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REVIEW

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Phyto-pharmacological perspective of Silymarin: A potential prophylactic or therapeutic agent for COVID-19, based on its promising immunomodulatory, anti-coagulant and anti-viral property

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Coronavirus disease 2019 (COVID-19) triggered by a new viral pathogen, named severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2), is now a global health emergency. This debilitating viral pandemic not only paralyzed the normal daily life of the global community but also spread rapidly via global travel. To date there are no effective vaccines or specific treatments against this highly contagious virus; therefore, there is an urgent need to advocate novel prophylactic or therapeutic interventions for COVID-19. This brief opinion critically discusses the potential of Silymarin, a flavonolignan with diverse pharmacological activity having antiinflammatory, antioxidant, antiplatelet, and antiviral properties, with versatile immune-cytokine regulatory functions, that able to bind with transmembrane protease serine 2 (TMPRSS2) and induce endogenous antiviral cytokine interferon-stimulated gene 15, for the management of COVID-19. Silymarin inhibits the expression of host cell surface receptor TMPRSS2 with a docking binding energy corresponding to −1,350.61 kcal/mol and a full fitness score of −8.11. The binding affinity of silymarin with an impressive virtual score exhibits significant potential to interfere with SARS-CoV-2 replication. We propose in-depth pre-clinical and clinical review studies of silymarin for the development of anti-COVID-19 lead, based on its clinical manifestations of COVID-19 and multifaceted bioactivities.

KEYWORDS
ACE2, anticoagulant, COVID-19, immunomodulatory, ISG15, pro-inflammatory-cytokine, SARS-COV-2, Silymarin, TMPRSS2

1 | INTRODUCTION

The severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2), a novel coronavirus (nCoV) of zoonotic origin was reported from the Wuhan province of China for the first time in December 2019. Currently, the fatal outcome of this viral infection has taken 23.68 lakhs of human lives and infected 10.80 million people globally (https://www.worldometers.info/coronavirus update, February 10, 2021). In spite of the global effort to understand the virus and a deluge of publications, the detailed viral biology, life cycle, pathophysiology, and immunopathological response of the host to this highly contagious virus are poor. So far, no specific prophylactic or therapeutic antiviral agent or effective vaccine is available, which has forced the world to go for complete lockdown for several months. Further, the scenario has become grim due to increasing mortality triggered by an unpredictable pathophysiological response like hyperinflammatory disorders, blood clots, pulmonary embolism, thrombosis, and cytokine storm-driven organ damage (Garg, Prabhakar, Malhotra, & Agarwal, 2020; Helms et al., 2020; Penman et al., 2020). The immunopathological response of CoV-2 is unprecedented and discordant

toward the host defense with symptoms-based clinical manifestation and immuno-genomic variation (Toyoshima, Nemoto, Matsumoto, Nakamura, & Kiyotani, 2020; Severe Covid-19 GWAS Group, 2020). So far, the clinical management of COVID-19 patients is based on a trial-and-error basis with re-purposed antiviral drugs like ritonavir-lopinavir (protease inhibitors), remdesivir (adenosine analog), the anti-protozoal hydroxy-chloroquine (endosomal inhibitor) and so on (Penman et al., 2020). However, in acute cases, patients sometimes fail to recover due to nonspecific drug binding, adverse drug reaction, and co-morbid conditions including organ malfunctioning (Carter, ThiLanAnh, & Notter, 2020; Renu, Prasanna, & Gopalakrishnan, 2020; Zumla, Chan, Azhar, Hui, & Yuen, 2016). Many COVID-19 patients with co-morbid or hyperactive and pre-existing immuno-compromised conditions have had a lethal outcome (Li et al., 2020; Renu et al., 2020). Additionally, patients with pre-existing blood coagulopathy may suffer from pulmonary or vascular stroke, due to an underlying mechanism that could probably mimic sepsis-like syndrome and disseminated intravascular coagulation (Al-Samkari et al., 2020; Coppola et al., 2020; Lemke & Silverman, 2020).

In this review, we will discuss the potential of commercially available silymarin, and its Food and Drug Administration (FDA) recommended ingredients silybin and silibinin against SARS-CoV-2 infection, particularly to control the pathophysiological response of COVID-19 patients. The present discussion will also highlight the rationality for re-purposing silymarin for COVID-19 patients due to its versatile beneficial role against several pathophysiological disorders.

2 | BRIEF DESCRIPTION OF FLAVONOLIGNAN-BASED SILYMARIN

Silymarin is a standardized extractive fraction derived from the seeds of the traditionally used medicinal plant "milk thistle" or *Silybum marianum* of the family Asteraceae. The plant is native to the Mediterranean region including Crete, Greece, Iran, and Afghanistan. Chemically silymarin is a polyphenolic flavonolignan complex of seven closely related derivatives (silybin A, silybin B, isosilybin A, isosilybin B, silychristin, isosilychristin, and silydianin) with one flavonoid taxifolin. The yield of silymarin is 65%–80% to total crude extract (Corchete, 2008; Kroll, Shaw, & Oberlies, 2007). Silibinin, an active molecule of silymarin, exists as a mixture of two diastereomers, silybin A, and silybin B, in an approximately equimolar ratio. It is used as a supportive treatment in any liver diseases due to its excellent hepatoprotective activities (Gillessen & Schmidt, 2020). Moreover, it exerts chemoprotective effect from environmental toxins, elicits antiinflammatory, antioxidant, and immunomodulatory activity (Esmaeil, Anaraki, Gharagozloo, & Moayedi, 2017), and protects from UV-induced photo-carcinogenesis, UVB-induced epidermal hyperplasia, sunburn, and repair UV-induced DNA damage (Balouchi, Gharagozloo, Esmaeil, Mirmoghtadaei, & Moayedi, 2014; Singh & Agarwal, 2009). It has a promising antiproliferative effect against human prostate adenocarcinoma, estrogen-dependent and independent breast carcinoma, colon carcinoma, ecto-cervical carcinoma, and small and non-small cell lung carcinoma (Bhatia, Zhao, Wolf, & Agarwal, 1999; Hogan, Krishnegowda, Mikhailova, & Kahlenberg, 2007). Chemically this bio-active flavonolignan derivative is known as (2*R*,3*R*)-3,5,7-trihydroxy-2-[(2*R*,3*R*)-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydro-1,4-benzodioxin-6-yl]-2,3-dihydrochromen-4-one. The structures of the bioactive flavonolignan pharmacophores of silymarin extract have been presented in Figure 1. All of them pass the Lipinski rule of five descriptors: molecular weight, hydrogen donors and acceptor, and log P suggesting it as the good candidate of the oral drug for future therapy (Liptnski, Lombardo, Dominy, & Feeney, 2001; https://pubchem.ncbi.nlm.nih.gov/compound/31553).

Contemporary literature reveals that silymarin modulates virus-specific and nonspecific T-cell proliferation as a potential immunomodulator (Johnson, He, Osuchowski, & Sharma, 2003), and elicits antiinflammatory effects via suppression of IFN-γ and IL-10 production (Adeyemo et al., 2013). Moreover, silymarin had significant antiviral potential (Liu, Jassey, Hsu, & Lin, 2019), and is a well-known hepatoprotective, antioxidant, and anticoagulant with promising antiinflammatory activity (Delmas, 2020). Silymarin along with its derivatives, natural and chemical, have shown profound antiinflammatory activity by significant suppression of TNF-α, IL-6, IFN-γ, and IL-4 from bronchoalveolar lavage and lung macrophages in asthma and chronic obstructive pulmonary diseases (Dobiasová et al., 2020; Nasab, Saghazadeh, & Rezaei, 2020). Currently, a potential antiinflammatory drug Acalabrutinib has been recruited for the treatment of lung injury in severe COVID-19 patients with hypoxia and fibrotic damage, caused by the massive hyper inflammation due to activation of macrophage and endothelial cells from cytokine storm (Roschewski et al., 2020). It is interesting to note that silymarin has better protection (73.29% reduction) against inflammatory cytokine IL-1β (Toklu et al., 2008), compared to the standard antiinflammatory agents like Acalabrutinib, which demonstrates a 50% reduction of IL-1β in the CLP mice model. Current literature reveals that the suppressive activity of silymarin and acalabrutinib against pro-inflammatory cytokines TNF-α and IL-6 are interestingly comparable (O'Riordan et al., 2019). Silymarin not only suppresses the induction of TNF-α but also reduces its serum concentration along with the IL1β, IFN-γ, and other pro-inflammatory cytokines (Nazemian et al., 2010). Further, it represses the mitogen-activated protein kinase (MAPKs) ERK1/2 and P38 activities and release of Th1-related cytokine IL-2, associated with T-cell proliferation, which may help in immunosuppression (Gharagozloo et al., 2013) to control the organ damage triggered by cytokine storm during acute COVID-19 (Estrada, 2020; Robba, Battaglini, Pelosi, & Rocco, 2020).

The antiinflammatory activity of silymarin is quite analogous with the standard FDA-approved drug Baricitinib, used for the treatment of Rheumatoid arthritis (RA) and now recommended as COVID-19 therapy (Tong et al., 2019; Richardson et al., 2020). It is noteworthy that silymarin and one of its active constituent silibinin have been approved for the non-randomized single-arm clinical trial in RA patients (Shavandi et al., 2017), due to its inhibitory activity on TNF-α and other pro-inflammatory biomarkers of RA (Dupuis et al., 2018).

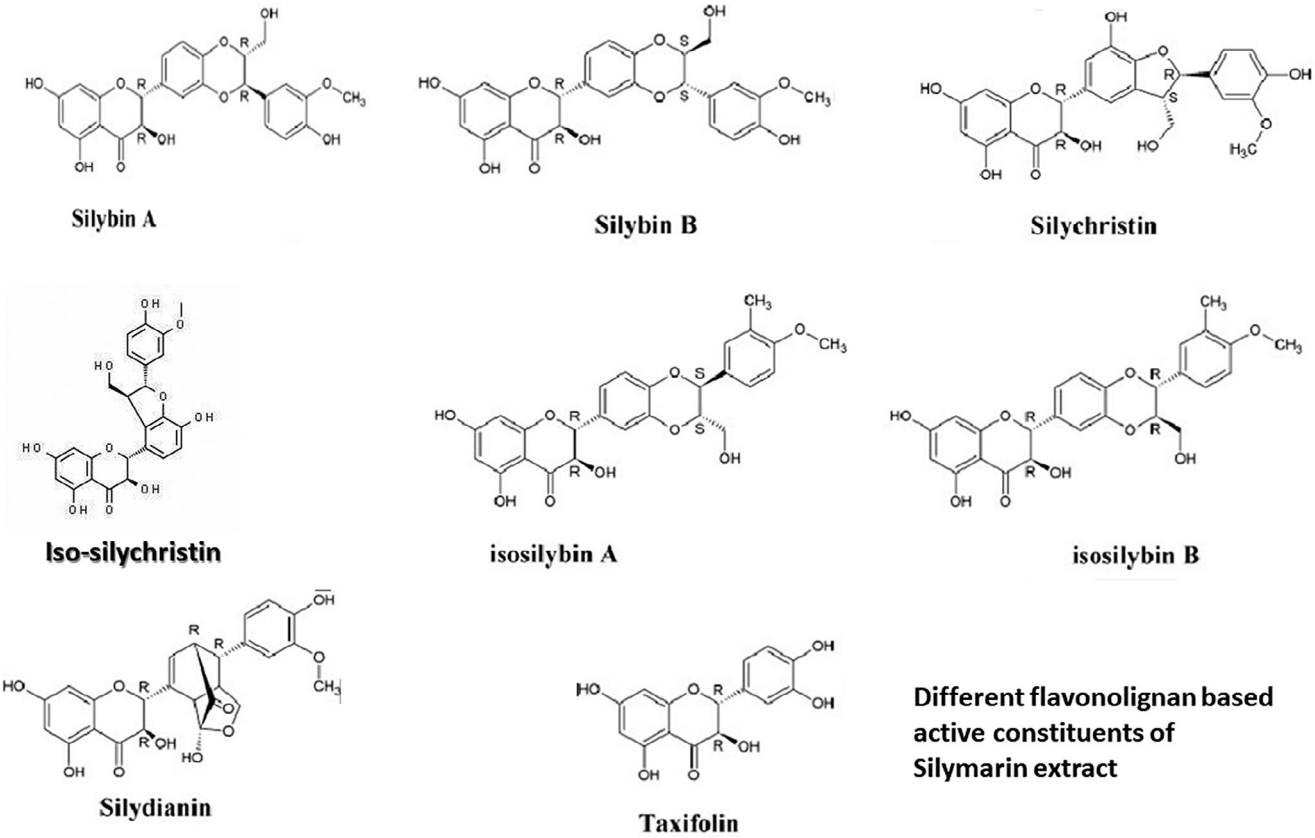

FIGURE 1 The structures of the different bioactive molecules of flavonolignans derivatives derived from the silymarin

Interestingly, it was reported that a high dose of silymarin can induce the expression of the interferon-stimulated gene (ISG) 15 (Adeyemo et al., 2013), an antiviral cytokine (Farrell, Broeze, & Lengyel, 1979; Swatek et al., 2018) induced by IFN-γ to promote the innate immune response and thereby stimulate the NK cells during influenza virus infection. Papain-like protease (PLpro), an essential enzyme of Coronaviruses, process viral polyproteins into a replicase complex for maturation, and release of new virion for viral spread (Harcourt et al., 2004; Lim, Ng, & Liu, 2000), and cleave host proteins to suppress the production of IFN-1 that provide antiviral immune responses to the host (Bailey-Elkin et al., 2014; Frieman, Ratia, Johnston, Mesecar, & Baric, 2009). A recent report illustrates that PLpro of SARS-CoV-2 cleaves off interferon-1 (IFN-1) stimulated gene-15 (ISG15) by cellular proteases rapidly than other SARS viruses leading to greater inhibition of TNF-α production with the reduced innate immune response of the host (Freitas et al., 2020). Upon infection PLpro of SARS-CoV-2 helps to cleave ISG-15 from interferon responsive factor-3 (IRF3) and attenuates IFN-1 responses; while inhibition of CoV-2 PLpro with GRL-0617 impairs virus-induced CPE, fosters antiviral IFN pathway, and reduce viral replication in infected cells, a dual strategy to suppress infection and promote antiviral immunity (Shin et al., 2020). Therefore, PLpro inhibitory activity of silymarin may be investigated to confirm whether ISG-15 mediated cytokine stimulation of silymarin can supersede the inhibitory action of PLpro during coronavirus infection. Furthermore, it needs to be verified whether signaling of IFN-γ and IL-4 cytokines relies on JAKs (Janus Kinases) to inhibit ACE2 expression at the transcriptional level in vitro, which may promote inhibition of infection, replication, and release of SARS-CoV-1 in Vero cells (De Lang, Osterhaus, & Haagmans, 2006). Moreover, it is reported that the variation of the doses of silymarin can modulate IFN-γ to control infection (Gharagozloo et al., 2010; Karimi, Vahabzadeh, Lari, Rashedinia, & Moshiri, 2011; Neha, Jaggi, & Singh, 2016). So, in the initial stage of infection, a higher dose of silymarin may boost the innate immune system through NK cells (McCarty & Block, 2006), which may help to tackle CoV-2 infection also.

Silymarin also blunts the enzymatic activity of cathepsin-B in ovarian cancer (Momeny et al., 2016); where overexpression of cathepsin B induces elevated production of TNF-α and IL-6, followed by inflammation. Moreover, silymarin alleviates the activity of both cathepsin B and cathepsin L along with the oxidative stress in TNBS-induced colitis in rat models (Bayramoglu et al., 2019). Cathepsin L, an endosomal cysteine protease, could be explored as an attractive drug target for CoV-2 infection, as it prevents the entry of SARS-CoV (Simmons et al., 2005) and regulates the host's innate and adaptive immune response related to tumor progression (Jakoš, Pišlar, Jewett, & Kos, 2019). So, silymarin mediated cathepsin L inhibitory activity may need to be validated in SARS-CoV-2 infection model. The overactivity of cathepsin L promotes the fusion and release of viral RNA after endocytosis of SARS-CoV, resulting in progressive infection

(Shah et al., 2010). Thus, silymarin can be tested for the inhibitory potential of cathepsin L to reveal its anti-SARS-CoV-2 activity (Liu, Luo, Libby, & Shi, 2020). Perhaps, silymarin mediated alleviation of upregulated inflammatory cytokines TNF-α, IL-6, and IL-8 and reduction of autophagic bronchial epithelial cells through ERK–MAPK pathway triggered by cigarette smoking (Ahmed, Moussa, Eldemerdash, Zakaria, & Abdel-Gaber, 2019; Li et al., 2016; Zhang, Wang, Cao, Wang, & Wu, 2017) may help to treat the pulmonary disorders in severe acute lung infection caused by the SARS-CoV-2.

The morbidity and mortality caused by COVID-19 infection have been intensified due to overactivated humoral immune response (Zhao et al., 2020), the formation of micro-thrombi in the blood vessel leading to blood clotting, viral laden antigen–antibody complexes, and severe allergic shock, along with the probable hypoxemic injury in vital organs like lungs, heart, brain, and kidneys (Garg et al., 2020). Such clinically uncontrolled manifestations can be managed by promoting cellular immunity with the reduced humoral response, prevention of hypoxemic, and reperfusion of injuries caused by a virus-driven cytokine storm. Here, silymarin could help to control such pathophysiological aberration due to its antiviral, immunomodulatory, antiinflammatory, antioxidant, antiplatelet, and anticoagulant properties (Abenavoli et al., 2018; Neha et al., 2016) in a sensible and phased manner. Further, the SARS-CoV-2 related ARDS, caused by massive elevation of inflammatory cytokines TNF-α, IL-1β, IL-6, and IL-32 in hyper-inflamed host cells (Spinelli, Conti, & Gadina, 2020), can be managed by silymarin may due to its attenuating activity on JAK overexpression (Agarwal, Tyagi, Kaur, & Agarwal, 2007; Richardson et al., 2020), as JAK-STAT3 inhibition may mediate the suppression of inflammatory cytokines and respiratory distress (Zegeye et al., 2018). Silibinin, another component of silymarin, is known to down-regulate the expression of TMPRSS2 in cell membrane surface in androgen deficient prostate cancer (Farooqi, Mansoor, Ismail, & Bhatti, 2010). Further, TMPRSS2 is reported to be an essential enzyme for cleavage of CoV-2 Spike protein(s) necessary for viral attachment with the host cell ACE2 receptor to enter into the host cell (Hoffmann et al., 2020). Hence, we hypothesized that silymarin could prevent the spread of SARS-CoV-2 infection by down-regulating the expression of TMPRSS2 receptors. This hypothesis is supported by our in silico docking study, which suggests that silymarin has a strong binding affinity to the homologous catalytic site of TMPRSS2 at its active site (Table 1). Further, our docking study revealed that the good binding affinity of silymarin is due to strong hydrogen bond interaction with the catalytic amino acid residues of the extracellular homological domain of TMPRSS2 receptor (PDB ID: 1Z8G) with minimized energy of −133.6 kcal/mol (Table 1). Our results also showed that silymarin had better interaction with the active site pocket of TMPRSS2, the second important host cell entry receptor of SARS-COV-2, for binding in comparison to standard TMPRSS2 inhibitor camostat mesylate. These findings may support that silymarin could block the host-virus interaction and further cellular infection by arresting the endosomal uptake of the virion. Over-expression of TMPRSS2 in response to SARS-CoV-2 during entry also plays a key role in immunopathology via upregulation of inflammatory chemokine and/or cytokine

TABLE 1 Binding affinity profile of Silymarin identified from Discovery studios software after the virtual screening via in silico molecular docking against homological active site of host transmembrane serine protease 2 enzyme (PDB ID: 1Z8G) and its drug-like properties

Phytocompound name	Plant source	Phytochemical empirical formula	Binding energy (kcal/Mol) (assessed in iGEMDOCK	Binding energy (kcal/Mol) (assessed in Swiss dock) FULL FITNESS score	3CLpro residues interacting with phytochemical through bonding and other interactions	M.W. gm/Mol	LogP value	No. of H-bond donor	No. of H-bond acceptor	No. of rotatable bond	Pubchem ID
Silymarin	*Silybum marianum* (*Milk thistle*)	$C_{25}H_{22}O_{10}$	−133.6	−1,350.61/−8.11	ARG-130,HIS-240,ARG-397, GLU-398,LYS-335[hydrogen bond] GLN-129,LYS-335,ASP-395, PHE-396,ARG-397,GLU-398,TRP-399,[Vander walls hydrophobic covalent bond]	482.4	2.4	5	10	4	1548994
Camostat mesilate (potent serine protease inhibitor)	–	$C_{21}H_{26}N_4O_8S$	−116.1	−1,478.50/−8.12	GLU-92, GLN-129, GLU-398, TRP-399[hydrogen bond] GLU-92, GLN-129, ARG-130, ASP-395, PHE-396, ARG-397, GLU-398, GLU-398, TRP-399[Vander walls hydrophobic covalent bond]	494.5	1.66	3	9	9	5284360

responses to intranasal and upper respiratory tract stimulation. Pieces of evidence accumulated so far suggest that severity in COVID-19 is related to hyper-inflammatory conditions triggered by diverse mediators, cytokines, chemokines, and related inflammatory factors (Tay, Poh, Renia, MacAry, & Ng, 2020), that could induce the TMPRSS2 gene to facilitate the infection followed by cytokine storm mediated organ damage. So, blocking of this receptor could function as a putative antiinflammatory receptor-based drug target against this novel coronavirus infection (Gkogkou, Barnasas, Vougas, & Trougakos, 2020; Iwata-Yoshikawa et al., 2019). Silymarin also attenuates the bronchial epithelial inflammation in chronic obstructive pulmonary diseases (COPD) of cigarette smokers due to elevated inflammatory cytokines TNF-α, IL-8, and IL-6 in response to autophagy and over-activation of ERK/p38 MAPK pathway. Interestingly, the pathophysiology of this signaling crosstalk can be suppressed by silymarin (Li et al., 2016). Moreover, silymarin exhibits antiviral activity against several virul diseases including Chikungunya virus (CHIKV) infection at an IC_{50} of 30 μg/ml by inhibiting viral replication via down-regulating viral nsp1, nsp3, and E2E1 proteins (Lani et al., 2015); and against the zoonotic RNA virus, the Mayaro infection by attenuating the virus-induced oxidative stress (Camini et al., 2018). Moreover, silymarin has a potent inhibitory activity (IC_{50}: 15.2 ± 3.53 μg/ml) against Enterovirus-71 with a selectivity index (SI) of 10.53, compared to baicalein (Lalani, Anasir, & Poh, 2020). Silybin, an active constituent of silymarin, demonstrated a significant reduction of HCV load at 20 mg/kg within 16 days of treatment by reducing the baseline mean viral load in Phase-3 clinical trials, without any adverse effect (Rendina et al., 2014). Song and Choi (2011) showed that silymarin at 100 μg/ml has potent antiviral activity against influenza virus A/PR/8/34 with 98% protection at an IC_{50} of 11.12 μg/ml. These reports collectively suggest that silymarin could be explored further for developing lead against SARS viruses, including novel coronavirus, as it elicited two-fold higher antiviral activity than the standard antiflu drug, oseltamivir (Tamiflu) in the influenza infection model (Song & Choi, 2011).

Recently it was observed that acute COVID-19 patients often die of blood clots due to induced pulmonary lung coagulopathy followed by internal hemorrhage mediated stroke (Connors & Levy, 2020; Menezes-Rodrigues et al., 2020). Thus, silymarin might be successfully used to manage and recover SARS-CoV-2 induced thrombotic pulmonary embolism associated strokes, due to its antiplatelet activity that may prevent coagulation. Further, silymarin treatment is found to reverse the blood clot formation in cardiac ischemia (Bijak, 2017; Bijak, Dziedzic, & Saluk-Bijak, 2018; Menezes-Rodrigues et al., 2020), lung and brain injury (Toklu et al., 2008), and thus, may help in the management of blood clot-induced severity at the acute inflammatory stage to reduce mortality; and increased recovery rate of COVID-19 patients with high d-dimer value. An earlier report suggested that the key flavonolignan derivative silybin of silymarin inhibited the blood coagulation factors thrombin and FXa (Bijak, 2017; Bijak, Ponczek, & Nowak, 2014).

Furthermore, a recent in silico study suggests that silymarin may block the protein–protein interaction between viral spike glycoprotein and host cell receptor ACE2 by binding with the receptor-binding domain (RBD) of spike protein at the RBD-ACE2 interface to control SARS-CoV infection (Ubani et al., 2020; Unni, Aouti, & Balasundaram, 2020). Thus, the clinical potency of silymarin could be investigated against mild, moderate, and acute infection caused by SARS-CoV-2, depending on the severity of the disease (Diao et al., 2020). Levels of circulating cytokines and T cell counts may help to select rational doses of silymarin, due to its well-known immunomodulatory potential, via suppression of CD4 T-cell activation with IL-2 and IFN-γ production at lower doses (Gharagozloo et al., 2010). Thus, the reviewed scientific literature so far discussed, and our in silico molecular docking study collectively suggests that silymarin could be used for possible therapeutic benefit in the management of COVID-19 patients due to its high safety index with significant antiinflammatory, anticoagulant, immune-modulatory, and antiviral response (Esmaeil et al., 2017) as illustrated in Figure 2. This may be achieved by varying the clinical doses of silymarin having a high therapeutic index with a broad margin of safety window (Wu, Lin, & Tsai, 2009). Therefore, the preclinical and clinical investigation of silymarin against COVID-19 needs to be validated in suitable models to design a potential therapeutic agent against super spreading SARS-CoV-2 mediated pandemic and restoring global normalcy. Summarized Table 2 suggested that in-vitro dose ranging from 50 to 100 μg/ml of silymarin had a promising antiviral response with more than 90% growth inhibition against Chikungunya, Mayaro, and influenza A viruses. Furthermore, 50–100 mg/kg of silymarin reduced the expression of inflammatory bio-markers very significantly and protected the lung injury in rat model at 200 mg/kg. So, its combination therapy with other FDA-approved safe and low doses of antiinflammatory and antiviral drugs need to be clinically investigated for formulating safer therapeutic armaments against COVID-19. The methodology of carried out research has been included in Table 2 for a better understanding of the discussion on the proposed theme.

3 | CLINICAL FEATURES OF SILYMARIN

It is interesting to report that a randomized placebo-controlled trial has been initiated to evaluate the clinical consequence in COVID-19 pneumonia in Phase-3, succeeding administration of silymarin. COVID-19 patients with pneumonia have been receiving standard care as per the Ministry of Health Protocol of Treatment + Silymarin (oral 420 mg/day in three divided doses) against a placebo control. Various clinical outcomes, such as time in days the patient was intubated for discharge or death whichever came first, were assessed up to 28 days. Several days patient remained positive to RT-PCR CoV-2 swab test at post-treatment, and any adverse events whether related to medication or not, have been carried out (US National Library of Medicine; *ClinicalTrials.gov*; Cairo University, Giza, Cairo, Egypt 12,613; Principal Investigator: Khaled Salem, MSc, First Posted: May 19, 2020. 08 pages; Last Update Posted: August 18, 2020-Identifier: NCT04394208 Silymarin in COVID-19 Pneumonia [SCOPE]. A randomized placebo-controlled trial to assess the clinical outcome in COVID-19 Pneumonia following administration of Silymarin owing to its role as a p38 MAPK pathway inhibitor and its antiviral, antiinflammatory, and antioxidant effects—May 19, 2020; Last Update

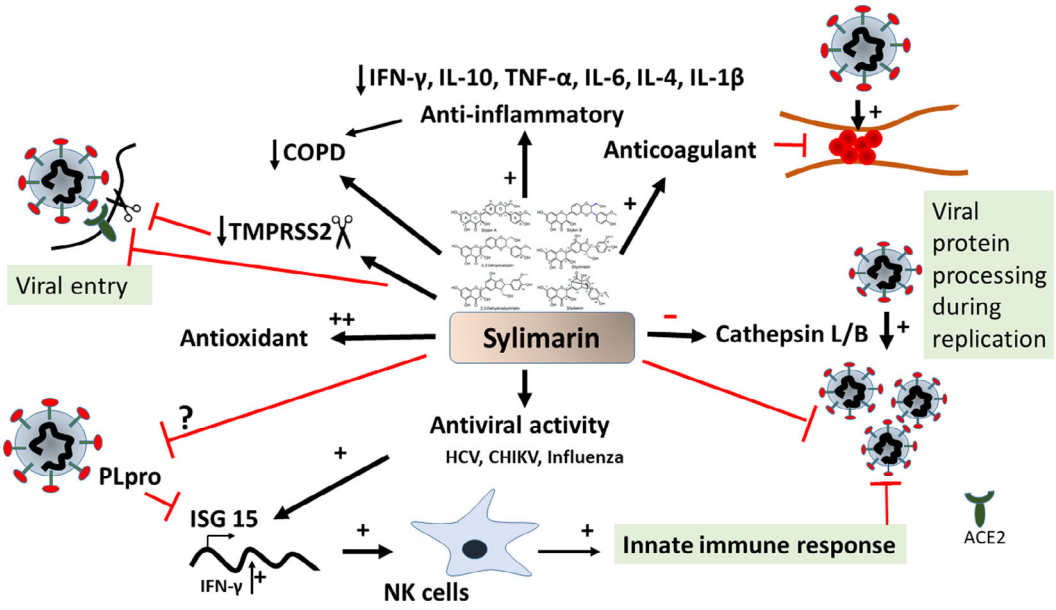

FIGURE 2 Multi-facets of silymarin action. Established and probable pharmacological roles of silymarin have been depicted. A "+" indicates a stimulatory effect, whereas a "−" an inhibitory role. A down arrow indicates a reduction in expression levels. The red dash sign indicates steps where silymarin could stop a particular step in SARS-CoV-2 pathogenesis. Details are in the text [Colour figure can be viewed at wileyonlinelibrary.com]

Posted: August 18, 2020 Pages-08. https://clinicaltrials.gov/ct2/show/NCT04394208).

Moreover, the interventional randomized clinical trial was successfully conducted for assessing the immunomodulatory activity in Beta-Thalassemia patients. Treatment with 140 mg oral dose three times a day, 7 days a week showed significant proliferation of T cells with stimulation of B and NK cells, along with the upregulated IL-2, IL-4, and IFN-gamma in the supernatant of activated T cells (US National Library of Medicine, Marjan Gharagozloo, Isfahan University of Medical Sciences, Sponsor: the Shiraz University of Medical Sciences-2012, US National Library of Medicine; *ClinicalTrials.gov* Identifier: NCT01752153- Immunomodulatory Effects of Silymarin in patients with beta-Thalassemia Major- December 19, 2012, 08 pages-https://clinicaltrials.gov/ct2/show/NCT01752153).

In a randomized clinical trial (Phase-2) of chronic hepatitis C patient's silymarin significantly prevented the associated liver disorders and reverse hepatic lesions with improved quality of life of the patients those used it as a dietary supplement (US National Library of Medicine, National Center for Complementary and Integrative Health (NCCIH)—January 31, 2002, Last Update Posted: August 18, 2006-Identifier: NCT00030030—Evaluating Silymarin for Chronic Hepatitis C-https://clinicaltrials.gov/ct2/show/NCT00030030). Another interventional non-randomized study to evaluate the safety and efficacy of intravenous silibinin (iSIL), a major constituent of silymarin extract, on hepatitis C virus (HCV) load in 20 HCV-HIV co-infected patients demonstrated a significant decline in HCV-RNA after 2 weeks of iSIL treatment at 20 mg/kg.(U.S. National Library of Medicine-ClinicalTrials.gov-Information provided by University of Zurich-March 22, 2013, Last Update Posted: March 5, 2015-NCT01816490—THISTLE-The HIV-HCV Silibinin Trial (THISTLE) —March 22nd, 2013–July 15, 2015; 08 pages. https://clinicaltrials.gov/ct2/show/NCT01816490). Moreover, the IdB 1016 phytosome complex containing the active constituent of silymarin, oral silybin, and phosphatidylcholine, significantly improves liver enzyme levels in serum and viral load in chronic HCV patients in Phase-2 randomized placebo-controlled trial with doses of 314 mg, 624 mg, and 942 mg three times daily (tid) for different stages of fibrosis (US National Library of Medicine-ClinicalTrials.gov National Center for Complementary and Integrative Health (NCCIH)—March 4, 2003 Last Update Posted: August 18, 2006—Identifier: NCT00055445-IdB 1016 Treatment for Hepatitis C Disease-March 4, 2003–August, 2006–08 pages-https://clinicaltrials.gov/ct2/show/NCT00055445).

Although the milk thistle treatment in chronic HCV infection failed as the conventional antiviral therapy but showed quite encouraging results for attenuation of viral load and liver marker enzymes in Phase-2 randomized trial. Results on oxidative stress, apoptosis, and fibrogenesis of the patients, compared to placebo control, suggest rigorous trials of botanical products of milk thistle (US National Library of Medicine-ClinicalTrials.gov, National Center for Complementary and Integrative Health (NCCIH)—March 20, 2008; Last Update Posted: March 18, 2013—Identifier:NCT00680342-Phase II Trial of Silymarin for Patients With Chronic Hepatitis C Who Have Failed Conventional Antiviral Treatment (SyNCH)-March 20, 2008–February, 2012–08 pages-https://clinicaltrials.gov/ct2/show/NCT00680342). Further, a recent review on diverse phytopharmaceutical suggests that silymarin can also be investigated for its ability to block the interaction of viral spike protein with the host cell receptors Furin, along with TMPRSS2 (Palit et al., 2020).

TABLE 2 Biological studies of Silymarin (SM) and its active constituents as an anti-COVID-19 agent based on the reported protective and therapeutic activities on inflammation, hyper-immune sensitization and viral diseases

					Results	
Author	**Year**	**Duration of studies**	**Species**	**Parameters**	**In vitro IC_{50}/CC_{50} (selectivity index)**	**In vivo effect**
Adeyemo et al., 2013	2013	T-cell proliferation assay for 16 h on drug exposure.	Viral hepatitis C infection caused by HCV, an RNA virus of the Flaviviridae family.	PBMC derived T-cell proliferation	IC_{50} ranged from 10–50 μg/dl of SM for different individuals against T-cell proliferation.	—
Adeyemo et al., 2013	2013	In-vivo treatment for 20 weeks.	HCV mice model.	*Candida albicans* induced in-vivo T cell proliferation assay.	—	*Candida* specific T-cell IFN-γ decreased from a mean 256 to 114 SFU/10^6 PBMC at 700 mg three times a day for 20 weeks in HCV infected mice.
Toklu et al., 2008	2008	10 days oral treatment with Silymarin	Cecal ligation and perforation (CLP)-induced sepsis for lung and brain injury & damage in rat model.	Levels of serum pro-inflammatory cytokines (tumor necrosis factor-alpha, interleukin-1β, and IL-6), lactate dehydrogenase (LDH) activity and tissue glutathione, as well as malondi-aldehyde and myelo-peroxidase activity, and the survival rate of rat.	—	Silymarin (50 mg/ kg, p.o.) decreased TNF-α (68.12%), IL-6 (73.69%), IL-1β (73.29%), LDH (66.12%), MPO level in brain (33.73%) and lungs (48.55%). Increased total anti-oxidant capacity (5.2-fold), 62.5% rat survived for long term.
Nazemian et al., 2010.	2010	210 mg/day silymarin (in three divided doses) for 2 months.	Peritoneal dialysis patient and normal healthy control.	Serum TNF-α	—	20% decreased in serum TNF-α level on responded dialysis patient, compared to healthy control.
Dupuis et al., 2018.	2018	Treated for 48 h.	Female RA patients	miR-155 expression in serum T lymphocytes for epigenetic role in RA.	—	Suppressed 50% expression of miR-155 for auto-immunomodulation of epigenetic key modulator in RA patient.
Gharagozloo et al., 2013	2013	72 h incubation for cell proliferation assay.	althy volunteers.	MAPKs' activity of cell lysate from activated naive CD4+ T cells. MAPKs' activity (ERK1/2 and P38) and Th1-related cytokines (IL-2, TNF-α, IFN-γ) Inhibit ERK1/2 and P38 pathway activation.	100 μM silymarin inhibited 40% T-cell proliferation from PBMC cells. At 100 μM it suppressed 92.31, 91.94, 71.43 & 84% production of IL-2, IFN-γ, TNF-α and G-CSF inflammatory Th1 cytokines released from activated PBMC.	—
Gharagozloo et al., 2010	2010	CD4+ T-cell proliferation assay was conducted from splenocytes incubated with Silymarin for 72 h. Supernatants from splenocytes untreated or treated with αCD3 mAb and silymarin for 72 h were assessed for IL-2 and IFN-γ.	CD4+ splenocytes IL-2 and IFN-γ production from CD4+ T-cell assay.	C57/Bl6 mice-derived splenocytes.	100 μM inhibited 99% CD4+ T cell proliferation from mouse splenocytes activated by α-CD3 mAb. 50 μM inhibited 73.34 and 49.58% production of IFN-γ and IL-2 by CD4+ T cells treated with α-CD3 mAb splenocytes.	—

TABLE 2 (Continued)

Author	Year	Duration of studies	Species	Parameters	Results	
					In vitro IC_{50}/CC_{50} (selectivity index)	In vivo effect
Bayramoglu et al., 2019	2019	Silymarin treatment started after 7 days of induction of colitis by TNBS and its analysis; and sacrifice was carried out after end of 7 days of silymarin treatment.	Sprague–Dawley rats of experimental colitis model induced by TNBS.	Tissue levels of malondialdehyde (MDA), cathepsin L, and cathepsin B; and activity of myeloperoxidase (MPO) enzyme.	—	50 mg/kg silymarin reduced the tissue MDA, MPO, Cathepsin B, and Cathepsin L level by 41.5%, 48.43%, 53.15%, and 25.04% respectively, compared to experimental colitis-induced rat. However, a higher dose (100 mg/kg) did not show the promising impact on tissue bio-marker of inflammatory bowel inflammation in colitis.
Li et al., 2016	2016	After exposure of cigarette smoke (CS) for 4 weeks, silymarin pretreated all animal groups sacrificed for analysis.	CS-induced male BALB/c mice model for evaluation of bronchial inflammation attenuating activity by the treatment of silymarin via intraperitoneal route of silymarin.	Analysis of inflammatory cytokines TNF-α, IL-6, IL-8 and autophagy in bronchial epithelia cell in COPD airway disease model pretreated with silymarin (SM).	—	SM at 50 mg/kg i.p. pre-treatment reduced the inflammatory cytokines TNF-α & KC from BALF of CS exposed COPD lung disorders by 39.2 and 45.71 respectively.
Zhang et al., 2017	2017	The mice received silybin (50, 100 mg/kg), once per day for 3 consecutive days before LPS sensitization.	LPS-induced acute lung injury model in mice for evaluating NF-κB and NLRP3 inflammatory marker by silybin treatment of intragastric (i.g.) route once per day for 3 consecutive days before LPS sensitization.	Level of NF-κB, NLRP3 and inflammatory cytokine from bronchoalveolar lavage fluid (BALF), blood plasma and tissue sample.	—	It demonstrates 72% suppression of total cells in BALF and 86.84% reduction of TNF-α, 90% mRNA expression of IL-6 serum at 100 mg/kg dose. Inhibits the activation of NLRP3 inflammasome in THP-1 cells by reducing the production of intracellular ROS at 100 mg/kg dose.
Ahmed et al., 2019	2019	Silymarin treated orally for 7 days at 200 mg/kg following HCL- induced acute lung injury.	Experiment carried out in adult inbred Sprague–Dawley male rats (about 3 months old), initially weighing an average of 180–200 g.	WBCs with differential count, oxidative stress parameters, Bcl-2, TGF-β, COX-2, Nrf-2, heme oxygenase-1 along with lung tissue histopathology with immuno-. Histochemical expression of survivin and PCNA were investigated.	—	Silymarin attenuated the histopathological lung injury with further up-regulation of Nrf-2 and HO-1 mRNA; and decreased the inflammatory and fibrotic parameters. It also upregulated the anti-apoptotic and the proliferation parameters and protected 43.75% of lung injury in an animal model.
Camini et al., 2018	2018	Mayaro virus infected HepG2 cells were treated with silymarin (3.125–100 μg/ ml) for 48 h post- infection.	Executed the plaque assay for antiviral activity of Chikungunya virus in Vero cells.	Inhibition assay of cytopathic effect, viral replication, and plaque reduction were used; and MAYV-induced ROS, MDA and carbonyl protein, were determined.	SL inhibits the relative viability of the Mayaro virus by 50% at 100 μg/ ml.	—

(Continues)

TABLE 2 (Continued)

Author	Year	Duration of studies	Species	Parameters	Results	
					In vitro IC_{50}/CC_{50} (selectivity index)	In vivo effect
Lani et al., 2015	2015	A cytopathic effect (CPE) inhibition assay was undertaken on infected Vero cells treating with silymarin for 48 h.	In vitro anti-CHIKV activity using a CHIKV replicon cell line and a clinical isolate of CHIKV of central/east African genotype.	Cytopathic effect and virus yield inhibition. Expression of nsP1, nsP3, and E2E1 proteins responsible for viral replication were determined.	100 μg/ml of silymarin suppressed the activity of Rluc marker expressed by the CHIKV replicon by 93.4%.	–
Lalani et al., 2020	2020	SI was exposed for 1 h at 37°C, in DMEM for EV-A71 antiviral efficacy in RD.	EV-A71 sub genotype B4 strain 41 (5,865/SIN/00000) was used for evaluation of antiviral efficacy.	CC_{50}, virucidal index (IC_{50})	IC_{50} against EV-A71 is 15.2 ± 3.53 μg/ml with SI of 10.53. It blocks both attachment and entry of EV-A71 to normal mammalian Vero cells.	–
Rendina et al., 2014	2014	Randomized, double-blind placebo-controlled, phase 2 trial of parallel group, was conducted for 14 consecutive days with silymarin.	The phase 3 trials have been conducted on 20 HCV patients.	Viral load in liver and bilirubin level in serum were calculated after treatment with silymarin.	–	Viral load reduced by 2.30 ± 1.32 in silibinin group compared to the placebo group, and bilirubin level has been improved in the treated group.
Song & Choi, 2011	2011	After incubation at 37°C in 5% CO_2 for 2 days, the morphology of influenza A infected cells was observed in microscope. The antiviral effect was determined by SRB method using CPE reduction.	Influenza A/PR/8/34 virus in MDCK cells was cultured and incubated in the presence of 100 μg/ml of silymarin.	Anti-influenza A/PR/8/34 virus activity, by inhibiting viral mRNA synthesis.	98% protection against influenza A/PR/8/34 viral strain with 100 μg/ml of silymarin.	–
Bijak et al., 2014	2014	Absorbance values were monitored every 12 s for 10 min in the presence of silybin using ELISA reader.	Amidolytic activity of factor Xa was determined spectroscopically by ELISA reader.	Velocity of reaction (mOD/min) for each absorbance curve for chromogenic reaction between Fxa and its substrate in the presence of silybin in respect to its IC_{50} value.	IC_{50} at 750 μM substrate concentration) for inhibition of FXa amidolytic activity by silybin was 35 μM.	–

4 | CONCLUSION

Currently, there is no promising officious treatment accessible for the highly contagious SARS CoV-2. Thus, identifying novel and fruitful treatments is imperious and would be of great benefit to patients. However, numerous clinical trials are ongoing globally to discover active drugs for COVID-19 treatment, and no drug has been proclaimed to be fruitful for curing COVID-19 so far. Silymarin is appreciated for its various immune-pharmacological and cytokine regulating effects and appears as a hopeful phytopharmaceutical for the management of COVID-19 (Figure 2). More significantly, derivatives of silymarin like silybin, and silibinin have promising antiviral responses that might also be a good option. However, some vital points such as exact dosing and time course of therapy based on the severity of the disease manifestation need to be addressed for fixing the proper treatment modality to control viral infection. Additionally, the biological function of silymarin is consolidated from in-vitro findings, animal experiments, and clinical data on related diseases may not correspond with clinical efficacy in humans. Therefore, the precise clinical curative or even prophylactic use, the optimal dose and course of treatment must be assessed following suitable preclinical and clinical study.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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