BIOMEDICAL SCIENCE

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

Lismayana Hansur[1,2], Jeanne Adiwinata Pawitan[3,4,5]

ABSTRACT

Objective: **To discuss the potential and molecular mechanism of Carthamus tincorius 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.**

Methods: **We search Web of Science, PubMed, and Scopus using keywords: Carthamus tinctorius, adjuvant, cytokine storm, COVID-19, SARS-Cov-2, acute lung injury (ALI), and Acute Respiratory Distress Syndrome (ARDS), on 11 September 2020, and 18 December 2020.**

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 tincorius 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 worldwide[1]. 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 production[2].

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 stimulates apoptosis of T-cells, so T-cells may not be directly killed by SARS-CoV-2[3]. 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-stage[4]. Interleukin (IL)-6 is one of the cytokines that contributes to host defense against infections and tissue injuries. However, excessive synthesis of 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 injuries[5].

Carthamus tinctorius contains active constituents, including quinochalcones 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 properties[6,7].

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 ARDS[8]. It was also used as adjuvant in antiretroviral therapy in patients

Received on February 22, 2021 and accepted on April 20, 2021

1) Doctoral Program in Biomedical Science, Faculty of Medicine, Universitas Indonesia
Jakarta, Indonesia
2) Faculty of Medicine, Universitas Muhammadiyah Makassar
Makassar, Indonesia
3) Department of Histology, Faculty of Medicine, Universitas Indonesia
Indonesia
4) Stem Cell Medical Technology Integrated Service Unit,
Dr. Cipto Mangunkusumo General Hospital/
Faculty of Medicine, Universitas Indonesia
Jakarta, Indonesia
5) Stem Cell and Tissue Engineering Research Center,
Indonesia Medical Education and Research Institute (IMERI),
Faculty of Medicine, Universitas Indonesia
Jakarta, Indonesia
Correspondence to: Jeanne Adiwinata Pawitan
(e-mail: jeanneadiwip@gmail.com)

with HIV/AIDS[9], and cancer therapy to treat some malignant and multi drug resistant (MDR) cancers[10]. 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, where 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 safflor yellow A (SYA), hydroxysafflor yellow A (HSYA), and anhydrosafflor yellow B (AHSYB). 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[11].

The potentials 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)[4,12-24].

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[25-27].

Sars-Cov 2 is one of respiratory viruses that are known to induce ROS-generating enzymes, including nicotinamide adenine dinucleotide phosphate oxidases (NADPH oxidases, Nox) that disturb antioxidant defenses[27]. NADPH oxidase 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[14,15]. A study showed that HSYA suppressed the generation of induced ROS through direct binding inhibition[28]. Several studies showed that HSYA could reduce oleic acid and LPS acute lung injuries[23,28]; therefore using HSYA as an adjuvant to alleviate Sars-Cov 2 induced SLI is very potential. Therapeutic interventions using HSYA as adjuvant to standard therapy might reduce inflammation and progression into chronic lung disease.

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[23], and showed effects on cerebral ischemia-reperfusion injury in rats, which was related to anti-inflammatory and antioxidant pathways[29]. 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 reaction; therefore HSYA has pleiotropic effects by inhibiting pro-inflammatory signaling, while activating antioxidant defense mechanisms[8]. 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[30], 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 levels[31]. As many studies showed that HSYA had antioxidant properties and could inhibit the ROS pathway, while oxidative stress is one of the trigger 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, IL-12, IFN-α, and TNF-α[4,15,22,32-35], which preferentially targeting lung tissue[4,22,32]. HSYA has a protective effect on LPS-induced inflammation, including acute respiratory distress syndrome (ARDS) by blocking the TLR4/NF-κB pathway[4,15,16,19,36]. 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 body weight in treatment groups[24]. HSYA suppressed the expression of myeloid differentiation factor 88 (Myd88), intercellular adhesion molecule (ICAM)-1, TNFα, IL-1β, and IL-6 at mRNA and protein level and inhibited the adhesion of leukocytes to A549 cells[15].

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)-β[16]; another study showed that HSYA alleviated bleomycin-induced increase in mRNA level of TNF-α, IL-1β and TGF-β1 in lung homogenates[22]. 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[33]. 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[37]. 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[35]. Sun *et al*[19] 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[36]. As various studies showed that HSYA inhibited TNF α, NF-κB 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)[38], SARS-CoV-2 spike protein attachment to TLR4 in TLR4 pathway causes phosphorylation of MyD88 (Fig 1). In addition, 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-κB)/p65 and inhibitory kappa B (IκB)-α induced pro inflammatory cytokine expression that might mediate 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 [4,12-24]

Model	Condition	HSYA mechanism of action	Relation with **COVID-19** signaling pathway	Ref
Mice	ARDS	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	TNF-α, IL-1β, IL-6, TLR4	[4]
Human fetal lung broblasts (MRC-5 cells)	TGF-β1-induced MRC-5 activation	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	MAPK	[12]
Male Sprague-Dawley rats	GEE mediated lung injury	Therapeutic effect due to enhanced PKA activity and platelet activation inhibition	platelet activation inhibition	[13]
Human fetal lung fibroblasts (MRC-5 cells)	TNF-α induced inflammation	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	TNF-α, TNFR1, NF-κB	[14]
Alveolar epithelial A549 cells	LPS induced inflammation	Suppression of TLR-4, MyD88, ICAM-1, TNFα, IL-1β, 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	TLR-4, MyD88, TNFα, IL-1β IL-6 NF-κB, MAPK	[15]
Mice	LPS induced ALI	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.	TNF-α, IL-1β, IL-6, IFN-β, TLR4, MyD88, TRIF, IRF3, NF-κB	[16]
HSAECs	PAF induced inflammation	Reduction in IL-1β, IL-6, and TNF- 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	NF-κB, IL-1β, IL-6, TNF-α	[17]
Male Hartley guinea pigs	Ovalbumin-Induced Asthma	Inhibition of JNK MAPK, p38 MAPK, ERK MAPK, and IκBα 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	IκBα, TNF-α, IFN-γ, IL-2, IL-1β, IL-6,	[18]
Male Kunming mice	LPS induced pulmonary inflammation	Decrease in NF-κB p65 nuclear translocation; inhibition of TNF-α, IL-1β and IL-6 mRNA expression; increase in IL-10 gene expression	TNF-α, IL-1β IL-6	[19]
Mice	Bleomycin-induced pulmonary fibrosis	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	TNF-α, IL-1β, IL-6; NF-κB	[20]
Rat	Chronic obstructive pulmonary disease	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	MAPK	[21]
Male C57BL/6 mice	Bleomycin-induced lung injury	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	TNF-α, IL-1β, TGF-β1, NF-κB	[22]
Male Sprague–awley rats	Oleic acid-induced ALI	Increase in antioxidant enzyme activities; inhibition of inflammatory response via cAMP/PKA signaling pathway	Antioxidant enzymes	[23]
Rats	ALI and chronic pulmonary fibrosis	Reduction in TNF-α, IL-1 IL-1β, and IL-6 mRNA expression, and NF-κB p65 positive cell number	NF-κB, TNF-α, IL-1 IL-1β, and IL-6	[24]

ARDS = Acute respiratory distress syndrome, TNF = tumor necrosis factor, IL = interleukin, TGF = transforming growth factor, TLR = Toll-like receptor, CD = cluster differentiation, mRNA = messenger RNA, NF-κB = nuclear factor kappa B, ERK/MAPK = extracellular signal-regulated kinase/mitogen activated protein kinase, SBE = Smad binding element, GEE = gasoline engine exhaust, PKA = protein kinase A, TNFR = tumor necrosis factor receptor, AP = activator protein, LPS = lipopolysaccharide, MyD = myeloid differentiation factor, ICAM = intercellular adhesion molecule, ALI = acute lung injury, MPO = myeloperoxidase, IFN = interferon, TRIF = TIR-domain-containing adapter-inducing interferon-β, IRF = interferon regulatory factor, IκB = inhibitory kappa B, HSAECs = human small airway epithelial cells, PAF = platelet activating factor, PKC = protein kinase C, JNK = c-Jun-N-terminal kinase, Ig = immunoglobulin, cAMP = cyclic AMP.

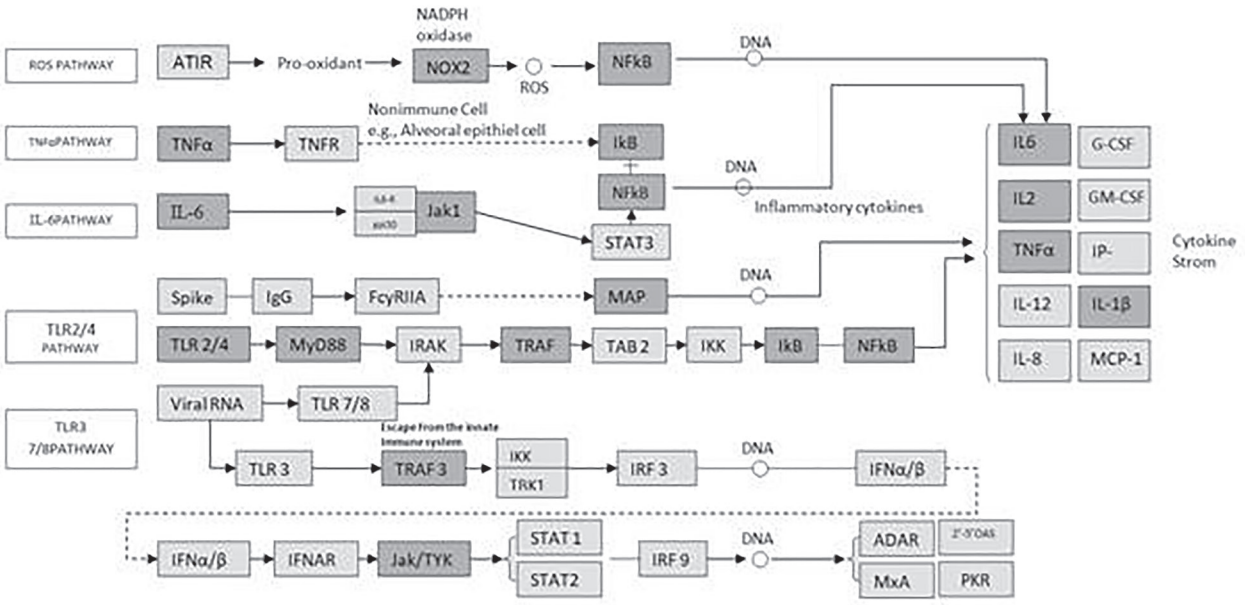

Figure 1: Molecular pathways in covid-19 based on Kyoto Encyclopedia of Genes and Genomes (KEGG)[38]

Dark grey boxes show the effect of HSYA in inhibition and down-regulation of cytokines to avoid cytokine storm. The flowchart in Figure 1 shows all significant steps that are involved in the covid-19 molecular pathway[38]. All studies on HSYA that are related to Covid-19 pathway cytokine inhibition are labeled with dark grey color.

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[39].

Song *et al*[15] 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[4]. Yang *et al* study[35] 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*[40] 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[41]. 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[38], attachment of viral RNA to TLR3 leads to activation of TRAF3 followed by activation of IKK and IRF3 as a transcription factor that leads to DNA activation for IFNα/β production, followed by activation of IFN receptor and activation of JAK/STAT for antiviral production (Fig.1).

Yu *et al* study[42] 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-κB/p65, and inhibitory kappa B (IκB)-α[16]. Moreover, HSYA treatment decreased NF-κB p65 nuclear translocation and inhibited p38 MAPK. These findings suggested that HSYA effectively inhibited LPS-induced inflammation in A549 human alveolar epithelial cells[15]. Further, Liu *et al* study[14] showed that HSYA could compete with TNF-α to bind with tumor necrosis factor receptor (TNFR) 1 and prevent binding of TNFR1 to TAK1-TAB2 complex. In addition, HSYA could also inhibit the activation of NF-κB 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-κB/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[43]. 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[44,45].

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[13]. 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[17], inhibited PAF-induced human bronchial smooth muscle activation, which lead to breathing difficulties, by targeting the PAF receptor (PAFR)[46], and inhibited PAFR binding[47]. Moreover, HSYA had a strong effect on cerebrovascular vasodilatation and significantly decreased platelet aggregation, blood viscosity, and thrombus formation[48]. 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 forma-

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 ALI/ARDS, and their 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.

ACKNOWLEDGEMENT

This work was supported by a research grant from the Ministry of Research, Technology and Higher Education of the Republic of Indonesia, Hibah Penelitian Pengembangan 2019, contract no.NKB-1804/UN2.R3.1/HKP.05.00/2019.

REFFERENCES

1. World Health organization. WHO Coronavirus Disease (COVID-19) Dashboard. WHO Web Page 2020. [Assessed 2020 Sept 11]. Available from: https://covid19.who.int
2. Chousterman BG, Swirski FK, Weber GF. Cytokine storm and sepsis disease pathogenesis. Semin Immunopathol 2017; 39: 517-28.
3. Chen C, Zhang XR, Ju ZY, He WF. Advances in the research of mechanism and related immunotherapy on the cytokine storm induced by coronavirus disease 2019. Zhonghua Shao Shang Za Zhi 2020; 36: 471-5.
4. Zhang Y, Song L, Pan R, Gao J, Zang B-X, Jin M. Hydroxysafflor Yellow A Alleviates Lipopolysaccharide-Induced Acute Respiratory Distress Syndrome in Mice. Biol Pharm Bull 2017; 40: 135-44.
5. Tanaka T, Narazaki M, Kishimoto T. Immunotherapeutic implications of IL-6 blockade for cytokine storm. Immunotherapy 2016; 8: 959-70.
6. Zhou X, Tang L, Xu Y, Zhou G, Wang Z. Towards a better understanding of medicinal uses of Carthamus tinctorius L. in traditional Chinese medicine: A phytochemical and pharmacological review. J Ethnopharmacol 2014; 151: 27-43.
7. Delshad E, Yousefi M, Sasannezhad P, Rakhshandeh H, Ayati Z. Medical uses of Carthamus tinctorius L. (Safflower): a comprehensive review from Traditional Medicine to Modern Medicine. Electron Physician 2018; 10: 6672-81.
8. Patel VJ, Biswas Roy S, Mehta HJ, Joo M, Sadikot RT. Alternative and Natural Therapies for Acute Lung Injury and Acute Respiratory Distress Syndrome. Biomed Res Int 2018; 2018. 2476824.
9. Cheng B-H, Zhou X, Wang Y, *et al*. Herb-drug interaction between an anti-HIV Chinese herbal SH formula and atazanavir in vitro and in vivo. J Ethnopharmacol 2015; 162: 369-76.
10. Wu JY-C, Yu Z-L, Fong W-F, Shi Y-Q. Chemotherapeutic activities of Carthami Flos and its reversal effect on multidrug resistance in cancer cells. African J Tradit Complement Altern Med 2013; 10: 36-40.
11. Wang Y-P, Guo Y, Wen P-S, *et al*. Three Ingredients of Safflower Alleviate Acute Lung Injury and Inhibit NET Release Induced by Lipopolysaccharide. Mediators Inflamm 2020; 2020: 2720369.
12. Pan R, Zhang Y, Zang B, Tan L, Jin M. Hydroxysafflor yellow A inhibits TGF-β1-induced activation of human fetal lung fibroblasts in vitro. J Pharm Pharmacol 2016; 68: 1320-30.
13. Wang C, Wang C, Ma C, Huang Q, Sun H, Zhang X, Bai X. Hydroxysafflor yellow A of Carthamus tinctorius attenuates lung injury of aged rats exposed to gasoline engine exhaust by down-regulating platelet activation. Phytomedicine 2014; 21: 199-206.
14. Liu S, Wang Y, Wen H, Sun X, Wang Y. Hydroxysafflor Yellow A Inhibits TNF-α-Induced Inflammation of Human Fetal Lung Fibroblasts via NF-κB Signaling Pathway. Evid Based Complement Alternat Med 2019; 2019: 4050327.
15. Song L, Zhu Y, Jin M, Zang B. Hydroxysafflor yellow a inhibits lipopolysaccharide-induced inflammatory signal transduction in human alveolar epithelial A549 cells. Fitoterapia 2013; 84: 107-14.
16. Liu Y-L, Liu Y-J, Liu Y, *et al*. Hydroxysafflor yellow A ameliorates lipopolysaccharide-induced acute lung injury in mice via modulating toll-like receptor 4 signaling pathways. Int Immunopharmacol 2014; 23: 649-57.
17. Guo X, Zheng M, Pan R, Zang B, Jin M. Hydroxysafflor Yellow A Suppresses Platelet Activating Factor-Induced Activation of Human Small Airway Epithelial Cells. Front Pharmacol 2018; 9: 859.
18. Zheng M, Guo X, Pan R, Gao J, Zang B, Jin M. Hydroxysafflor Yellow A Alleviates Ovalbumin-Induced Asthma in a Guinea Pig Model by Attenuateing the Expression of Inflammatory Cytokines and Signal Transduction. Front Pharmacol 2019; 10: 328.
19. Sun CY, Pei CQ, Zang BX, Wang L, Jin M. The ability of hydroxysafflor yellow a to attenuate lipopolysaccharide- induced pulmonary inflammatory injury in mice. Phyther Res 2010; 24: 1788-95.
20. Jin M, Wu Y, Wang L, Zang B, Tan L. Hydroxysafflor Yellow A Attenuates Bleomycin-induced Pulmonary Fibrosis in Mice. Phyther Res 2016; 30: 577-87.
21. Wang Y, Xue C, Dong F, *et al*. Hydroxysafflor yellow a attenuates small airway remodeling in a rat model of chronic obstructive pulmonary disease. Biol Pharm Bull 2014; 37: 1591-8.
22. Wu Y, Wang L, Jin M, Zang BX. Hydroxysafflor yellow A alleviates early inflammatory response of bleomycin-induced mice lung injury. Biol Pharm Bull 2012; 35: 515-22.
23. Wang C, Huang Q, Wang C, *et al*. Hydroxysafflor yellow A suppress oleic acid-induced acute lung injury via protein kinase A. Toxicol Appl Pharmacol 2013; 272: 895-904.
24. Jin M, Wang L, Wu Y, Zang BX, Tan L. Protective effect of hydroxysafflor yellow A on bleomycin- induced pulmonary inflammation and fibrosis in rats. Chin J Integr Med 2018; 24: 32-9.
25. Cecchini R, Cecchini AL. SARS-CoV-2 infection pathogenesis is related to oxidative stress as a response to aggression. Med Hypotheses. 2020; 143: 110102.
26. Baqi HR, Farag HAM, El Bilbeisi AH, Askandar RH, El Afifi A. Oxidative Stress and Its Association with COVID-19: A Narrative Review. Kurdistan J Appl Res 2020: 97-105.
27. Khomich OA, Kochetkov SN, Bartosch B, Ivanov A V. Redox biology of respiratory viral infections. Viruses 2018; 10(8): 392.
28. Xu X, Guo Y, Zhao J, Wang N, Ding J, Liu Q. Hydroxysafflor Yellow A Inhibits LPS-Induced NLRP3 Inflammasome Activation via Binding to Xanthine Oxidase in Mouse RAW264.7 Macrophages. Mediators Inflamm 2016; 2016: 8172706.
29. Xu H, Liu W, Liu T, *et al*. Synergistic neuroprotective effects of Danshensu and hydroxysafflor yellow A on cerebral ischemia-reperfusion injury in rats. Oncotarget 2017; 8: 115434-43.
30. He Y, Liu Q, Li Y, *et al*. Protective effects of hydroxysafflor yellow A (HSYA) on alcohol-induced liver injury in rats. J Physiol Biochem 2015; 71: 69-78.
31. Chen M, Wang M, Yang Q, *et al*. Antioxidant effects of hydroxysafflor yellow A and acetyl-11-keto-β-boswellic acid in combination on isoproterenol-induced myocardial injury in rats. Int J Mol Med 2016; 37: 1501-10.
32. Costela-Ruiz VJ, Illescas-Montes R, Puerta-Puerta JM, Ruiz C, Melguizo-Rodríguez L. SARS-CoV-2 infection: The role of cytokines in COVID-19 disease. Cytokine Growth Factor Rev 2020; 54: 62-75.
33. Cheng B-F, Gao Y-X, Lian J-J, *et al*. Hydroxysafflor yellow A inhibits IL-1β-induced release of IL-6, IL-8, and MMP-1 via suppression of ERK, NF-κB and AP-1 signaling in SW982 human synovial cells. Food Funct 2016; 7: 4516-22.
34. Lv Y, Qian Y, Fu L, Chen X, Zhong H, Wei X. Hydroxysafflor yellow A exerts neuroprotective effects in cerebral ischemia reperfusion-injured mice by suppressing the innate immune TLR4-inducing pathway. Eur J Pharmacol 2015; 769: 324-32.
35. Yang G, Zhou X, Chen T, *et al*. Hydroxysafflor yellow A inhibits lipopolysaccharide-induced proliferation and migration of vascular smooth muscle cells via Toll-like receptor-4 pathway. Int J Clin Exp Med 2015; 8: 5295-302.
36. Wu W, Jin M, Tong J, Wang X, Zang B. Inhibitory effect of hydroxysafflor yellow A against PMN activation induced by LPS. Yao Xue Xue Bao 2011; 46: 153-7.
37. Chen T-T, Du Y-J, Liu X-L, Zhu H-B. Inhibitory action of hydroxysafflor yellow A on inflammatory signal transduction pathway related factors in rats with cerebral cortex ischemia. Yao Xue Xue Bao 2008; 43: 570-5.
38. Molecular pathways in covid-19. In: Kyoto Encyclopedia of Genes and Genomes. [Assessed 2020 Sept 11]. Available from: (KEGG). https://www.genome.jp/kegg-bin/show_pathway?hsa05171
39. Pelaia C, Tinello C, Vatrella A, De Sarro G, Pelaia G. Lung under attack by COVID-19-induced cytokine storm: pathogenic mechanisms and therapeutic implications. Ther Adv Respir Dis 2020; 14: 1753466620933508.
40. Wang J, Wang P, Gui S, *et al*. Hydroxysafflor Yellow A Attenuates the Apoptosis of Peripheral Blood CD4(+) T Lymphocytes in a Murine Model of Sepsis. Front Pharmacol 2017; 8: 613.
41. Chauhan AJ, Wiffen LJ, Brown TP. COVID-19: A collision of complement, coagulation and inflammatory pathways. J Thromb Haemost 2020; 18(9): 2110-7.
42. Yu L, Liu Z, He W, *et al*. Hydroxysafflor Yellow A Confers Neuroprotection from Focal Cerebral Ischemia by Modulating the Crosstalk Between JAK2/STAT3 and SOCS3 Signaling Pathways. Cell Mol Neurobiol 2020; 40(8): 1271-81.
43. Zhang S, Liu Y, Wang X, *et al*. SARS-CoV-2 binds platelet ACE2 to enhance thrombosis in COVID-19. J Hematol Oncol 2020; 13: 120.
44. Younes Z, Florian P, Isabelle A, *et al*. Platelets Can Associate With SARS-CoV-2 RNA and Are Hyperactivated in COVID-19. Circ Res 2020; 127: 1404-18.
45. Francesco T, Gianluca S, Stefania C, *et al*. Platelets Promote Thromboinflammation in SARS-CoV-2 Pneumonia. Arterioscler Thromb Vasc Biol 2020; 40: 2975-89.
46. Guo X, Zheng M, Pan R, *et al*. Hydroxysafflor yellow A (HSYA) targets the platelet-activating factor (PAF) receptor and inhibits human bronchial smooth muscle activation induced by PAF. Food Funct 2019; 10: 4661-73.
47. Zang B, Jin M, Si N, Zhang Y, Wu W, Piao Y. [Antagonistic effect of hydroxysafflor yellow A on the platelet activating factor receptor]. Yao Xue Xue Bao 2002; 37: 696-9.
48. Sun Y, Xu D-P, Qin Z, *et al*. Protective cerebrovascular effects of hydroxysafflor yellow A (HSYA) on ischemic stroke. Eur J Pharmacol 2018; 818: 604-9.