis. The data were expressed as mean±standard deviation. The comparison among three groups adopted one-way analysis of variance, and the comparison between two groups adopted LSD-t test. A value of $P < 0.05$ was considered statistically significant.

3 Results

3.1 Liver function indexes

At 24 h after sepsis modeling, the serum AST and ALT levels in sepsis and echinacoside groups were significantly higher than those in control group, respectively ($P < 0.05$). Compared with sepsis group, each index in echinacoside group was obviously decreased ($P < 0.05$) (Figure 1).

3.2 Liver injury score and hepatocyte apoptosis rate

As shown in Figure 2, compared with control group, the liver injury score and hepatocyte apoptosis rate in sepsis and echinacoside groups were obviously increased, respectively ($P < 0.05$), and each index in echinacoside group was obviously lower than that in sepsis group ($P < 0.05$).

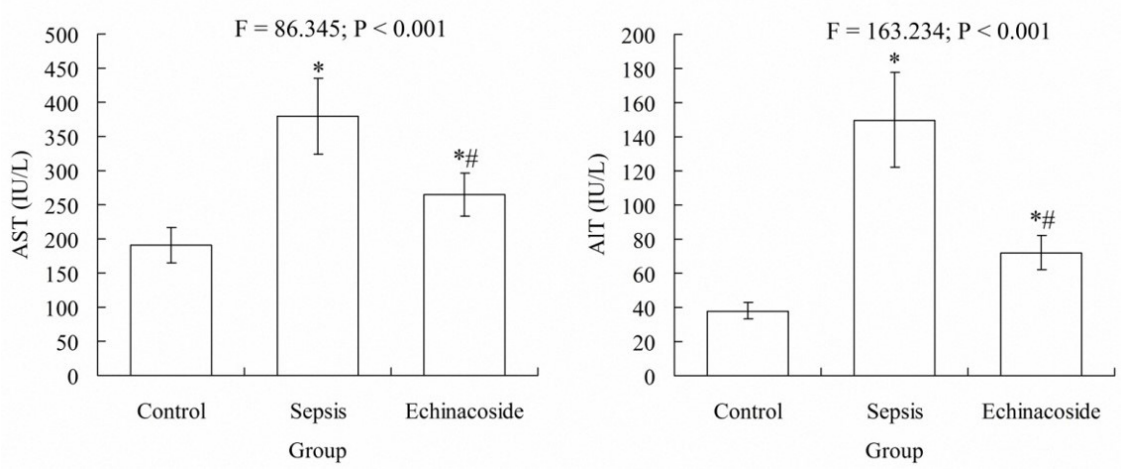

Figure 1. Liver function indexes in three groups (n = 15). *P < 0.05 *vs.* control group; #P < 0.05 *vs.* sepsis group. AST, aspartate aminotransferase; ALT, alanine aminotransferase.

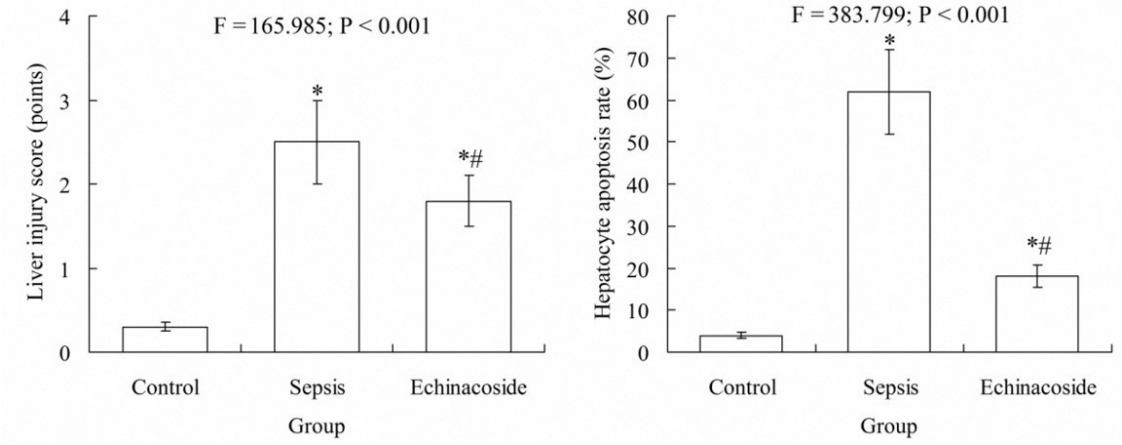

Figure 2. Liver injury score and hepatocyte apoptosis rate in three groups (n = 15). *P < 0.05 *vs.* control group; #P < 0.05 *vs.* sepsis group.

3.3 Inflammatory indexes

Figure 3 presented that, the serum MCP-1, TNF-α, IL-6 and IL-1β levels in sepsis and echinacoside groups were significantly higher than those in control group, respectively (P < 0.05). Compared with sepsis group, each index in echinacoside group was obviously decreased (P < 0.05).

3.4 Oxidative stress indexes

As shown in Figure 4, compared with control group, in sepsis and echinacoside groups the liver tissue catalase, SOD and GSH-Px levels were obviously decreased, respectively (P < 0.05), and the MDA level was obviously increased, respectively (P < 0.05). Compared with sepsis group, in echinacoside group the catalase, SOD and GSH-Px levels were obviously increased, respectively (P < 0.05), and the MDA level was obviously decreased (P < 0.05).

3.5 Nrf2 and HO-1 protein expressions

As presented in Figure 5, the Nrf2 and HO-1 protein expression levels in liver tissues in sepsis and echinacoside groups were significantly lower than those in control group, respectively (P < 0.05). Compared with sepsis group, each index in echinacoside group was obviously increased (P < 0.05).

4 Discussion

Sepsis is common in patients with severe burns, trauma, septic shock, etc.. The liver is an important organ for material metabolism and detoxification, and it is also one of the most frequently affected organs for sepsis patients. Liver dysfunction and liver injury are closely related to sepsis, and have been the powerful predictors of high mortality in severe sepsis patients (Nesseler et al., 2012; Kim & Choi, 2020). In this study, echinacoside was extracted from *Cistanche tubulosa* (Schenk) R. Wight, and its protective effect on liver injury in sepsis rats was investigated. Results showed that, at 24 h after sepsis modeling, compared with sepsis group, in echinacoside group the serum AST and ALT levels were obviously decreased, and the liver injury score and hepatocyte apoptosis rate were also obviously decreased. This indicates that, the echinacoside pretreatment can alleviate the liver injury and reduce the liver dysfunction in sepsis rats.

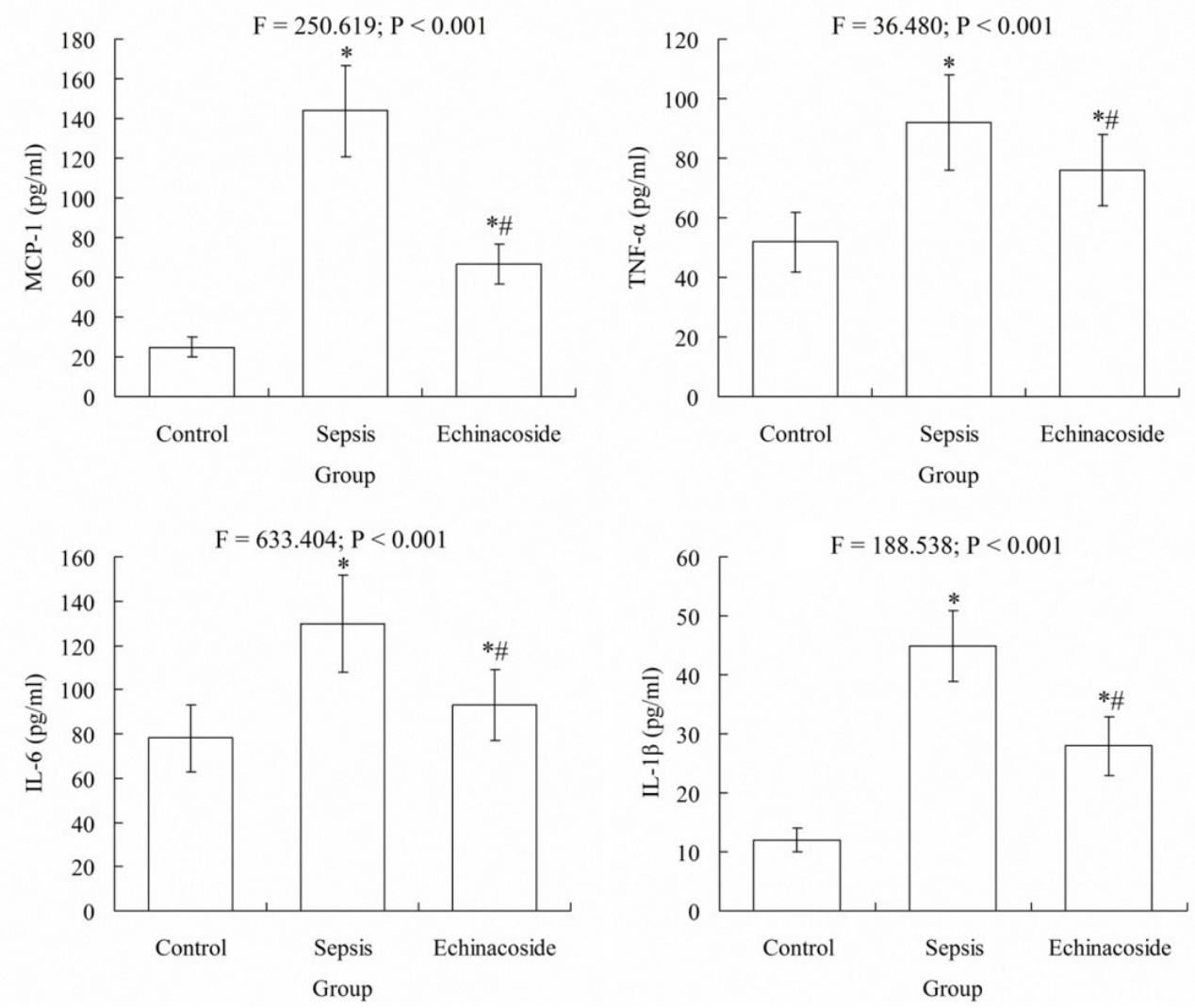

Figure 3. Inflammatory indexes in three groups (n = 15). *P < 0.05 *vs.* control group; #P < 0.05 *vs.* sepsis group. MCP-1, monocyte chemoattractant protein-1; TNF-α, tumor necrosis factor α; IL-6, interleukin 6; IL-1β, interleukin 1β.

Main cause of liver injury in sepsis is the excessive production of inflammatory mediators. MCP-1 belongs to the chemokine family and can activate the monocytes/macrophages. It is an important proinflammatory cytokine (Singh et al., 2021). TNF-α is one of the most important inflammatory mediators, which can activate the inflammatory cascade reaction and participate in regulating the apoptosis pathway and inducing multi-tissue cell damage (Alizadeh Zeinabad & Szegezdi, 2022). IL-6 is a multifunctional cytokine, which can induce the production of other harmful inflammatory factors, increase the liver load, and participate in the liver injury (Unver & McAllister, 2018). IL-1β is also a proinflammatory cytokine. It binds to the receptors of cells and participates in the immune response, thus leading to the tissue damage (Lopez-Castejon & Brough, 2011). In our study, compared with sepsis group, in echinacoside group the serum MCP-1, TNF-α, IL-6 and IL-1β levels decreased significantly. This indicates that, the echinacoside treatment may reduce the inflammatory response, thus alleviating the liver injury in sepsis rats.

Oxidative stress is another pathogenesis of liver injury in sepsis (Xu et al., 2020). Under normal conditions, the production of reactive oxygen species (ROS) in the body and the antioxidant protection are in dynamic balance. When stimulated by infection, ischemia reperfusion injury and other factors, the body state is unbalanced, and the production of ROS increases, causing the oxidative stress injury (Filomeni et al., 2015). Catalase, SOD and GSH-Px are important antioxidant enzymes that can antagonize ROS to maintain the oxidative balance of cells themselves. MDA is the main degradation product of lipid peroxidation. Its content can indirectly reflect the damage degree of body cells attacked by ROS (Lin et al., 2019). In our study, compared with sepsis group, in echinacoside group the liver tissue catalase, SOD and GSH-Px levels increased significantly, and the MDA level decreased significantly. It is suggested that echinacoside treatment can reduce the degree of oxidative stress, which may be related to its alleviation of liver injury in sepsis rats.

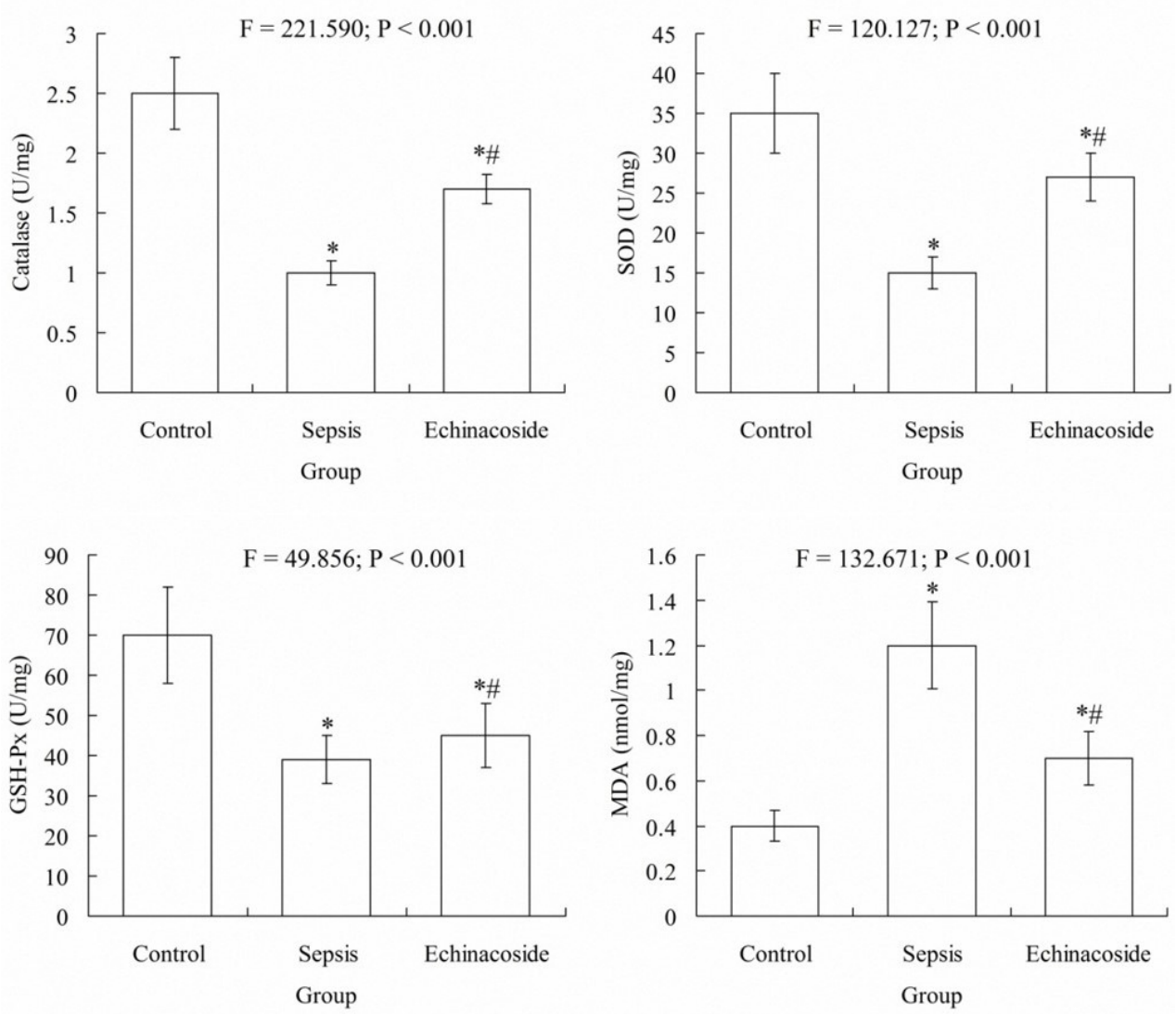

Figure 4. Oxidative stress indexes in three groups (n = 15). *P < 0.05 *vs.* control group; #P < 0.05 *vs.* sepsis group. SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde.

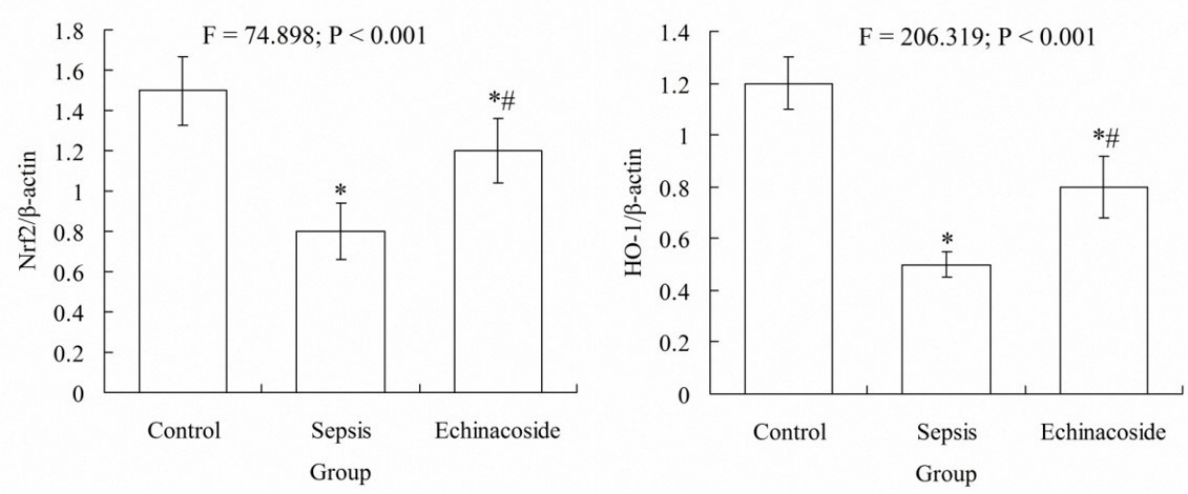

Figure 5. Nrf2 and HO-1 protein expressions in three groups (n = 15). *P < 0.05 *vs.* control group; #P < 0.05 *vs.* sepsis group. Nrf2, nuclear factor erythroid 2-related factor 2; HO-1, heme oxygenase 1.

Nrf2/HO-1 signal pathway is the most important anti-oxidative stress pathway in the body, which plays an important role in the occurrence and development of liver injury in sepsis (Wu et al., 2017). Nrf2 is the strongest transcription factor that regulates oxidative stress. When the cells are stimulated by oxygen free radicals, Nrf2 can be phosphorylated and uncoupled from the specific receptors. Then, it is translocated to the nucleus, and initiates the expression of downstream antioxidant genes such as HO-1 (Bouvier et al., 2017). HO-1 is the rate limiting enzyme in the process of heme degradation, which degrades heme into biliverdin, carbon monoxide and free iron. It can regulate the oxidative stress and inflammatory response through Nrf2 nuclear translocation (Loboda et al., 2016). Results of this study showed that, compared with sepsis group, the liver tissue Nrf2 and HO-1 protein expression levels in echinacoside group were obviously increased. It is suggested that, the echinacoside can increase the antioxidant level by activating Nrf2/HO-1 signal pathway, thus alleviating the liver injury in sepsis rats.

5 Conclusion

In conclusion, echinacoside from *Cistanche tubulosa* (Schenk) R. Wight can inhibit the inflammatory response, activate the Nrf2/HO-1 signal pathway, and reduce the oxidative stress, thus alleviating the liver injury in sepsis rats. This study has provided an experimental basis for preparation of echinacoside and its application to prevention of liver injury in sepsis.

Conflict of interest

The authors declare that there is no conflict of interest.

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