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Original article

# The Effect of *Carthamus tinctorius* in Combination with Dexamethasone on Coagulation Markers in Mice Induced by SARS-CoV2 Spike Protein

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## Abstract

**Background and objectives:** COVID-19 is a respiratory condition frequently followed by an inflammatory reaction in addition to respiratory symptoms, which disrupts the blood coagulation process. One drug candidate known to have dual anti-inflammatory and anticoagulating effects is hydroxysafflor yellow A, an active compound found in the flowers of *Carthamus tinctorius*. Therefore, this study analysed the effect of *C. tinctorius* ethanol extract in combination with dexamethasone on coagulation biomarkers in mice induced with the SARS-CoV2 spike protein. **Method:** Flowers of *Carthamus tinctorius* were extracted with 98% ethanol. The concentrated extract was used in the study. Twenty-five Balb/c mice, comprising five healthy controls and twenty mice were induced with SARS-CoV2. The SARS-CoV2-induced mice were then randomized to receive seven days of treatment, which consisted of vehicle or dexamethasone 2.5 mg/kg BW, dexamethasone 2.5 mg/kg BW + *C. tinctorius* extract 400 mg/kg BW, or dexamethasone 2.5 mg/kg BW + *C. tinctorius* extract 800 mg/kg BW. Subsequently, the lungs and blood of the mice were analysed. **Results:** SARS-CoV2-induced mice increased all the coagulation markers studied. Treatment with dexamethasone alone or in combination with *C. tinctorius* extract 400 mg/kg BW did not cause reductions in D-dimer, plasminogen activator inhibitor-1, lactate dehydrogenase, platelet-to-leucocyte ratio, or neutrophil-to-leucocyte ratio. However, dexamethasone co-treatment with *C. tinctorius* extract at 800 mg/kg BW resulted in the normalization of D-dimer, PAI-1, and NLR in SARS-CoV2-induced mice. **Conclusion:** The administration of high dose of *C. tinctorius* flower extracts (800 mg/kg BW) in combination with dexamethasone showed the benefit of minimizing the coagulation disorder in SARS-CoV2.

**Keywords:** *Carthamus tinctorius*, COVID-19; hydroxysafflor yellow A; inflammation; thrombosis

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## Introduction

Coronavirus disease 19 (Covid-19) is a virus-borne illness caused by the severe acute

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respiratory syndrome coronavirus (SARS-CoV-2). This virus may be transmitted from person to person by close contact and droplets. Fever, headache, and cough are some of the clinical symptoms that people infected with SARS-CoV-2 experience [1,2]. In contrast, individuals with severe symptoms are typically accompanied by shortness of breath when the respiratory tract is inflamed. In addition to inflammation, there are coagulation abnormalities, namely thrombosis, related to mortality and morbidity in COVID-19 patients [3]. Previous research has revealed that the inflammatory process connects with coagulation status and is strongly connected to patient severity in COVID-19 patients [4]. In addition to disease severity, thrombosis and high levels of coagulation markers are critical prognostic factors in COVID-19 patients [5-7]. The renin-angiotensin-aldosterone system (RAAS) overactivation is related to inflammation in SARS-CoV2 patients [8].

Current COVID treatment techniques are based on the severity of the patient and the stage of the illness. Antiviral, anti-inflammatory, and symptomatic medications are prescribed [9-11]. Anticoagulant medications are not currently the standard of care for COVID-19 patients. The drug of choice currently recommended for all COVID-19 patients requiring oxygenation and ventilation, by the WHO panel is dexamethasone [9]. However, several studies have demonstrated that corticosteroids alone might not be enough for COVID-19 patients. Combined use of corticosteroids and anticoagulants might benefit COVID-19 patients with moderate to severe COVID-19 to prevent disease complications and improve clinical outcomes [12-14]. Thus, discovering a novel pharmacological treatment candidate with anti-inflammatory and anticoagulant properties, in combination with a corticosteroid might be a favourable strategy for COVID-19 patients.

Hydroxysafflor yellow A, an active chemical identified in *Carthamus tinctorius* Linn., is one therapeutic candidate with anti-inflammatory and anticoagulant properties [15,16]. *Carthamus tinctorius* or safflower can be found in regions with low precipitation, specifically in Southern Asia, China, or India [17]. The flower extracts were empirically and experimentally utilized as antiviral and anti-inflammatory medicine [15,17]. *Carthamus tinctorius* has been demonstrated in studies to influence the immune system, to act as an antioxidant, anti-aging, anti-hypoxia, anti-fatigue, anti-inflammatory, anti-fibrosis of the liver, anticancer, analgesic, and antithrombotic agent [18-20]. Research on murine macrophage cell models have shown that, *C. tinctorius* extract acts as an anti-inflammatory agent by inhibiting the NF-κB signalling pathway and activating the Nrf-2/HO-1 signalling pathway [20]. A study in a blood stasis rat model has revealed that *Carthamus tinctorius* extract improved plasma viscosity, and erythrocyte aggregation index [21]. Another study on $FeCl_3$-induced thrombosis model mice found that administering *C. tinctorius* extract had an antithrombotic effect [22]. Therefore, this research aimed to investigate the effect of the *Carthamus tinctorius* flower extract in combination with dexamethasone on coagulation indicators in mice induced with the SARS-CoV2 spike protein.

## Material and Methods

### Ethical considerations

The present study was authorized by the Faculty of Medicine Universitas Indonesia with the number KET-908/UN2.F1/ETIK/PPM.00.02/ 2021. The animals were treated following the guidelines for laboratory animal care and use at the Animal Research and Facility, Department of Microbiology, Faculty of Medicine Universitas Indonesia.

### Chemicals

SARS-CoV2 spike S protein recombinant antigen (UniProt Number P0DTC2, molecular weight 138 kDa) was obtained from Sigma Aldrich, Singapore (Cat no AGX819). ELISA Kit for D-dimer (Cat no EM0979) and PAI-1 (Cat no EM1262) were from Finetest, China. Spectrophotometer kit to determine the concentration of lactate dehydrogenase was from Randox Laboratories, Ireland (Cat No LD3842). Ethanol, methanol and all other chemicals were of the highest purity grade from Merck, Germany.

### Plant material

The flowers of *Carthamus tinctorius* were taken from the Bone area of South Sulawesi, Indonesia during the flowering season of 2021. The plant had been identified by the Taxonomic Laboratory of Universitas Negeri Makassar, Indonesia, and a voucher specimen was placed at the National Research and Innovation Agency, Herbarium Bogoriense Laboratory, Cibinong, Indonesia

(with voucher number of B-573/V/DI.05.07/11/ 2021).

Two hundred grams of *Carthamus tinctorius* flower powders were macerated for 24 hours in 98% ethanol. Subsequently, the mixture underwent filtration, leading to the segregation of the residue and the filtrate. The residual sediment was subjected to repeated maceration up to three times. The resulting filtrate was concentrated using a rotary evaporator over a water bath maintained at 55 $^{o}$C. The concentrated extract was then used for the treatment of the SARS-CoV2-induced mice.

**SARS-CoV2 induced mice**

This was experimental research in Balb/c mice, aged 12 – 15 weeks, induced with spike protein S of SARS-CoV2. The animals were placed into five groups of six mice. Twenty mice were induced by spike S recombinant protein SARS-CoV2 15 µg in 50 µL of saline, followed by a 100 µL air injection intratracheally. SARS-CoV2 induction was implemented from previous Hansur et al. [23]. The twenty SARS-CoV2-induced mice were then randomized to receive vehicle only or dexamethasone 2.5 mg/kg BW per oral (p.o.) or dexamethasone 2.5 mg/kg BW (p.o.) + CT extract (*Carthamus tinctorius*) at 400 mg/kg BW p.o. or dexamethasone 2.5 mg/kg BW p.o. + CT extract at 800 mg/kg BW p.o., twenty-four hour after the spike protein induction. The treatment was given for seven days. The mice were sacrificed on the eighth day, and lung and blood samples were collected.

**Hematologic analysis**

An animal hematology analyser measured platelet, lymphocyte, and neutrophil levels from whole blood samples.

**Quantification of D-dimer, plasminogen activator inhibitor-1 (PAI-1), and lactate dehydrogenase (LDH)**

The concentration of D-Dimer and PAI-1 in the blood plasma sample was determined by comparing the optical density value of the sample on an ELISA reader to a standard curve.

The lactate dehydrogenase concentration from serum samples was determined spectrophotometrically at a wavelength of 365 nm using a kit according to the manufacturer's procedure.

**Analysis of hydroxysafflor A**

**Sample preparation from extract**

The *Carthamus tinctorius* extract sample was prepared by diluting 1 mg of dry extract in 5 mL of 50% methanol, followed by filtration through a 0.2 µm syringe filter, and centrifugation at 14000 rpm for 5 minutes. Afterward, 50 µL of the sample was then mixed with 100 µL of methanol before being injected to the UPLC system.

**Sample preparation from serum**

Sample from mice serum was prepared by adding 1 mL to 250 µL of serum, which was subsequently centrifuged at 14000 rpm for 5 minutes at 5 °C. The supernatant was then transferred to another tube and evaporated at 55 °C using TurboVap under nitrogen until dry. Afterwards, 50% methanol was used to rehydrate the dried extract. For MS/MS detection, a 7 µL aliquot was put into the UPLC machine.

**Sample preparation from lung tissues**

Ten mg of sample was homogenized in 250 µL phosphate buffer serum 0.1 M with pH 7.4. Afterward, 1 mL of $CH_3OH$ (methanol) was added and vortexed for 30 s, which was subsequently centrifuged at 14000 rpm for 5 minutes at 5 °C. A 500 µL supernatant was then transferred to another tube and evaporated using TurboVap under $N_2$ at 55 °C until dry. The dried extract was reconstituted with 50% methanol. Seven µL aliquot was injected into the UPLC machine for MS/MS detection.

Ten mg of sample was homogenized in 250 µL of 0.1 M $Na_2HPO_4$ (disodium hydrogen phosphate) buffer solution with a pH of 7.4. After that, 1 mL of methanol was added and vortexed for 30 seconds before centrifuging at 14 000 rpm for 5 minutes at 5°C. The supernatant (500 µL) was transferred to another tube and evaporated under $N_2$ at 55 °C using TurboVap until dry. Methanol at a concentration of 50% was used to rehydrate the dried extract. For MS/MS detection, 7 µL aliquot was placed into the UPLC machine.

**UPLC-MS/MS system for hydroxysafflor yellow A analysis**

The concentrations of hydroxysafflor yellow A in *C. tinctorius* extract, as well as blood and lung tissue of the mice, were assessed using UPLC-MS/MS technique, with a lower limit of quantification (LLOQ) value of 0.20 ng/mL. The

liquid chromatography system employed a Poroshell$^{TM}$ 120 EC-C18 column, 2.7 µm, 4.6 x 50 mm, as the stationary phase at ambient temperature. The mobile phase used consisted of $CH_3OH$ : $NH_4CH_3CO_2$ 5 mM (85:15). The electrospray ionization process (ESI) was employed with negative mode at m/z parent 611 and m/z daughter 491 (Figure 1). The quantification of hydroxysafflor yellow A in the extract, serum, and pulmonary tissue was accomplished through a standard curve, whereby the response value of the specimen was calculated with that of the reference curve. Before analysing the study samples, method validation was conducted to ensure accuracy and precision.

**Statistical analysis**

One-way ANOVA was used to examine group differences, followed by post-hoc multiple comparisons using Fisher's Least Significant Difference (LSD). When the p-value was larger than 0.05, the difference between groups was deemed significant.

## Results and Discussion

The content of hydroxysafflor yellow A in *Carthamus tinctorius* flower extracts was detected to be 0.63% (w/w) or equal to 0.63 mg hydroxysafflor yellow A / 100 mg extracts after sample analysis in UPLC-MS/MS.

In prior studies, diverse concentrations of hydroxysafflor yellow A from *Carthamus tinctorius* have been obtained using different solvents and extraction techniques, with values ranging from 0.023% to 14.564% [15,24,25]. Zong et al. employed alcohol extraction methods and obtained a yield of hydroxysafflor yellow A that closely resembled ours at 0.584% [26].

Our study used two doses of *Carthamus tinctorius* extracts, 400 mg/kg BW and 800 mg/kg BW, corresponding with the administration of hydroxysafflor yellow A of 2.53 mg/kg BW and 5.06 mg/kg BW.

Up to date, limited studies have evaluated the anticoagulant effects of hydroxysafflor yellow A. So far, several ex vivo studies, but none *in vivo*, have demonstrated the protective effects of hydroxysafflor yellow A on blood coagulation markers [27,28]. An *in vivo* study has investigated the protective effects of *C. tinctorius* extracts at 100 mg/kg BW and 200 mg/kg BW on hemorheological disorders in epinephrine-induced rats [21]. Li et al. showed that the administration of *Carthamus tinctorius* may offer the added advantage of reducing blood viscosity, thereby enhancing blood fluidity [21]. In our study, we used higher doses compared to Li et al. assuming that SARS-CoV2-induced coagulation disorders would need higher doses than that of epinephrine-induced rats.

D-dimer, PAI-1, and lactate dehydrogenase are markers often used to evaluate the coagulation system in COVID-19 patients [29-31]. Studies have shown that SARS-CoV2 often disrupts the coagulation pathway, including fibrinolysis activity [32].

The significantly increased D-dimer levels in individuals with COVID-19 indicate a high likelihood of hyperfibrinolysis mediated by plasmin. The process of fibrinolysis is a crucial and dynamic mechanism that involves the maturation and degradation of fibrin clots. Maintaining homeostasis in the normal physiologic state is primarily attributed to fibrinolysis inhibitors, specifically plasminogen activator inhibitor-1 (PAI-1) and α2-antiplasmin. Imbalances in the proportional levels of activators and inhibitors involved in the process of fibrinolysis can lead to a range of irregularities, encompassing excessive cleavage of fibrin through plasmin-mediated mechanisms (known as hyperfibrinolysis) to a complete cessation of fibrinolysis [7,30,33].

In the present study, we found a marked increase in D-dimer and PAI-1 concentrations, a slight increase in lactate dehydrogenase mice, and a small increase in lactate dehydrogenase levels in SARS-CoV2-induced mice. Treatment with dexamethasone or dexamethasone + *Carthamus tinctorius* 400 mg/kg BW did not improve the three coagulation markers in SARS-CoV2-induced mice. However, combining dexamethasone and *C. tinctorius* extract at 800 mg/kg BW normalized D-dimer, PAI-1, and lactate dehydrogenase concentrations (Figure 2).

Lactate dehydrogenase levels reverted to normal after treatment with 800 mg/kg BW dexamethasone and *Carthamus tinctorius* extract. Nonetheless, this was not of statistical significance. This was resulted from a modest elevation of the levels of LDH in SARS-CoV-2 groups (about 10%).

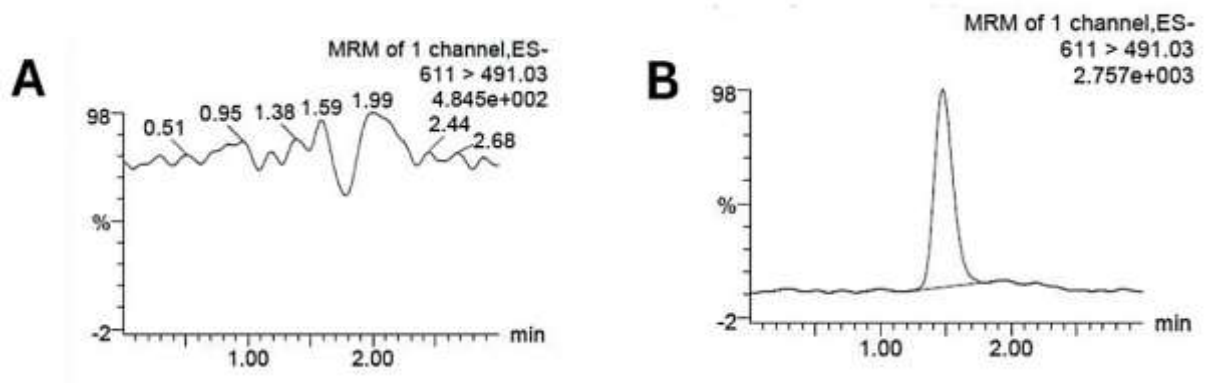


**Figure 1**. Chromatogram of (A) blank homogenates from lung tissues UPLC-MS/MS; (B) spike hydroxysafflor yellow A in lung tissue sample injected to UPLC-MS-MS apparatus with electrospray ionization process (ESI) negative mode at m/z parent 611 and m/z daughter 491

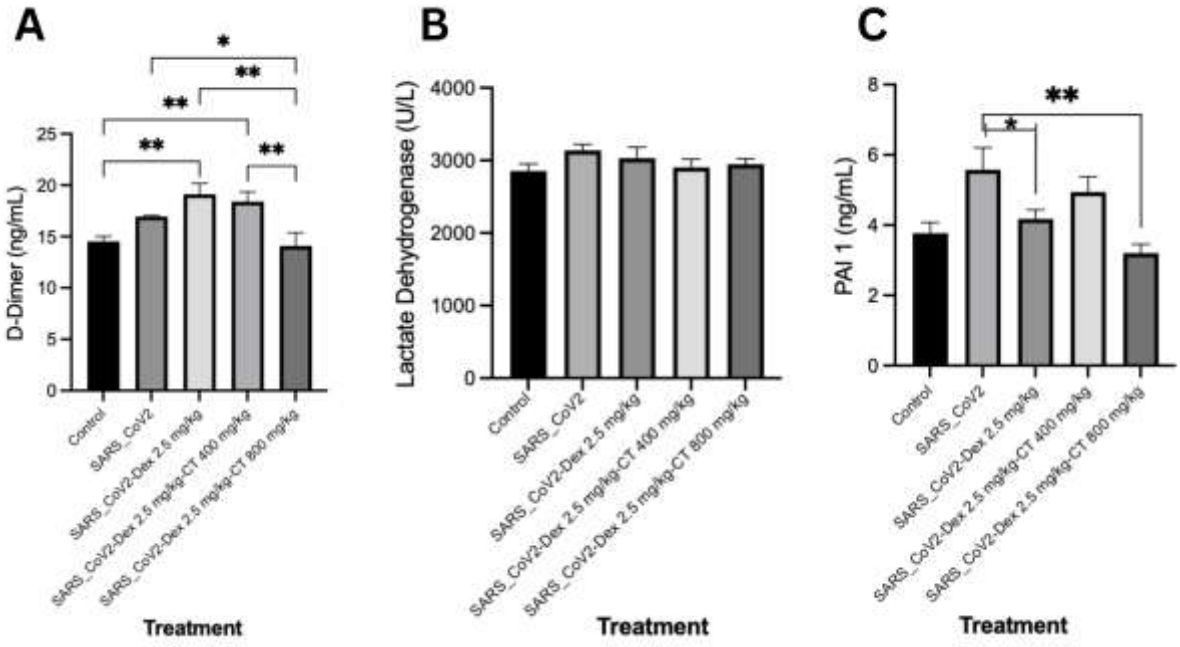


**Figure 2.** The levels of (A) D-dimer; (B) lactate-dehydrogenase; (C) Plasminogen activator inhibitor-1 (PAI-1) of healthy control or SARS-CoV2 induced mice treated with dexamethasone or the combination of dexamethasone + *Carthamus tinctorius* (CT) extract; * p<0.05; ** p <0.001; Dex: dexamethasone 2.5 mg/kg BW; CT 400: *Carthamus tinctorius* extract 400 mg/kg BW; CT 800: *Carthamus tinctorius* extract 800 mg/kg BW

Nonetheless, in humans, studies had found that even 20% increase of LDH levels in severe COVID-19 patients, may increase the risk of comorbidities and mortalities up to 6-times [34,35]. In the present study, treatment with dexamethasone alone resulted in a 3% decline of LDH levels. However, combining dexamethasone and *Carthamus tinctorius* in SARS-COV2-induced mice resulted in a decrease of 8-9% LDH levels. Although dexamethasone is recognized as a standard treatment in moderate to severe COVID-19 cases, in our study, dexamethasone alone did not diminish D-dimer, albeit it lowered PAI-1. The timing of steroid therapy in SARS-CoV2 infection might explain the phenomenon. According to research conducted by Rubio et al., the administration of dexamethasone during the early stages of SARS-

CoV-2 infection has been associated with the development of severe COVID-19, resulting in is defined by a significant increase in D-dimer levels [36]. Thus, adding *Carthamus tinctorius* extract at 800 mg/kg BW might benefit dexamethasone in normalizing D-dimer and PAI-1 in SARS-CoV2-induced mice.

Our findings indicated a rise in platelet count, neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR) in mice induced with SARS-CoV2. Dexamethasone alone or in conjunction with *Carthamus tinctorius* extract at 400 mg/kg BW or 800 mg/kg BW did not significantly alleviate platelet count or PLR. However, the administration of dexamethasone in conjunction with *C. tinctorius* at the dose of 800 mg/kg BW resulted in a decrease in the NLR, which approaches the value in the healthy control group (Figure 3).

The predominant entry route for SARS-CoV-2 is through the respiratory system, specifically the lungs. Within the pulmonary system, a localized coagulation mechanism, also known as broncho-alveolar hemostasis, collaborates with immune cells to combat infection. The acute phase response that has been marked includes platelets and fibrinogen. Thus, platelets and fibrinogen are acknowledged as the indicators of hypercoagulability [37,38]. Furthermore, COVID-19 patients have been found to exhibit evidence of pulmonary microthrombi during post-mortem examinations [39]. There are indications that the distinct respiratory patterns observed in COVID-19 patients may imply that microthrombi plays a crucial role in the clinical decline of the disease [40]. In cases of mild COVID-19, the microthrombi are degraded by the robust fibrinolytic activity in the pulmonary system, facilitating efficient gas exchange. An increase in D-dimer levels reflects this process. The activation of the pulmonary coagulation system is significantly pronounced in patients with severe illness [32].

Platelet counts usually increase in infected individuals, mainly in the early stages. In SARS-CoV2-infected patients, platelet counts are virtually never very low, even in the critically ill [37]. Previous studies in COVID-19 patients demonstrated that individuals exhibiting increased platelet count experienced extended hospital stays and unfavourable clinical outcomes [41]. A notable increase in platelet count might be resulted from cytokine storm, though the fundamental mechanisms are yet to be elucidated [42].

Our study showed that platelet counts increased in SARS-CoV2 infected mice, and treatments with dexamethasone and *C. tinctorius* extract of both doses did not reduce the platelet counts. However, in our study, the increase in the treatment did not cause extreme thrombocytosis that might lead to other coagulopathy and thrombosis.

A previous *ex vivo* studies in the a non-infectious model using a blood sample from rabbit plasma had demonstrated the effect of hydroxysafflor yellow A, the main compound of *C. tinctorius*, showed anticoagulant effect by inhibiting platelet aggregation and thrombosis formation as well as reducing blood viscosity [27,28].

In addition to the platelet count, recently, the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) have been established as markers of systemic inflammatory response in the bloodstream [43]. An imbalance in the levels of neutrophils and lymphocytes has been observed in association with the advanced stage of COVID-19. This phenomenon could be associated with the manifestation of a cytokine storm where neutrophils assume a crucial role as effector cells. The correlation between neutrophil and lymphocyte can be demonstrated by the neutrophil-to-lymphocyte ratio, which has the potential to serve as an early indicator of the disease's severe course [44,45]. Additionally, PLR has been used as an indicator of a pro-inflammatory state and a prognostic factor for the progression of COVID-19. Numerous studies have investigated the correlation between COVID-19 diagnosis and mortality rates in individuals afflicted [45,46].

In COVID-19 patients, a high dose of dexamethasone is needed to reduce the excessive systemic inflammation by lowering neutrophil response and impeding the neutrophil plugs in the lungs and other inflamed organs [47]. A study showed that lower doses of dexamethasone give different protection against inflammation in COVID-19 patients [48]. In our study, only the combinations of dexamethasone and the high dose of *C. tinctorius* extract (800 mg/kg BW) could have a beneficial effect in lowering NLR. Our results are in line with our findings in D-dimer and PAI-1.

The administration of *Carthamus tinctorius* extract at doses of 400 and 800 mg/kg

BW with dexamethasone led to a lung tissue concentration of 0.353 ng of hydroxysafflor yellow A /100 mg lung tissues and 0.638 ng of hydroxysafflor yellow A /100 mg lung tissues, respectively (Figure 4). Nevertheless, the presence of hydroxysafflor yellow A was not discernible in the circulatory system.

Hydroxysafflor A is classified as a Biopharmaceutics Classification System (BCS) III drug and exhibits limited oral bioavailability [15]. In a previous study in normal and diabetic cardiomyopathy mice, administration of hydroxysafflor yellow A at 60 mg/kg BW resulted in a maximum concentration (Cmax) of 2.41 μg/mL and 4.08 μg/mL, respectively [49]. In contrast, in our study, blood and lung samples taken 24 hours after seven days of consecutive administration of *Carthamus tinctorius* at 400 and 800 mg/kg BW or 2.53 mg/kg BW and 5.06 mg/kg BW, resulted in a detectable concentration of hydroxysafflor yellow A in the target tissues, but not in the plasma. However, the small amount of hydroxysafflor yellow A in the pulmonary tissues is enough to alleviate the increase in coagulation markers in SARS-CoV2-induced mice.

A pharmacokinetic study of hydroxysafflor yellow A administered intravenously in rats revealed a volume of distribution of 0.29 L/kg and a half-life of 0.83 hours (about 50 minutes). The findings indicated that hydroxysafflor yellow A had an extensive volume of distribution (1.16 L in 250 grams of rats) [50]. The volume of rats' whole blood WAS around 64 mL per kg (or 16 mL in 250 grams of rats) [51]. Thus, the fact that hydroxysafflor yellow A has a volume of distribution of 1160 mL in a 250-gram rats and a short half-life shows that the hydroxysafflor yellow A is rapidly distributed to body tissues, which is why it was undetectable in the systemic circulation 24 hours after the last drug administration. In the current study, it was demonstrated that hydroxysafflor yellow A was well distributed to pulmonary tissues.

Although hydroxysafflor yellow A is known to be the main component in *Carthamus tinctorius* extract, many other components are found in the plant's flower petals. In addition to hydroxysafflor yellow A, *C. tinctorius* also contains alkaloids, flavonoids, lignans, organic acids, and polyacetylenes [52].

Altogether, the substances in safflower attributed to its anti-inflammatory and anti-coagulative properties in the present model of SARS-CoV2-induced mice.

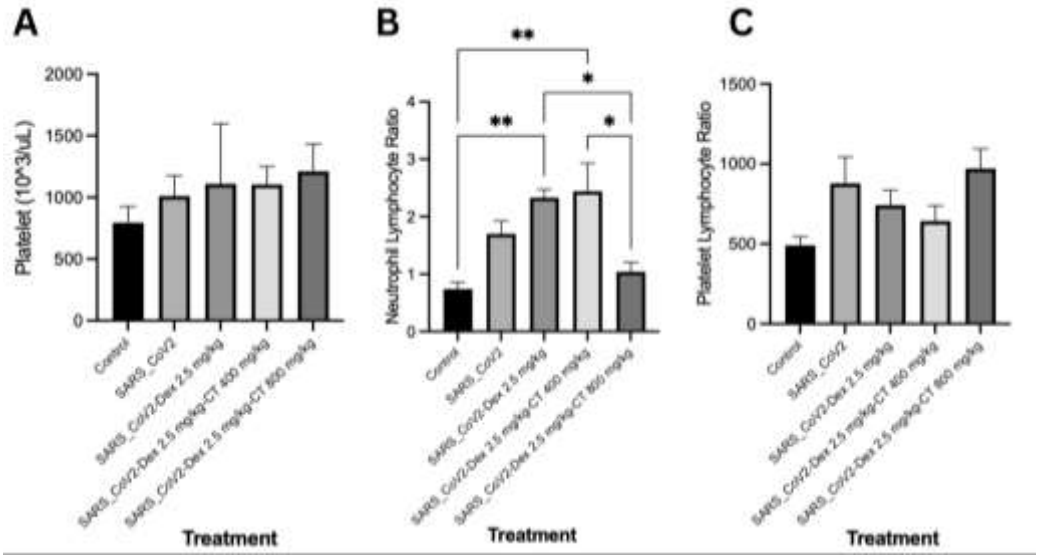


**Figure 3.** (A) Platelet count; (B) neutrophil-to-lymphocyte ratio (NLR); (C) Platelet-to-lymphocyte ratio (PLR) of healthy control or SARS-CoV2 induced mice treated with dexamethasone or the combination of dexamethasone + *Carthamus tinctorius* (CT) extract; * $p<0.05$; ** $p<0.001$; Dex: dexamethasone 2.5 mg/kg BW; CT 400: *Carthamus tinctorius* extract 400 mg/kg BW; CT 800: *Carthamus tinctorius* extract 800 mg/kg BW

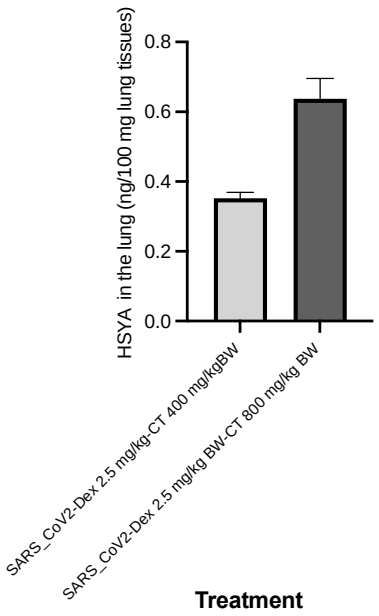


**Figure 4.** Concentrations of hydroxysafflor yellow A (HSYA) in the lung tissues of SARS-CoV2-induced mice after treatment with dexamethasone in combination with *Carthamus tinctorius* extract 400 mg/kg BW or 800 mg/kg BW. Dex: dexamethasone 2.5 mg/kg BW; CT 400: *Carthamus tinctorius* extract 400 mg/kg BW; CT 800: *Carthamus tinctorius* extract 800 mg/kg BW

Our results suggest that further studies with *C. tinctorius* flower extracts may benefit in alleviating the risk of coagulopathy in SARS-CoV2 individuals. Further preclinical as well as clinical studies regarding its mechanism of action, identifications of active compounds, and dose selections are needed for *Carthamus tinctorius* extracts to be a candidate as adjunctive treatment to dexamethasone in coagulation disorders caused by SARS-CoV2 infections.

## Conclusion

The administration of high doses of *C. tinctorius* flower extracts (800 mg/kg BW) combined with dexamethasone can minimize the coagulation disorder in SARS-CoV2-induced mice. The effect can be explained mainly by the anti-inflammatory effect of one of its active compounds, hydroxysafflor yellow A, on the pulmonary tissues.

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## Author contributions

Yan Tirta Indra Kurniawan dan Lismayana Hansur carried out the experiment; Yan Tirta Indra Kurniawan, Beti Ernawati Dewi and Melva Louisa wrote the initial manuscript; Melva Louisa, Beti Ernawati Dewi, Jamal Zaini and Ari Estuningtyas supervised the project. All the authors provided feedback and approved the final manuscript.

## Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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## Abbreviations

BCS: biopharmaceutics classification system; Cmax: maximum concentration; CT: *Carthamus tinctorius* extract; Dex: dexamethasone, ESI: electrospray ionization; HSYA: hydroxysafflor yellow A; LDH: lactate dehydrogenase; LLOQ: lower limit of quantification; NF-κB: nuclear factor kappaB; NLR: neutrophil to lymphocyte ratio; PAI-1: plasminogen activator inhibitor type 1; PLR: platelet to lymphocyte ratio; UPLC-MS/MS: ultra-high performance liquid chromatography tandem mass spectrometry