

***Camellia sinensis* L. Extract Suppresses Inflammation on Acute Respiratory Distress Syndrome Cells Models via Decreasing IL-1 β , IL-6 and COX-2 Expressions**

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Abstract

Acute Respiratory Distress Syndrome (ARDS) is one of the clinical manifestations in severe COVID-19 patients characterized by acute inflammation resulting in respiratory failure and death. *Camellia sinensis* L. or green tea extract has many beneficial secondary metabolites including polyphenols which have anti-inflammatory and anti-viral roles. This study was aimed to investigate the activity of green tea extract (GTE) as an anti-inflammatory on ARDS model cells. In this study, rat lung alveolar type II epithelial cells line (L2) was induced by lipopolysaccharide (LPS) to mimic the inflammation process in ARDS. These cells were then treated with GTE to determine the GTE effectiveness in reducing the inflammation. The GTE polyphenol constitutions were first confirmed using Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS). The cytotoxic assay was conducted using MTS assay kit to determine the safe range of GTE concentrations that used in the next assay. The effectiveness of the GTE was determined by measuring pro-inflammatory cytokines (IL-1 β and IL-6) and inflammation-mediated enzymes (COX-2), both of which were by ELISA method. Furthermore, this study measured the ACE-2 and TMPRSS-2 gene expressions by qRT-PCR method. The results showed that GTE treatment significantly reduced pro-inflammatory cytokines IL-1 β , IL-6, inflammation-mediated enzyme COX-2, and significantly increased ACE-2 and TMPRSS-2 mRNA expressions. In short, green tea extract possesses the potential to alleviate inflammation.

Keywords: Acute respiratory distress syndrome, *Camellia sinensis*, Cytokines, Inflammation

Introduction

Acute Respiratory Distress Syndrome (ARDS) is one of clinical manifestations in severe COVID-19 patients characterized by acute inflammation results in respiratory failure and death [1]. Lung inflammation is proposed to start in the alveolar epithelial cell type 2 (AEC-II), then causes alveolar tissue collapse. Subsequently, various signalling pathways and cytokines will be activated and secreted by the affected cells [2]. Damaged immune cells release these cytokines, which leads to hyperinflammation and increases the risk of death since it frequently affects lung function [3]. The virus causes lung cytokine storms that cause hyperinflammation or ARDS. Hyperinflammation is the basis for the development of specific anti-inflammatory therapy, such as an anti-Interleukin-6 (anti-IL-6) or anti-IL-1 [4].

According to previous research, the main entry of the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is the binding of virus to the Angiotensin-Converting Enzyme-2 (ACE-2) receptors [5]. Additionally, the protein spike (S) of the virus also plays an important role in priming Trans-Membrane Protease Serine-2 (TMPRSS-2) [6]. ACE-2 also plays a protective role in ARDS and has the potential to be developed as an ARDS therapeutic medication [7]. The ACE-2 protect rat Pulmonary Microvascular Endothelial Cells (PMVECs) from LPS-induced apoptosis and inflammation via suppressing c-Jun N-terminal Kinases (JNK) and Nuclear Factor-KappaB (NF-kB) activation [8]. When

infection-caused inflammation occurs in the respiratory tract, high levels of inflammation mediator cytokines would be secreted. Thus, the inflammation process involves various cytokines such as IL-1 β , IL-6, IL-8, IL-10, Cyclooxygenases (COX)-2, and Tumor Necrosis Factor (TNF)- α [9,10]. Inhibiting and controlling the level of inflammatory mediators can be used as a new therapeutic approach for ARDS treatment, as pharmacological treatment is not yet available [11].

Plant-based products that have anti-inflammatory properties seem to be effective and safe for treating inflammation. Green tea (*Camellia sinensis* L.) has been reported to have anti-inflammatory activities through its polyphenols. Tea will act as an anti-inflammatory agent by inhibiting the secretion of various pro-inflammatory cytokines [12]. Based on the numerous *in vitro* and *in vivo* studies, they have suggested the biological properties of green tea and polyphenols have antioxidant, antiobesity, anticancer, antibacterial, anti-inflammatory, and antiviral activities [13-16]. *C. sinensis* extract exerts anti-inflammatory potential due to downregulate of NF- κ B and Mitogen-Activated Protein Kinase (MAPK) pathways in macrophages, the green tea treatments could suppress the release of proinflammatory cytokines (TNF- α , IL-6 and IL-1 β) and the mRNA expression levels of inducible Nitric Oxide Synthase (iNOS), COX-2 [17]. *C. sinensis* extract treatment also has antiviral activity through suppressing proinflammatory cytokines gene expression levels (IL-6, TNF- α and IL-1 β) [18]. In *in vitro* and *in vivo* studies, LPS is the most common inducer used to induce inflammation. Lipopolysaccharide (LPS) stimulation could mimic the inflammatory responses as seen in lung injury [19]. Therefore, LPS was used in this study to evoke the condition of inflammation.

The aim of this study was to determine the effect of green tea extract (GTE) on rat lung cells line (L2) induced by as an ARDS model toward levels of IL-1 β , IL-6, COX-2, gene expression of ACE-2 and TMPRSS-2.

Materials and methods

Camelia sinensis L. extraction

GTE was produced by PT. Fathonah Amanah Shidiq Tabligh (Depok, Indonesia) (Batch 00107201057). As a solvent, 70 % ethanol was used in the extraction and its processed under Good Manufacturing Practice (GMP) standard by The Indonesian Food and Drug Authority (BPOM). After the extraction process, GTE was added with lactose and stored at 25 \pm 2 $^{\circ}$ C [20,21].

LC-MS/MS analysis

GTE compound contents were analyzed using LC-MS method with Hypersil Gold specifications (150 mm \times 2.1 mm \times 1.9 μ m). TSQ Quantum Access MS/MS Triple Q (quadrupole) mass spectrometer with Electrospray Ionization (ESI) was controlled by TSQ Tune software, which operated with a positive charge [22].

Cell culture

Rat L2 lung epithelial cells (ATCC $^{\circ}$ CCL-149TM) was obtained from Aretha Medika Utama, Biomolecular and Biomedical Research Center, Bandung, West Java, Indonesia. The cells were grown in complete medium consisted of Minimum Essential Medium Alpha (MEM- α) (Biowest, L0475-500), 10 % Fetal Bovine Serum (FBS) (Biowest, S1810-5000), 1 % Antibiotic-antimycotic (ABAM) 100X (Biowest, L0009), and 0.1 % Gentamicin (Gibco, 15750078). To induce inflammation, LPS *Escherichia coli* O55:B5 (Sigma-Aldrich, L4516-1MG) was used. The cells were induced with 4 μ g/mL LPS incubated for 18 h in 37 $^{\circ}$ C, 5 % CO $_2$ incubator, then treated with GTE that was diluted in DMSO to reach the final concentration 1.56, 3.13 and 6.25 μ g/mL and incubated for 24 h. To determine the levels of IL-6 and COX-2, supernatant cells were used and analyzed using ELISA kit. ACE-2 and TMPRSS-2 genes expressions were determined using qRT-PCR method [23].

Cell viability assay

This assay was conducted referring to manufacturer's protocol. Briefly, the cells were grown in 96 well-plates in density of 8 \times 10 3 cells/cm 2 and incubated for 24 h. Subsequently, the medium was replaced with new medium made of 180 μ L complete medium with 20 μ L LPS 1 μ g/mL. Afterward, the cells were incubated at 37 $^{\circ}$ C, 5 % CO $_2$ for 16 to 18 h. After incubation, previous medium was discarded, then 180 μ L new medium and 20 μ L of sample was added to each well and incubated at 37 $^{\circ}$ C, 5 % CO $_2$ for 24 h. Untreated cells were seeded in 180 μ L fresh medium and 20 μ L of sample used as controls. Then, the cells were incubated at 37 $^{\circ}$ C, 5 % CO $_2$ for 24 h. After incubation, 20 μ L of MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (Abcam, ab223881) was added to each

well and incubated for 3 h. The absorbance was read at 490 nm using a spectrophotometer (Multiskan GO and Microplate Spectrophotometer, Thermo Scientific N12391) [23].

Protein assay

This assay was employed to measure total protein, then used to assess cytokine level by mg protein in ELISA assay. The standard solution was used Bovine Serum Albumin (BSA) to determine the total amount of protein. BSA solution (2000 µg/mL) was prepared by dissolving 2 mg BSA (Sigma, A9576) in 1000 µL of ddH₂O. Then a series of dilutions was made from 2000 to 0 µg/mL. The standard solution and the sample were added 20 µL to each well. After that, Quick Start Dye Reagent 1X (Biorad, 500-0205) was immediately added 200 µL to each well and incubated for 5 min at room temperature. The absorbance was read at 595 nm [24,25].

Quantification of IL-6, IL-1β, and COX-2 level

The supernatant cells were harvested and tested using ELISA kit to determine the levels of pro-inflammatory cytokines level in the cell L2. The IL-6, IL-1β, and COX-2 levels were determined using ELISA kit (Elabscience, E-EL-R0015, E-EL-H0109, E-EL-R0792 respectively) [21].

Quantification of ACE-2 and TMPRSS-2 genes expression

The cells were harvested and tested using qRT-PCR method to determine the levels genes expressions of ACE-2 and TMPRSS-2. Total RNA was isolated and purified using Direct-zol RNA Miniprep Plus Kit (Zymo, R2073), the procedure was according to the protocol of manufacture. Then, the synthesis of complementary-DNA was carried out using *SensiFAST cDNA synthesis kit* (Bioline, BIO-65054) and the procedure was according to the protocol. PCR included priming at 25 °C for 5 min, reverse transcription at 46 °C for 20 min, RT inactivation at 95 °C for 1 min, and optimization step at 4 °C. After that, for gene expression analysis of ACE-2 and TMPRSS-2 were carried out used SYBR NO-ROX kit (Bioline, BIO-98005) and primer [22]. The primer sequences were shown in **Table 1**.

Table 1 Primer sequences of genes.

Gene	Primer sequence (5'-3')	Product size (bp)	Annealing (°C)	Cycle	Reference
ACE-2 rat	F: AACAAGCACAGACTACAATCGT R: ACGGTTTGATCTCTTTGAAGGT	248	58	40	NM_001012006.2
TMPRSS-2 rat	F: TATGAGAAAGGGAAGACCTCAG R: ATACTGTCACATTCCCGTATACTC	277	60	40	NM_130424.3
GAPDH rat	F: 5'-TCAAGATGGTGAAGCAG-3' R: 5'-ATGTAGGCCATGAGGTCCAC-3'	217	57	40	NM_001289726

Statistical analysis

The experimental results were presented as mean and standard deviation. For statistical analysis of data, SPSS 26.0 software was used. One-way analysis of variance was used for the comparison among groups and Tukey HSD post hoc test. Statistical significance was defined as $p < 0.05$.

Results and discussion

Results

LC/MS-MS result

LC/MS-MS was employed to analyze compounds contained in GTE. The results of the measurements are presented as peaks. Each peak conveys a certain compound and is determined by referring a previous study. The result of the LC/MS-MS for the GTE contained quercetin, myricetin, 6-prenylaringenin, and 8-prenylaringenin. LC/MS-MS chromatogram can be seen on **Figure 1**.

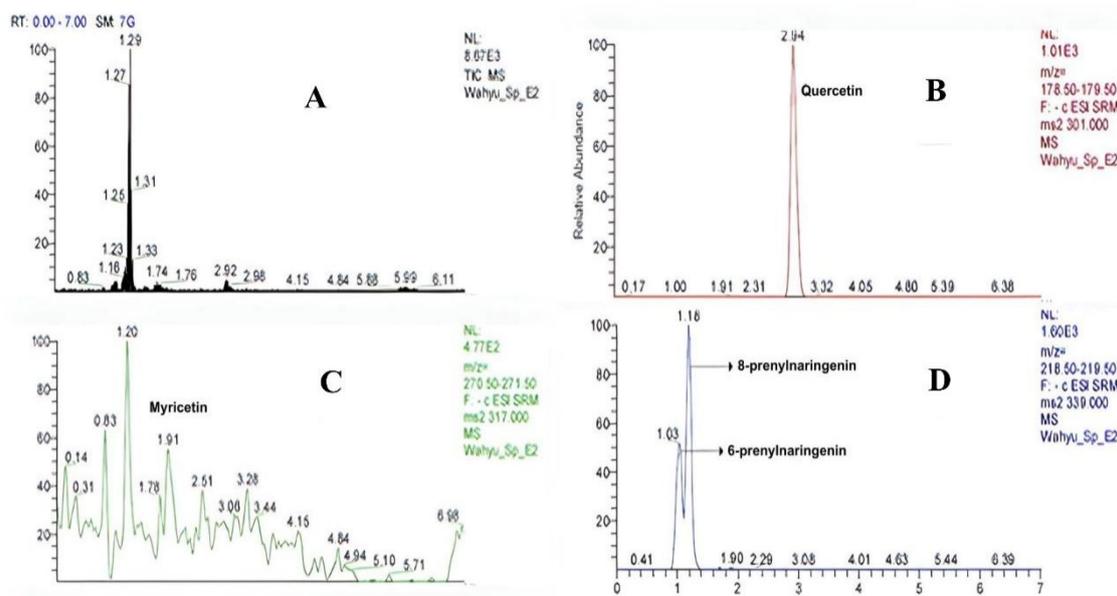


Figure 1 Chromatogram showed the relative abundance over time in minutes in each m/z, total ionic chromatography (TIC) of A) GTE, B) Quercetin, C) Myricetin, D) 6-prenylnaringenin and 8-prenylnaringenin.

Figure 1 showed that all 4 compounds were found in the extract according to the detection of the parent and daughter ion of each compound. It could be seen when scanning for the parent and daughter ion of quercetin (179-301) [26], myricetin (271-317) [26], 6-prenylnaringenin (219-399) [27], and 8-prenylnaringenin (219-399) [28].

Cytotoxic assay

Applying extract towards cells possibly has 2 effects, therapeutic or cytotoxic. Each effect might appear as certain concentration treatment. Therefore, it is necessary to evaluate the safe and efficient concentration through cytotoxic assay before it is used for further analysis. In this study, cytotoxic assay was conducted using MTS assay kit. The result of the cytotoxic assay is presented on **Figure 2**.

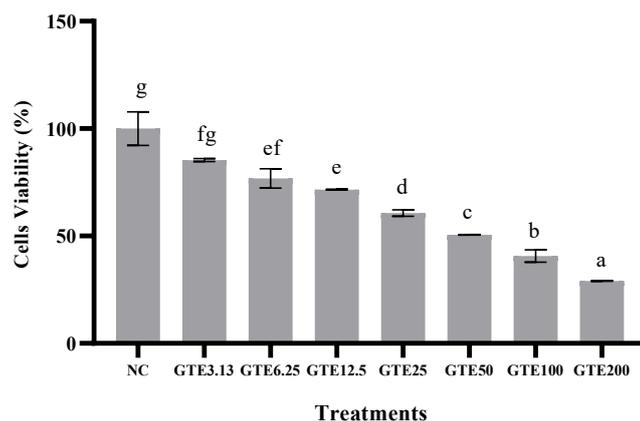


Figure 2 Effect of various GTE concentration toward cell viability in LPS-induced rat lung cells as an ARDS cells model. The data is presented as mean value \pm standard deviation. NC: Negative Control (untreated cell), PC: Positive Control (LPS-induced rat lung cells), GTE 3.13: PC + GTE 3.13 μ g/mL, GTE 6.25: PC + GTE 6.25 μ g/mL, GTE 12.5: PC + GTE 12.5 μ g/mL, GTE 25: PC + GTE 25 μ g/mL, GTE 50: PC + GTE 50 μ g/mL, GTE 100: PC + GTE 100 μ g/mL, GTE 200: PC + GTE 200 μ g/mL. A different mark (a, b, c, d, e, ef, fg, g) indicates significant difference among treatments based on Tukey HSD post hoc test $p < 0.05$.

Figure 2 shows the percentage of cell viability corresponding to the treatment of various GTE concentrations, and seems dose dependent manner. GTE 3.13 $\mu\text{g/mL}$ generates highest cell viability and statistically is not significantly different from NC. An IC_{50} test was also run to further examined the cytotoxic analysis. The result showed that the concentration in which GTE will kill 50 % of the cells population is at 95.5 $\mu\text{g/mL}$. Therefore, it is safe to assume that the best concentration of GTE that would cause the least toxicity is 3.125 $\mu\text{g/mL}$ and further than that concentration would be too toxic on the cells.

IL-6, IL-1 β , and COX-2 Levels

Three concentrations with highest cell viabilities were used for further analysis, including ELISA assay. This assay was employed to measure protein levels of IL-6, IL-1 β , and COX-2. The measurement results represent inflammation occurrence among treatments groups as shown on **Figure 3**.

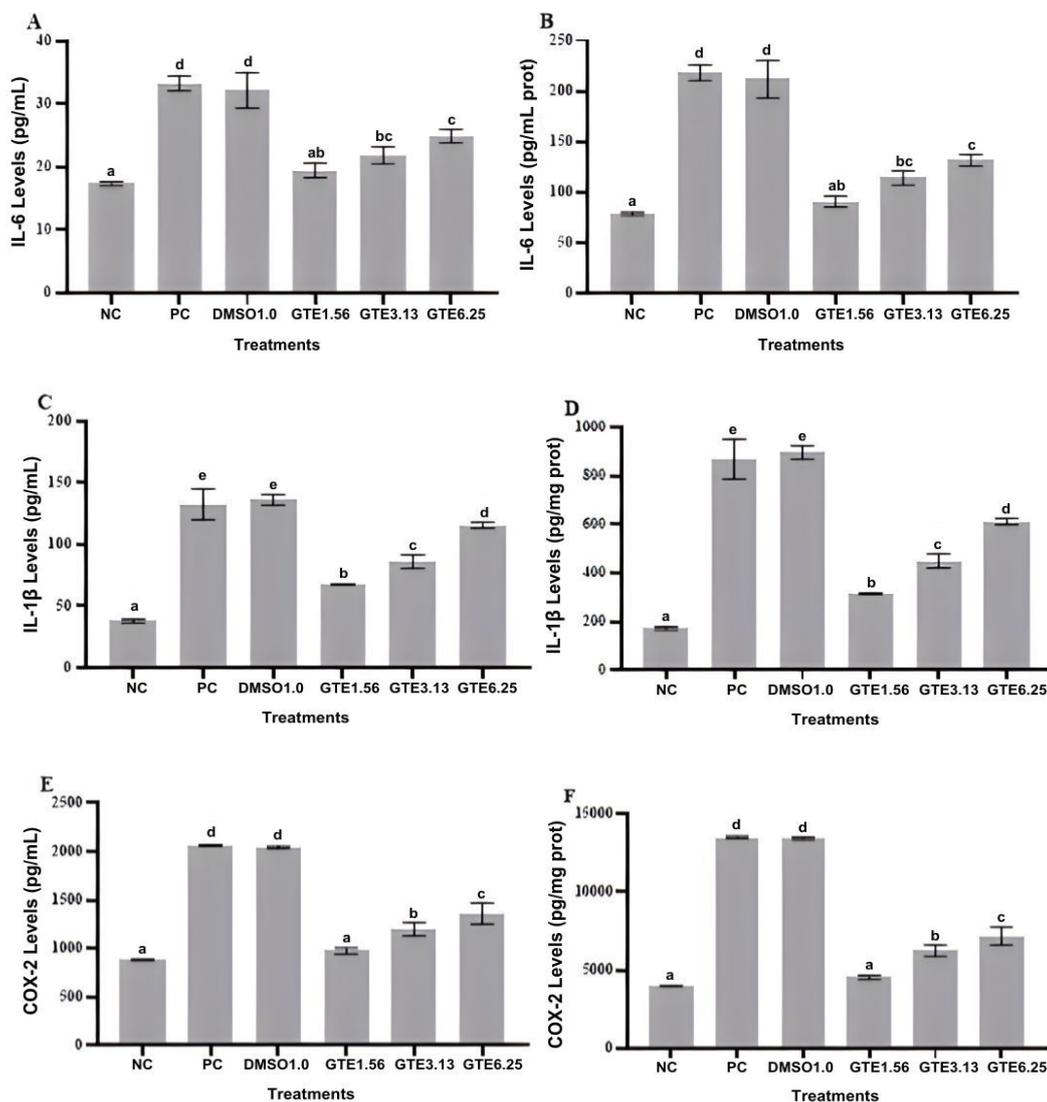


Figure 3 Effect of various GTE concentration toward level of IL-6, IL-1 β , COX-2 in LPS-induced rat lung cells as ARDS cells model. The data is presented as mean value \pm standard deviation. NC: Negative Control (untreated cell), PC: Positive Control (LPS-induced rat lung cells), DMSO1.0: PC + DMSO 1 %, GTE 3.13: PC + GTE 3.13 $\mu\text{g/mL}$, GTE 6.25: PC + GTE 6.25 $\mu\text{g/mL}$, GTE 12.5: PC + GTE 12.5 $\mu\text{g/mL}$, GTE 25: PC + GTE 25 $\mu\text{g/mL}$, GTE 50: PC + GTE 50 $\mu\text{g/mL}$, GTE 100: PC + GTE 100 $\mu\text{g/mL}$, GTE 200: PC + GTE 200 $\mu\text{g/mL}$. A different mark (a, ab, bc, d) significant different among treatment toward IL-6 levels (**Figures 3A** and **3B**), different mark (a, b, c, d, e) significant different among treatment toward IL-1 β levels (**Figures 3C** and **3D**), different mark (a, b, c, d) significant different among treatment toward COX-2 levels (**Figures 3E** and **3F**) based on Tukey HSD post hoc test $p < 0.05$.

Based on the **Figure 3** showed that three concentration treatments of GTE significantly lowered levels of IL-6, IL-1 β , and COX-2 compared to the PC. The GTE 1.56 $\mu\text{g/mL}$ was the most active to lower IL-6, IL-1 β , and COX-2 levels, and treatment of GTE 1.56 $\mu\text{g/mL}$ was comparable with NC.

ACE-2 and TMPRSS-2 gene expressions

Considering ACE-2 and TMPRSS-2 roles in various diseases such as kidney disease, inflammation in the lung, and ARDS, thus the levels of these genes represent ARDS occurrence in LPS-induced cells. The different of genes expression were observed among the groups and it conveyed GTE effect toward ARDS cells model, as shown on **Figure 4**.

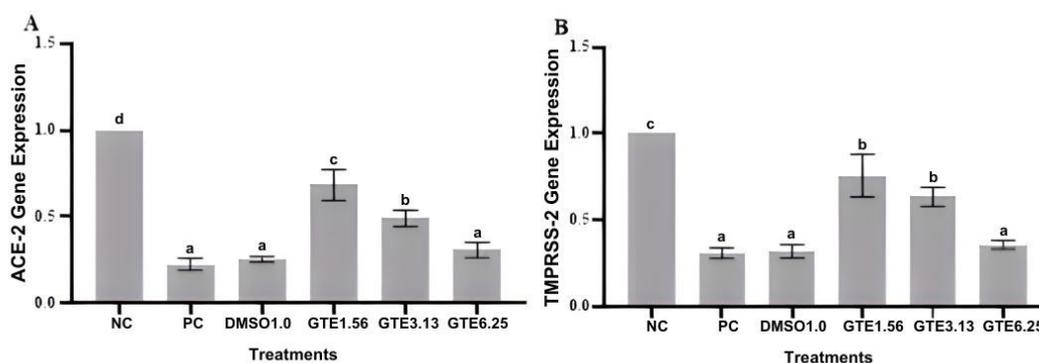


Figure 4 Effect of various concentration GTE toward gene expressions of ACE-2, TMPRSS-2.

The data is presented as means value \pm standard deviation. NC: Negative Control (untreated cell), PC: Positive Control (LPS-induced rat lung cells), DMSO1.0: PC + DMSO 1 %, GTE 3.13: PC + GTE 3.13 $\mu\text{g/mL}$, GTE 6.25: PC + GTE 6.25 $\mu\text{g/mL}$, GTE 12.5: PC + GTE 12.5 $\mu\text{g/mL}$, GTE 25: PC + GTE 25 $\mu\text{g/mL}$, GTE 50: PC + GTE 50 $\mu\text{g/mL}$, GTE 100: PC + GTE 100 $\mu\text{g/mL}$, GTE 200: PC + GTE 200 $\mu\text{g/mL}$. A different mark (a, b, c, d) significant different among treatment toward ACE-2 gene expression (**Figure 4A**), different mark (a, b, c) significant different among treatment toward TMPRSS-2 gene expression (**Figure 4B**), different based on Tukey HSD post hoc test $p < 0.05$.

According to **Figure 4**, ACE-2 and TMPRSS-2 genes expressions decreased by LPS induction and treatment of GTE 1.56 $\mu\text{g/mL}$ and GTE 3.13 $\mu\text{g/mL}$ significantly increased ACE-2 and TMPRSS-2 gene expression.

Discussion

ARDS is an acute inflammation which happens on the lungs and is associated with COVID-19 [29,30]. Considering its severity leads to death, there is an urgent need to find out the alternative drugs or supplements to help the ARDS condition. One of the more familiar anti-inflammatory agents is definitely green tea. Green tea has been found containing anti-inflammatory flavonoids such as quercetin and myricetin [8,31,32]. Additionally, recent in silico studies have proven that these flavonoids were able to help in suppressing the COVID-19 virus [34]. Thus, one of the first steps in this study is to find out the component of the GTE itself. Liquid chromatography with tandem mass spectrometry (LC-MS/MS) is an advanced separation and analysis technique developed in the past decades. This technique is known to yield better, more sensitive results than any other technique and so was used in this study [35,36].

Based on the chromatogram of **Figure 1**, all the target compounds of this study, quercetin, myricetin, 6-prenylnaringenin, and 8-prenylnaringenin are found in GTE. The target compounds are ones that have the highest peak as it is considered the most abundant in that specific mass over charge ratio since each daughter ion of any compound is supposed to be different. In this case quercetin's peak is at the retention time of 2.94 min, myricetin at 1.20 min, 6-prenylnaringenin at 1.03 min, and 8-prenylnaringenin at 1.18 min. The 6- and 8-prenylnaringenin are an isomer of each other, there is little difference in their mass to charge ratio. Therefore, LC is important in this case because we can also differentiate them by their retention time. *C. sinensis* leaves have an abundant of flavonols content including myricetin, quercetin, and kaempferol based on High Performance Liquid Chromatography (HPLC) analysis [37]. Based on Zhou *et al.* [38] study, some tea varieties have flavonoids like quercetin, myricetin and also differences of catechins. However, according to Fang *et al.* [36] the prenylated flavonoids have better activities because of their

ability to increase permeability of the cells. Thus, in this study 6- and 8-prenylnaringenin, which was usually attributed to the anti-inflammatory activities of hops tea were screened in GTE extract as well.

Determining cell viability is one of the most important first steps to screen potential compounds to ensure the compound used is not toxic to cells [40,41]. Based on the MTS assay results, 3.125 µg/mL of GTE yields the best cell viability. The result presented on **Figure 2** also shows how GTE concentration is inversely proportional to the cell's viability. This is due to the fact that GTE with its flavonoid contents has plenty health benefits, and also may be toxic when given in large concentrations [6]. The highest viability of all the treatments is 85.33 % which is not significantly different from cells without any treatments. On top of that, the IC₅₀ value from the cytotoxic assay is at 95.5 µg/mL. IC₅₀ value refers to a concentration of a compound that would be able to inhibit 50 % of cell growth in a population [42]. Determining the IC₅₀ value of GTE was done to reassure that the concentration used for the LPS-induced cells would not cause cytotoxicity that would interfere with the final results of the following assays. Based on the MTS assay result, it was decided that the concentration that would be used in the following assays were 6.25, 3.125, and 1.25 µg/mL and GTE with concentration of 3.125 µg/mL showed the highest cell viability compared to other concentration.

All assays conducted in this study featured positive and negative controls, as well as DMSO controls so that there could be real comparisons made between healthy cells, inflamed cells, and inflamed cells that were treated. DMSO treatment were established to ensure the effect of the treatment done in this study were truly based on the GTE content and did not have any significant interferences from the solvent of the treatment, which DMSO was the GTE solvent. As such, the best outcome for this control would have to be not significantly different from the positive controls. This outcome was shown in all our results, proving that the effect of the treatment came from the content of the GTE itself. Healthy cells would have less pro-inflammatory cytokines and less expression of pro-inflammatory genes [43,44]. From all the assays ran, it could be seen that this is true as well, rendering all the assays valid based on the control results.

The GTE treatments significantly reduced the level of IL-1β in LPS-induced cells. GTE 1.56 µg/mL was the best result due to the IL-1β almost similar with normal cells. This finding is in line with previous studies on GTE being able to help with inflammation by reducing pro-inflammatory cytokines like IL-1β [34,45,46]. It was tested that GTE contained flavonoids like quercetin and myricetin are able to inhibit the NF-κB pathway [47]. The presence of 6-PN and 8-PN were also studied to be able to help alleviate inflammation [48]. The increasing of IL-1β level as the improvement of GTE concentration is observed and possibly due to the toxic effect resulted by GTE high concentration [41]. This toxicity could cause oxidative stress in which cells would then overproduce on ROS. ROS has been known to be able to activate inflammatory pathways like NF-κB which is responsible for the production of several inflammatory cytokines including NF-κB [50]. Taking this into account, GTE could be a promising anti-inflammatory agent given that they are administered in the correct dose.

Besides containing myricetin and quercetin, GTE also contains epigallocatechin-3-gallate (EGCG) that can act as anti-inflammatory, the anti-inflammatory effects showed EGCG was broad-spectrum therapeutic in COVID-19. EGCG will decrease the production of pro-inflammatory cytokine such as IL-6 from immune cells (macrophages, neutrophils, and monocytes) [51] and inflammation-mediated enzyme such as COX-2 through the suppression of NF-κB activation [52]. The treatment of GTE for IL-6 and COX-2 showed that GTE with concentration of 1.56 µg/mL gave the best result due to being significantly lower than the positive control (**Figure 3**).

ACE-2 is a crucial component of the renin-angiotensin-aldosterone system (RAAS) that play role in respiratory homeostasis and cardiovascular systems [53]. The ectodomain S1 binds to the ACE-2 enzyme's peptidase domain, whereas the S2 is cleavage further by the host cell serine protease TMPRSS [53]. ACE-2-Ang-(1-7)-Mas axis has anti-inflammatory and antifibrotic effects on the respiratory system, as an anti-inflammatory and antioxidative stress [54]. Furthermore, GTE treatment needed to blocked of the renin-angiotensin system reduced the pulmonary injury by activating the ACE-2-Ang-(1-7)-Mas axis [54]. In Li *et al.* [7] study, ACE-2 overexpression significantly prevented LPS-induced lung damage and inflammation in rat lungs. ACE-2 prevents LPS-induced ARDS by blocking MAPKs and NF-κB signaling pathway and also protect against lung inflammation [7]. Epigallocatechin-3-gallate in *C. sinensis* increased sirtuins proteins which leads to decrease in pro-inflammatory gene transcription and NF-κB, protecting organisms from oxidative stress in autoimmune, respiratory, and cardiovascular diseases [55].

GTE and its compounds also can modulate the miRNAs expression in the control processing of inflammation [56]. The polyphenol content of GTE can reduce the expression of miRNAs host cells, this is important because specific miRNAs can actually increase the potential for exposure to the COVID-19 virus and can interact with it. There are some miRNAs whose regulation is modulated by polyphenols. Hsa-miR-1246 is homologous in sequence with ACE-2, thereby increase the potential for COVID-19 exposure

[57,58]. The green tea polyphenols may involve in decreasing pro-inflammatory gene expression by suppressing epigenetic factors such as miRNAs in response to inflammatory illnesses, primarily affecting immune cell function [59]. Quercetin, myricetin, and catechin also can induce Nuclear Factor-erythroid Related Factor-2 (NRF-2) which provides anti-inflammatory and anti-oxidant protection to tissues [60]. All of this supported the result of the study in which was shown that GTE were able to increase ACE-2 and TMPRSS-2 expressions. The concentration of 1.56 µg/mL GTE was the best concentration to increase ACE-2 and TMPRSS-2 expression compared to the other concentrations, 3.13 and 6.25 µg/mL (**Figure 4**). Furthermore, GTE and another natural agent may constitute a supportive treatment approach to modulating dysregulated miRs that involved in regulation of cytokines in various inflammation, and delivering advantages in severe respiratory illnesses [57].

Conclusions

GTE contains important secondary metabolites such as quercetin, myricetin, 6-prenylnaringenin, and 8-prenylnaringenin, which may help with COVID-19 inflammatory symptoms. The green tea extract treatment can decrease the pro-inflammatory cytokines IL-1 β , IL-6, inflammation-mediated enzyme COX-2, ACE-2 and TMPRSS-2 mRNA expressions.

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