The Potential of Hydroxysafflor Yellow A as an Adjuvant in COVID-19 Patients with Acute Respiratory Distress Syndrome

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ABSTRACT

Objective: To discuss the potential and molecular mechanism of Carthamus tinctorius derived hydroxysafflor yellow A (HSYA) as an alternative herbal adjuvant that may regulate various signaling pathways that might be related to the regulatory effects in COVID-19 patients with ALI and ARDS.


Results and Discussions: In COVID-19 patients, SARS-CoV-2 replication might be associated with hyper induction of pro-inflammatory cytokine, which is known as a cytokine storm, and may cause acute lung injury (ALI) that leads to Acute Respiratory Distress Syndrome (ARDS). Carthamus tinctorius derived HSYA were used in many studies, in vivo in animal models or in vitro in cell lines and showed inhibition of multiple inflammatory pathways that were involved in ALI and ARDS, which might occur in covid-19 patients. HSYA showed pleiotropic effects in regulating cytokine levels. It regulated TNF-α, IL-1β, IL-6, IFN-β, and showed protective effect by blocking TLR4, MyD88, TRIF, IRF3, NF-κB to avoid cytokine storm and prevent tissue damage. HSYA was showed to reduce oxidative stress-mediated damage, and down-regulate inflammatory cytokines. Further, it was relatively safe when studied as an adjuvant in HIV and cancer patients.

Conclusion: We supposed that HSYA could be used as an alternative adjuvant in COVID-19 patients with ARDS. However, clinical trials are needed to prove its efficacy in COVID-19 patients with ARDS.

KEY WORDS

cytokine storm, COVID-19, SARS-Cov-2, Carthamus tinctorius, HSYA

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a pandemic disease that is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is a single-strand RNA virus, and causes substantial morbidity. COVID-19 is very infectious, which in some persons might be lethal, and has caused 1.82 million deaths worldwide1. SARS-CoV-2 infects the upper respiratory tract and rapid virus replication may cause hyper induction of pro-inflammatory cytokine production, which results in highly elevated pro-inflammatory cytokines that is also known as ‘cytokine storm.’ The severity of infections is due to an activation cascade that will lead to an auto-amplifying cytokine production2.

Cytokine storm may cause a massive inflammatory cell infiltration in the lungs, which subsequently develop acute lung injury (ALI) that may lead to acute respiratory distress syndrome (ARDS), or multiple organ failure. In addition, high pro-inflammatory cytokines may stimulate apoptosis of T-cells, so T-cells may not be directly killed by SARS-CoV-23. ARDS is a severe and progressive respiratory failure due to pulmonary edema, leukocyte accumulation, and excessive deposition of the extracellular matrix that may cause massive fibrosis at a later-stage4. Interleukin (IL)-6 may lead to an activated vascular endothelial cells and coagulation pathway, but inhibits myocardial function. Therefore, some COVID-19 patients with cytokine storm might show clinical and laboratory characteristics such as myocardial ischemia, hyper coagulation status, thrombosis, and inflammation that may mediate various tissue injuries5.

Carthamus tinctorius contains active constituents, including quinones, chalcones and flavonoids. According to pharmacological and clinical studies, it is promising for amelioration of myocardial ischemia as it can help to dilate coronary artery. In addition, several studies showed its beneficial effect on coagulation, thrombosis, inflammation, toxicity, and cancer. Moreover it can modulate the immune system, has antioxidant, anti-aging, anti-hypoxia, anti-fatigue, anti-inflammation, anti-hepatic fibrosis, antitumor, and analgesic properties6. Carthamus tinctorius active compound, i.e. hydroxysafflor yellow A (HSYA) was studied in experimental models and was showed to inhibit multiple inflammatory pathways that were associated with ALI and ARDS7. It was also used as adjuvant in antiretroviral therapy in patients with HIV8.
with HIV/AIDS, and cancer therapy to treat some malignant and multi drug resistant (MDR) cancers. As a natural-product, HSYA has low toxicity, but the potentials and mechanisms of HSYA that may be involved in severe COVID-19 mitigation are not well understood. Therefore, this review aimed to discuss the potential and molecular mechanism of HSYA as an alternative herbal adjuvant that may regulate various signaling pathways that might alleviate symptoms in COVID-19 patients with ALI and ARDS, where we discussed Carthamus tinctorius active compounds and mechanism of molecular action, Carthamus tinctorius derived HSYA inhibition on reactive oxygen species (ROS) pathway, on tumor necrosis factor (TNF-α) and IL-2 pathway, on Toll-Like Receptor (TLR) 4 and TLR 3, 7/8 pathway, and inhibition on platelet activity.

MATERIAL AND METHODS

To write this narrative review, we searched electronic databases i.e. Web of Science, PubMed, and Scopus and we restricted between 2010 through 2020. The keywords used were: Carthamus tinctorius, adjuvant, cytokine storm, COVID-19, SARS-Cov-2, acute lung injury, ALI, Acute Respiratory Distress Syndrome and, ARDS. Searches were done on 11 September 2020, and 18 December 2020. Inclusion criteria were all papers in English about Carthamus tinctorius or HSYA in relation to its effects and mechanisms on cytokines and cytokine storm, ALI, and ARDS, while the exclusion criteria were articles that are not related to the topic.

RESULTS AND DISCUSSION

From 3 electronic databases, we got 61 articles from Pubmed, 35 articles from Scopus, and three articles from the web of science. The papers were sorted, and after elimination of duplication we got 81 articles. After excluding out of topic articles, forty-eight were selected to be included, which consisted of original and review articles concerning COVID-19, and studies on cell lines and animals, whereas Carthamus tinctorius active compound HSYA was used to alleviate ALI and ARDS. Among the selected articles, fourteen were addressing HSYA mechanism of action in alleviating ALI/ARDS, and its relation to COVID-19 signaling pathway; therefore they were tabulated in Table 1.

Carthamus tinctorius active compounds and HSYA mechanism of molecular action

Carthamus tinctorius or safflower contains three main active compounds. They are safflower yellow A (SYA), hydroxysafflor yellow A (HSYA), and anhydro-safflor yellow B (AHYSB). These main active compounds can alleviate lung injury that is induced by lipopolysaccharide (LPS). However, the exact mechanism on how these active compounds prevent neutrophil mediated inflammatory responses is not well understood, possibly by inhibition of neutrophil extracellular trap (NET) release.

The potential of Carthamus tinctorius active compounds to attenuate inflammation have been studied in cell cultures and animal models. Many studies have focused on investigating the natural compound HSYA in alleviating inflammation by inhibiting various signaling pathways that are also involved in COVID-19 signaling pathway (Table 1).4,15,20

HSYA inhibition on ROS pathway

Oxidative stress is one of the mechanisms in COVID-19 pathogenesis. Viral infected cells are critical events that are related to inflammation and subsequent tissue damage. In respiratory viral infection, there is an interaction between oxidative stress and cytokine over expression as a mechanism that facilitates tissue injury that may lead to organ failure.4,19

SARS-Cov-2 is one of respiratory viruses that are known to induce ROS-generating enzymes, including nicotinamide adenine dinucleotide phosphate oxidases (NADPH oxides, Nox) that disturb antioxidant defenses.15 NADPH oxide is a leading ROS source, and the NOX subunit plays an essential role in increasing the NADPH oxidase activity. NOX significantly increases nuclear factor (NF-κB) that acts as a transcription factor for cytokine expression, which may induce a cytokine storm (Fig. 1). HSYA treatment was showed to decrease NF-κB p65 nuclear translocation that inhibited the activation of the NF-κB signaling pathway.19,20 A study showed that HSYA suppressed the generation of induced ROS through direct binding inhibition.20 Several studies showed that HSYA could reduce oleic acid and LPS acute lung injuries15,24; therefore using HSYA as an adjuvant to alleviate Sars-Cov-2 induced SLI is very potential. Therapeutic interventions using HSYA as an adjuvant to standard therapy might reduce inflammation and progression of acute lung disease.23

Carthamus tinctorius natural products have been consumed for centuries without significant toxicity to alleviate ALI. One of the products, which is a flavonoid compound i.e. HSYA, was showed to reduce oxidative stress and inflammatory cytokine mediated damage,4,19,20 and showed effects on cerebral ischemia-reperfusion injury in rats, which was related to anti-inflammation and antioxidant pathways.20 The mechanism of action involves increase in antioxidant enzyme activity, reduction in oxidative stress-mediated damage, regulation of anti-inflammatory cytokine levels, and suppression of inflammatory response; therefore HSYA has pleiotropic effects by inhibiting pro-inflammatory signaling, while activating antioxidant defense mechanisms. Moreover, a study showed that HSYA effectively protected rat liver from long term injury, which was related to enhanced antioxidant capacity of liver tissues and inhibition of TGF-β1 expression and another study showed that HSYA significantly increased the expression of PGC-1α and Nrf2, the activity of the antioxidant enzyme SOD, and also decreased MDA and ROS levels15. As many studies showed that HSYA had antioxidant properties and could inhibit the ROS pathway, while oxidative stress is one of the triggers of cytokine storm in COVID-19, the use of HSYA as adjuvant in COVID-19 treatment might be beneficial.

HSYA Inhibition on TNF α, NF-κB and Interleukin pathways

In the most severe inflammatory conditions, the prognosis can be significantly aggravated by the over production of mainly pro-inflammatory cytokines such as IL-1, IL-6, IFN-α, and TNF-α,14,20,24 which preferentially targeting lung tissue, whereas HSYA has a protective effect on LPS-induced inflammation, including acute respiratory distress syndrome (ARDS) by blocking the TLR4/NF-κB pathway.15,16,19 Moreover, mRNA expression of TNF-α, IL-1β, and IL-6, and the number of NF-κB p65 positive cells were lower in HSYA doses of 53.3 and 80.0 mg/kg in treatment groups. HSYA exerted its anti-inflammatory activity by expression of myeloid differentiation factor 88 (Myd88), intracellular adhesion molecule (ICAM)-1, TNFα, IL-1β, and IL-6 at mRNA and protein level and inhibited the activation of leucocytes to A549 cells.4

A study showed that HSYA down-regulated both myeloperoxidase (MPO) level in lung tissues and inflammatory cytokine serum levels, including TNF-α, IL-1β, IL-6, and IFN (interferon)-γ, another study showed that HSYA alleviated bleomycin-induced increase in mRNA level of TNF-α, IL-1β and TGF-β1 in lung homogenates.20 In addition, HSYA significantly inhibited the expression of IL-6, IL-8, and matrix metalloproteinase (MMP)-1 in IL-1β-stimulated inflammatory pathway in human SW982 synovial cells.4,10 Another study showed that HSYA suppressed p65 binding activity, and pro-inflammatory cytokine transcriptions, including TNF-alpha, IL-1beta, and IL-6, while promoting mRNA expression of anti-inflammatory cytokine IL-10 in rats with cerebral cortex ischemia.10 In a study on LPS-induced proliferation and migration of vascular smooth muscle cells, there were down regulation of several key pro-inflammatory cytokines, including TNF-α, IL-6, and IL-8.16 Sun et al.16 showed inhibition in pro-inflammatory cytokine TNF-α, IL-1β, and IL-6 mRNA expressions and increase in anti-inflammatory cytokine IL-10 gene expression in LPS induced pulmonary inflammation in mice. Further, TNF-α and IL-6 mRNA expression elevations in LPS induced poly-morpho-nuclear (PMN) cell activation were inhibited.4 As various studies showed that HSYA inhibited TNF α, NF-kB and Interleukin pathways, which plays a role in the development of ARDS, the use of HSYA in COVID-19 patients might prevent or alleviate ARDS.

HSYA inhibition on TLR 4 pathway

Based on covid-19 pathway in Kyoto Encyclopedia of Genes and Genomes (KEGG), SARS-CoV-2 spike protein attachment to TLR4 in TLR4-RAC3-Ubl-RNF4 pathway causes activation of MyD88 signaling. There are interferon regulatory factor 3 (IRF3), and TIR-domain-containing interferon-β (TRIF) protein up-expressions, which are followed by translocation of nuclear factor kappa B (NF-kB)/p65 and inhibitory kappa B (IkB)-α induced pro inflammatory cytokine expression into cytoplasm. Inhibiting cytokine storm and tissue damage. When the lung is the key target of the cytokine storm, then severe acute respiratory syndrome is triggered that is responsible for the widespread clinical syndrome that is known as COVID-19. In some patients, SARS-CoV-2...
Table 1: Molecular action of Hydroxysafflor yellow A (HSYA) in cell lines and animal model studies

<table>
<thead>
<tr>
<th>Model</th>
<th>Condition</th>
<th>HSYA mechanism of action</th>
<th>Relation with COVID-19 signaling pathway</th>
<th>Ref</th>
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<tr>
<td>Mice</td>
<td>ARDS</td>
<td>Reduction in TNF-β, IL-1α, IL-6, TGF-β1, TLR4 and CD 14 expressions at the mRNA and protein levels; reduction in NF-κB levels; blocking of TLR4/NF-κB pathway, and TLR4 receptor</td>
<td>TNF-α, IL-1β, IL-6, TLR4</td>
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<td>Human fetal lung fibroblasts (MRC-5 cells)</td>
<td>TGF-β1-induced MRC-5 activation</td>
<td>Inhibition of Smad and ERK/MAPK signaling pathways through Smad2, Smad3, and ERK phosphorylation suppression → Smad2, Smad3 nuclear translocation inhibition, and Smad3 - SBE binding inhibition</td>
<td>MAPK</td>
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<td>Male Sprague-Dawley rats</td>
<td>GEE mediated lung injury</td>
<td>Therapeutic effect due to enhanced PKA activity and platelet activation inhibition</td>
<td>platelet activation inhibition</td>
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</tr>
<tr>
<td>Human fetal lung fibroblasts (MRC-5 cells)</td>
<td>TNF-α induced inflammation</td>
<td>Competition with TNF-α to bind with TNFR1; inhibition of TNFR1 binding to TAK1-TAB2 complex, and NF-κB signaling pathway activation; suppression of TGF-β1 promoter - AP-1 binding</td>
<td>TNF-α, TNFR1, NF-κB</td>
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<td>Alveolar epithelial A549 cells</td>
<td>LPS induced inflammation</td>
<td>Suppression of TLR-4, MyD88, ICAM-1, TNFα, IL-1β, IL-6, and IL-6 expression at mRNA and protein level; inhibition of leukocytes adhesion to A549 cells; decrease in NF-κB p65 nuclear translocation; inhibition of p38 MAPK phosphorylation</td>
<td>TLR-4, MyD88, TNFα, IL-1β IL-6 NF-κB, MAPK</td>
<td>[15]</td>
</tr>
<tr>
<td>Mice</td>
<td>LPS induced ALI</td>
<td>Lung tissues: down-regulation of MPO; serum: decrease in TNF-α, IL-1β, IL-6, and IFN-β levels; prevention of TLR4, MyD88 and TRIF protein up-regulation; prevention of phosphorylation of IRF3 and translocation of NF-κB/p65; inhibition of IκB-α.</td>
<td>TNF-α, IL-1β, IL-6, IFN-β, IL-8, IL-6 NF-κB, MAPK</td>
<td>[16]</td>
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<tr>
<td>HSAECs</td>
<td>PAF induced inflammation</td>
<td>Reduction in IL-1β, IL-6, and TNF-α expressions; decrease in monolayer permeability of HSAECs → restoration of cell-barrier function; inhibition of PKC, MAPK, and AP-1 expressions; inhibition of NF-κB activation</td>
<td>NF-κB, IL-1β, IL-6, TNF-α</td>
<td>[17]</td>
</tr>
<tr>
<td>Male Hartley guinea pigs</td>
<td>Ovalbumin-Induced Asthma</td>
<td>Inhibition of JNK MAPK, p38 MAPK, ERK MAPK, and IκB phosphorylation; inhibition of ovalbumin-induced elevations of Ig E, PAF, IL-1β, IL-6, IL-4, IL-5, and IL-13, and decreases in TNF-α, IFN-γ, IL-2, and IL-3</td>
<td>IκBα, TNF-α, IFN-γ, IL-2, IL-1β, IL-6</td>
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<td>Male Kunming mice</td>
<td>LPS induced pulmonary inflammation</td>
<td>Decrease in NF-κB p65 nuclear translocation; inhibition of TNF-α, IL-1β and IL-6 mRNA expression; increase in IL-10 gene expression</td>
<td>TNF-α, IL-1β IL-6</td>
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<td>Mice</td>
<td>Bleomycin-induced pulmonary fibrosis</td>
<td>Reduction in TNF-α, IL-1β, and IL-6 mRNA expression; decrease in number of NF-κB p65 positive cells at doses of 53.3 and 80.0 mg</td>
<td>TNF-α, IL-1β IL-1β, NF-κB</td>
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<tr>
<td>Rat</td>
<td>Chronic obstructive pulmonary disease</td>
<td>Decrease in collagen deposition and thickening of small airway wall; decrease in TGF-β mRNA and protein level → inhibition of p38 MAPK phosphorylation in lung tissues</td>
<td>MAPK</td>
<td>[21]</td>
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<tr>
<td>Male C57BL/6 mice</td>
<td>Bleomycin-induced lung injury</td>
<td>Decrease in mRNA level of TNF-α, IL-1β and TGF-β1 in lung homogenates; inhibition of NF-κB activation and p38 MAPK phosphorylation in lung tissue</td>
<td>TNF-α, IL-1β, TGF-β1, NF-κB</td>
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<td>Male Sprague-Dawley rats</td>
<td>Oleic acid-induced ALI</td>
<td>Increase in antioxidant enzyme activities; inhibition of inflammatory response via cAMP/PKA signaling pathway</td>
<td>Antioxidant enzymes</td>
<td>[23]</td>
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<tr>
<td>Rats</td>
<td>ALI and chronic pulmonary fibrosis</td>
<td>Reduction in TNF-α, IL-1 IL-1β, and IL-6 mRNA expression, and NF-κB p65 positive cell number</td>
<td>NF-κB, TNF-α, IL-1 IL-1β, IL-6</td>
<td>[24]</td>
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promotes a dysfunctional immune response that causes dysregulation of cytokine secretory pattern. Over production of cytokines causes hyper cytokinemia that underlies the hyper inflammatory state, which might lead to injury of alveolar epithelial and vascular endothelial cells, as well as to sustained lung infiltration by neutrophils and macrophages.

Song et al. study showed that HSYA suppressed the expression of TLR-4 and Myd88 at mRNA and protein level and inhibited the adhesion of leukocytes to A549 cells. Another study also showed that HSYA treatment reduced TLR4 at mRNA and protein levels. In addition, HSYA reduced collagen deposition in lung tissue.

Yang et al. study showed that HSYA inhibited LPS-induced vascular smooth muscle cell inflammatory pathways, at least partly, via inhibition of TLR-4/Rac1/Akt pathway. Therefore, the suppression of TLR4-mediated pathway might be a promising target for the treatment of ALI and ARDS that might occur in covid-19 patients. Further, a study by Wang et al. showed that HSYA was an effective therapeutic agent in preventing sepsis-induced apoptosis of CD 4(+)+ T lymphocytes, probably through its anti-inflammatory and anti-apoptotic effects. As various studies showed that HSYA inhibited TLR4 pathway, which plays a role in development of cytokine storm in COVID-19, HSYA theoretically might prevent or alleviate cytokine storm in COVID-19 patients.

**HSYA inhibition on TLR 3, 7/8 pathways**

COVID-19 is frequently accompanied by a hyper coagulation inflammatory state with micro-angiopathic pulmonary changes that can precede the diffuse alveolar damage that is characteristic of typical ARDS, which is seen in other severe pathogenic infections. The presence of ALI with alveolar-capillary membrane damage, increased vascular permeability, pulmonary edema, and respiratory failure, might be due to uncontrolled hyper inflammatory responses in the lungs. Its severe form, ARDS remains the primary mortality cause in COVID-19 critically ill patients. The ARDS is a critical and progressive respiratory failure that is characterized by early acute inflammation and late-stage massive fibrosis, including pulmonary edema, prominent leukocyte accumulation, and excessive extracellular matrix deposition. In COVID-19 KEGG pathway, attachment of viral RNA to TLR3 leads to activation of TRAF3 followed by activation of IKK and IRE3 as a transcrip- tion factor that leads to DNA activation for IFNa/β production, followed by activation of IFN receptor and activation of JAK/STAT for antiviral production (Fig.1).

Yu et al. study showed that HSYA treatment with a dosage of 8 mg/kg body weight or higher markedly down regulated the expression of JAK2-mediated signaling of inflammation due to focal cerebral ischemia. HSYA was supposed to provoke negative feedback signaling on activation of JAK2/STAT3, which was modulated by crosstalk between JAK2/STAT3 and SOCS3 signaling pathways that might contribute to its therapeutic roles against inflammation in cerebral ischemia. Another study showed that HSYA inhibited activation of IRF3, translocation of NF-kB/p65, and inhibitory kappa B (IkB)-α. Moreover, HSYA treatment decreased NF-kB p65 nuclear translocation and inhibited p38 MAPK. These findings suggested that HSYA effectively inhibited LPS-induced inflammation in A549 human alveolar epithelial cells.

Further, Liu et al. study showed that HSYA could compete with TNF-α to bind with tumor necrosis factor receptor (TNFR1) and prevent binding of TNFRI to TAK1-TAB2 complex. In addition, HSYA could also inhibit the activation of NF-kB and suppress the binding of TGF-β1 promoter to activator protein-1 (AP-1). Therefore, this study suggested that HSYA reduced TNF-α-induced inflammatory response and proliferation of MRC-5 human fetal lung fibroblasts through NF-kB/AP-1 signaling pathway. The effect of HSYA on inflammation and proliferation of human fetal lung fibroblasts provides a theoretical basis for HSYA as adjuvant in the treatment of COVID-19, which might develop severe pulmonary fibrosis.

**HSYA inhibition on platelet activity**

Hyper coagulation and thrombosis contribute to mortality of subjects suffering from severe COVID-19. SARS-CoV-2 might induce platelet activation by binding to platelet ACE2 that leads to thrombus formation. In addition, platelets might be associated with viral RNA and become activated as significant sources of inflammatory mediators, which lead to overwhelming thrombo-inflammation in COVID-19 patients. Platelets might be primed by SARS-CoV-2 to deliver pro-inflammatory and pro-coagulant mediators into systemic circulation. A study showed that HSYA could suppress platelet activity, improve lung function, increase lung permeability, and therapeutically effective for gasoline engine exhaust induced lung injury in rats, and acted by enhancing protein kinase (PK) A activity and inhibiting platelet activation. In addition, various studies showed HSYA beneficial effects, where HSYA reduced platelet-activating factor (PAF) induced inflammatory factor expression and restore PAF induced airway epithelial cell-barrier destruction, inhibited PAF-induced human bronchial smooth muscle activation, which lead to breathing difficulties, by targeting the PAF receptor (PAFR), and inhibited PAFR binding. Moreover, HSYA had a strong effect on cerebrovascular vasodilatation and significantly decreased platelet aggregation, blood viscosity, and thrombus formation. These findings demonstrated that HSYA had protective effects that prevent platelet activation and thrombus formation. Therefore, the use of HSYA as adjuvant in combination with COVID-19 standard therapy might target various deleterious aspects, i.e. various pathways that lead to cytokine storm, ALI/ARDS, lung fibrosis, and thrombus formation.
tion. However, studies to prove the safety and efficacy of HSYA as adjuvant in COVID-19 patients, and studies to determine its formulation and dose need to be conducted.

CONCLUSION

Carthamus tinctorius derived HSYA has been evaluated for its effectiveness against inflammation on cell lines and in vivo, including in ALL/MRL/MpJ mice, and the molecular pathways are well understood. Various study results suggested that Carthamus tinctorius might help to alleviate inflammation, prevent thrombus formation and promote lung tissue healing in severe COVID-19 with ARDS. However, clinical trials are needed to further claim its efficacy in covid-19 patients.

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