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# Effects of Haskap (*Lonicera caerulea* L.) Extracts against Oxidative Stress and Inflammation in RAW 264.7 Cells

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**ABSTRACT:** This study aimed to evaluate the antioxidant and anti-inflammatory activities of *Lonicera caerulea* L. ethanol extract (LCEE) and water extract (LCWE) *in vitro*. We primarily evaluated the improvement effect of LCWE and LCEE on hydrogen peroxide ($H_2O_2$)-induced oxidative damage and lipopolysaccharide (LPS)-induced inflammatory damage in RAW 264.7 cells by detecting oxidation-related indicators and inflammatory factors, respectively. Cellular studies showed that LCWE and LCEE increased superoxide dismutase and catalase antioxidant enzyme levels and decreased malondialdehyde and nitric oxide peroxide levels in $H_2O_2$-induced RAW 264.7 cells. Moreover, LCWE and LCEE decreased the secretion of inflammatory factors [e.g., interleukin (IL)-6, IL-1β, and tumor necrosis factor-α] in LPS-induced RAW 264.7 cells. In conclusion, LCWE and LCEE demonstrated excellent antioxidant and anti-inflammatory effects *in vitro*. However, LCWE was superior to LCEE, which may be related to its chemical composition and requires further research.

**Keywords:** anti-inflammatory, antioxidants, *Lonicera*, RAW 264.7 cells

## INTRODUCTION

Haskap (*Lonicera caerulea* L.), which belongs to the honeysuckle family, is an emerging small berry that is currently mainly grown in northeastern Asia and parts of North America (Rupasinghe et al., 2012). Since ancient times, the fruit of *L. caerulea* L. has been widely used in Russian, Chinese, and Japanese folk medicine (Vasantha Rupasinghe et al., 2018). Moreover, it is rich in anthocyanins, flavonoids, polysaccharides, vitamins, minerals, trace elements, and other active substances that can promote human health; thus, it has high medicinal value and health care functions (Celli et al., 2014; Khattab et al., 2015).

According to modern scientific research, natural products exert many physiological activities, including antioxidation and anti-inflammation. Natural non-plant materials such as mushrooms are widely used as traditional medicines in East Asian cultures. Because they are rich in terpenoids, mushrooms have been shown to exert anti-inflammatory effects, including decreasing interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, and nuclear factor kappa B (NF-κB) levels (Elsayed et al., 2014). As the oldest nutraceutical, honey has been shown to exhibit anti-inflammatory properties, including reducing TNF-α, cyclooxygenase-2, and NF-κB levels; however, the exact mechanism of honey's anti-inflammatory activity remains unknown (Vallianou et al., 2014). The typical examples of natural plant materials include various teas that originated in East Asia with a long history of medicinal use. For example, green tea contains terpenes such as resveratrol, anthocyanins, catechins, and paclitaxel, which exert beneficial effects against tumors, Alzheimer's disease, neurodegenerative diseases, and other diseases by mediating oxidation and inflammation (Tang et al., 2019). Recently, berries have shown a strong competitive advantage. According to previous studies, *L. caerulea* L. has antibacterial, anti-inflammatory, antioxidant, hypoglycemic, hepatoprotective, intestinal flora regulation, neurocognitive improvement, and other health care functions, and its potential health benefits are increasingly being emphasized (Wu et al., 2018; De Silva and Vasantha Rupasinghe, 2020; Gołba et al., 2020; Dayar et al., 2021). Therefore, in the present study, we established a hydrogen peroxide ($H_2O_2$)-induced RAW 264.7 cell oxidative stress model and lipopolysaccharide (LPS)-induced RAW 264.7 cell in-

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flammation model, and used cell viability, oxidation, and inflammation-related indicators to investigate the antioxidant and anti-inflammatory abilities of *L. caerulea* L. ethanol extract (LCEE) and water extract (LCWE).

## MATERIALS AND METHODS

### Sample preparation

To prepare LCEE, haskap berry powder (capacity: 100 g; barcode number: 0634158981777, HASKAPA Co., Ltd.) was added to absolute ethanol in accordance with a liquid-to-solid ratio of 20:1 (V:M). Thereafter, it was subjected to three ultrasonic extractions (40°C, 20 min), stored at 4°C for 12 h, and centrifuged (1,008 *g*, 10 min) to obtain the supernatant. To remove ethanol and water, the supernatant was rot-evaporated. Subsequently, it was freeze-dried for 48 h, ground into a powder, and sealed for use.

To prepare LCWE, double-distilled water was added to haskap berry powder in accordance with a liquid-to-solid ratio of 20:1 (V:M). Thereafter, it was ultrasonically treated (40°C, 20 min) and extracted three times in a water bath (95°C, 40 min). After vacuum filtration, the supernatant was collected by incubating at 4°C for 12 h. Subsequently, it was rot-evaporated to about 100 mL. Next, anhydrous ethanol was added to reach 80%, and the sample was stored at 4°C for 12 h and centrifuged (1,008 *g*, 10 min) to obtain a precipitate. This procedure was repeated twice, and the precipitate was dried (55°C, 48 h) and sealed for future use.

### Toxicity assessment of LCEE and LCWE on RAW 264.7 cells

RAW 264.7 cells were cultured in Dulbecco's modified Eagle' medium (DMEM, high glucose, containing 10% fetal bovine serum and 1% penicillin-streptomycin double antibody solution) in a saturated humid environment at 37°C and 5% carbon dioxide. The medium was changed every other day. Log phase cells were used in all experiments.

The RAW 264.7 cell suspension ($1\times10^4$ cells/mL) was seeded in a 96-well cell culture plate (60 μL cells+100 μL medium) and incubated at 37°C for 24 h until adherent. Next, 20 μL of LCEE or LCWE (200 μg/mL, normal group as control) was added, and the cultures were incubated for another 24 h period. Cell viability was detected using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) method.

MTT (5 mg/mL) was added to the tested cell sample, homogenized, and left to culture for 4 h. The upper supernatant was removed, 150 μL of DMSO was added, and the cells were shaken for 30 min at 37°C in the dark. Blue formazan crystals that formed were dissolved, and the optical density (OD) value was measured at 490 nm using an automatic microplate reader (Multiskan GO, Thermo Scientific) (Li et al., 2021). Each group had three replicates. Cell viability was calculated as follows:

$$\text{Cell viability}/\% = (A_s/A_c)\times100\%$$

where $A_s$ corresponds to the OD value of the LCEE (or LCWE) treatment group and $A_c$ corresponds to the OD value of the normal group without any treatment. The same MTT assay was used for the evaluation of cell viability.

### Improvement of $H_2O_2$-induced oxidative damage in RAW 264.7 cells by LCEE and LCWE

***Screening of $H_2O_2$ inducer concentration*:** The RAW 264.7 cell suspension ($1\times10^4$ cells/mL) was inoculated into a 96-well cell culture plate (60 μL cells+100 μL medium) and incubated at 37°C for 24 h. When cells were adherent, the cell cultures were exposed to 20 μL of $H_2O_2$ at different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 mmol/L) for 4 h to prepare different oxidative damage models. Cell viability was determined using the MTT method (Safaeian et al., 2015).

***Effects of LCEE and LCWE on the viability of RAW 264.7 cells induced by $H_2O_2$*:** Briefly, 20 μL of optimal $H_2O_2$ concentration was added to RAW 264.7 cells after the above adhesion step. Then, the cultures were incubated for 4 h to prepare the oxidative damage model. Finally, 20 μL of 200 μg/mL LCEE (or LCWE) was added to the cultures and incubated for 24 h. Cell viability was measured using the MTT method.

***Determination of malondialdehyde (MDA), nitric oxide (NO), superoxide dismutase (SOD), and catalase (CAT) levels in $H_2O_2$-induced RAW 264.7 cells treated with LCEE and LCWE*:** Briefly, the RAW 264.7 cell oxidative damage model was prepared using an optimal $H_2O_2$ concentration (ibid.). After the medium was removed from the RAW 264.7 oxidative damage cell model, 200 μL of LCEE and LCWE (200 μg/mL) was added to each well and mixed with 2 mL of fresh DMEM for 24-h culture. After sample treatment, the RAW 264.7 cells were removed from the supernatant medium and washed twice with precooled phosphate-buffered saline (0.1 mol/L). Then, the cells were scraped off using a cell scraper, mixed using a pipette, transferred to a 1.5-mL centrifuge tube, and centrifuged to remove the supernatant. The washing and centrifugation procedures were repeated twice. Next, 800 μL of saline solution was added, and the mixture was homogenized and used for the following experiments.

***Detection of MDA content by 2-thiobarbituric acid (TBA) method*:** The RAW 264.7 cell homogenate was added with 10% trichloroacetic acid (TCA) and centrifuged (4°C, 16,128 *g*, 15 min) to prepare the supernatant. Before being rap-

idly cooled and centrifuged, a mixture comprising 1.5 mL of the supernatant (the control group was mixed with 1.5 mL of 10% TCA) and an equal volume of 0.5% TBA (dissolved in 10% TCA) solution was prepared and placed in a boiling water bath for 30 min. The OD values of the supernatant at 532 and 600 nm were measured (Janero, 1990). The MDA concentration was calculated as follows:

$$\text{MDA concentration } (\mu\text{mol/L}) = [(A_{532} - A_{600}) \times V_1] / [(1.55 \times 0.1) \times V_2]$$

where $A_{532}$ and $A_{600}$ are the absorbance values at 532 and 600 nm wavelengths, respectively; $V_1$ is the volume of reaction solution (mL); $V_2$ is the volume of the extraction solution in the reaction solution (mL), and $1.55 \times 0.1$ is the micromolar absorption coefficient of MDA.

***Detection of NO content by Griess method*:** Fifty microliters of each standard application solution of $NaNO_2$ (0.195, 0.39, 0.78, 1.56, 3.125, and 6.25 mM) or culture supernatant with an equal volume of Griess reagent [0.1% *N*-(1-naphthyl) ethylenediamine, 1% sulfanilamide in 5% phosphoric acid] was combined and incubated at 37°C for 10 min. After mixing, the absorbance was measured at 540 nm. The NO content of the test sample was determined in accordance with the NO reaction standard curve obtained using the $NaNO_2$ standard solution (Sun et al., 2003).

***Detection of CAT enzyme activity using the ultraviolet absorption method*:** The RAW 264.7 cell homogenate was centrifuged (4°C, 1,792 *g*, 15 min) to obtain the supernatant (i.e., crude enzyme extract). Then, 0.2 mL of crude enzyme extract ($S_1$), inactivated crude enzyme extract ($S_0$), and phosphate buffer (pH 7.8) were added to 1.5 mL of phosphate buffer (pH 7.8) and 1 mL of distilled water. After preheating at 25°C, 0.6 mL of 0.1 mol/L $H_2O_2$ was added, and the time was recorded immediately. The absorbances were measured at 240 nm once per minute for 4 min (Zhang et al., 2009). The CAT enzyme activity was calculated as follows:

$$\text{CAT enzyme activity } (\mu \cdot \text{mL}^{-1}\ \text{min}^{-1}) = \Delta A_{240} / (0.1 \times V_1 \times t)$$

where $A_{240}$ is the difference of $As_0 - As_1$, $As_0$ is the absorbance value of the control tube added with the inactivated enzyme solution, $As_1$ is the absorbance value of the sample tube, 0.1 indicates that every 0.1 drop of $A_{240}$ is 1 unit of enzyme activity ($\mu$), $V_1$ is the crude enzyme extract solution for determining volume (mL), and t is the $H_2O_2$ concentration at the last reading time (min).

The SOD activity was measured using the EZ-SOD assay kit (Cat. No. DG-SOD400, DoGenBio Co., Ltd.).

### Improvement effect of LCEE and LCWE on the inflammatory injury of RAW 264.7 cells induced by LPS

***Establishment of LPS-injured RAW 264.7 cell inflammation model*:** Studies have shown that 1 μg/mL of LPS can induce RAW 264.7 cell inflammation (Xu et al., 2017). RAW 264.7 cells were cultured in a 6-well cell culture plate until adhesion. Afterward, 200 μL of 1 μg/mL LPS was added and incubated for 4 h to prepare the inflammatory injury model.

***Determination of IL-6, IL-1β, and TNF-α levels in LPS-induced RAW 264.7 cells treated with LCEE and LCWE*:** After the medium was removed from the RAW 264.7 cell inflammatory injury model, 200 μL of LCEE and LCWE (200 μg/mL) was added to each well, along with 2 mL of fresh DMEM to continue incubation for 24 h. Enzyme-linked immunosorbent assay was used to determine IL-6 (catalog number: M6000B), TNF-α (catalog number: MTA 00B), and IL-1β levels (catalog number: MLB00C) in accordance with the manufacturer's recommendations (Bio-Techne Corp.).

### Software and data analysis method

Data are presented as the mean±standard deviation. Data were plotted using Microsoft® Excel® 2016 MSO Edition software. Statistical analyses were conducted using SPSS version 20.0 (IBM Corp.), and statistical significance was considered at $P<0.05$. All experiments were repeated three times.

## RESULTS

### Effects of LCEE and LCWE on RAW 264.7 cytotoxicity

We evaluated the cytotoxicity of 200 μg/mL of LCEE and LCWE against RAW 264.7 cells (Fig. 1). RAW 264.7 cells were treated with either 200 μg/mL of LCEE or LCWE. Then, MTT assay was performed. The cell viability of RAW 264.7 cells treated with LCEE and LCWE

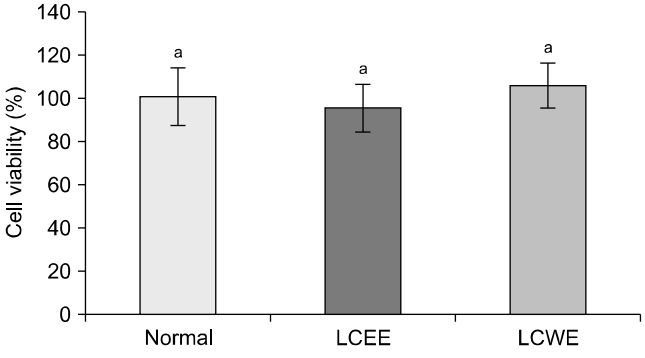



**Fig. 1.** Effects of 200 μg/mL of *Lonicera caerulea* L. ethanol extract (LCEE) (or *L. caerulea* L. water extract, LCWE) on the viability of RAW 264.7 cells. Mean values in the same bar graph (a) are not significantly different ($P>0.05$) according to Tukey's test.

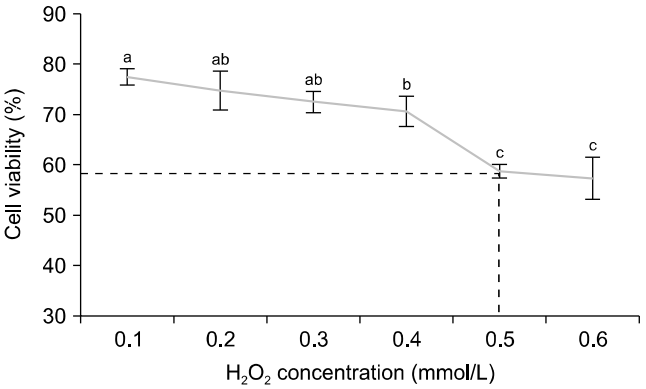



**Fig. 2.** Effects of different hydrogen peroxide ($H_2O_2$) concentrations on the cell viability of RAW264.7 cells. Mean values with different letters in the same line graph (a-c) are significantly different ($P$<0.05) according to Tukey's test.

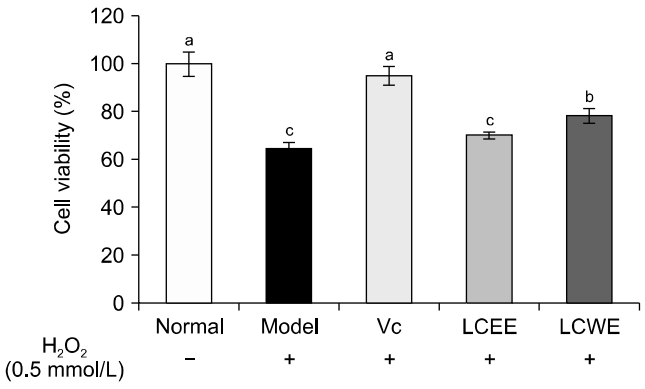



**Fig. 3.** Effects of *Lonicera caerulea* L. ethanol extract (LCEE) and *L. caerulea* L. water extract (LCWE) on the viability of hydrogen peroxide ($H_2O_2$)-induced RAW 264.7 cells. Mean values with different letters in the same bar graph (a-c) are significantly different ($P$<0.05) according to Tukey's test. Vc, vitamin C.

was 95.47% and 105.81%, respectively. Both treatments showed no significant difference compared with the control group ($P$>0.05), indicating that 200 μg/mL of LCEE and LCWE had no apparent lethal effect on RAW 264.7 cells. Therefore, 200 μg/mL of LCEE or LCWE was used in subsequent experiments.

### Screening of $H_2O_2$ concentration

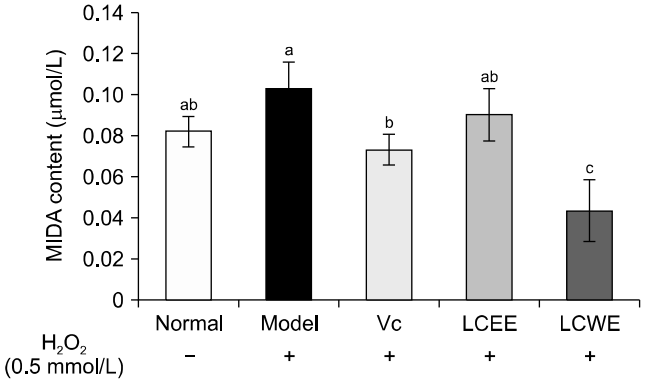
MTT assay showed that the viability of RAW264.7 cells exposed to $H_2O_2$ decreased in a time- and concentration-dependent manner (Fig. 2). The viability of RAW264.7 cells decreased after $H_2O_2$ treatment in a dose-dependent manner: 0.1 mmol/L 77.47%±1.69%, 0.2 mmol/L 74.73% ±3.87%, 0.3 mmol/L 72.46%±2.05%, 0.4 mmol/L 70.60% ±3.08%, 0.5 mmol/L 58.70%±1.32%, and 0.6 mmol/L 57.29%±4.19%. Notably, the viability of RAW264.7 cells significantly decreased after treatment with 0.5 mmol/L $H_2O_2$ ($P$<0.05). After careful consideration, 0.5 mmol/L was selected as the optimal induction dosage of $H_2O_2$ in this study.

### Effects of LCEE and LCWE on the viability of RAW 264.7 cells induced by $H_2O_2$

As shown in Fig. 3, the viability of RAW 264.7 cells after $H_2O_2$-induced injury significantly decreased (64.45%, $P$< 0.05) compared with control group (i.e., normal group) cells, indicating that the oxidative damage model had been successfully established. By contrast, compared with the group treated with $H_2O_2$ alone, the viability of RAW264.7 cells induced by $H_2O_2$ significantly improved after vitamin C (Vc) and LCWE treatment ($P$<0.05), whereas the viability of cells after LCEE treatment was not significant. In addition, the viability of RAW264.7 cells treated with the same concentration of LCWE (78.29%) was significantly higher than that treated with the same concentration of LCEE (70.07%), but lower than that treated with Vc (95.03%).

### Effects of LCEE and LCWE on MDA and NO content in RAW 264.7 cells induced by $H_2O_2$

As shown in Fig. 4, RAW 264.7 cells treated with $H_2O_2$ (0.5 mmol/L) for 4 h showed an approximately 1.25-fold



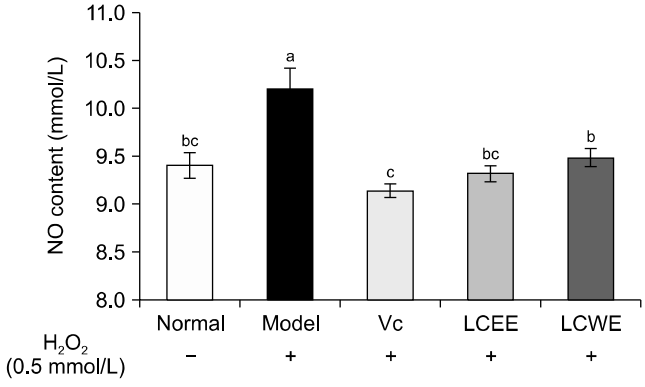




**Fig. 4.** Effects of *Lonicera caerulea* L. ethanol extract (LCEE) and *L. caerulea* L. water extract (LCWE) on the malondialdehyde (MDA) and nitric oxide (NO) content of hydrogen peroxide ($H_2O_2$)-induced RAW 264.7 cells. Mean values with different letters in the same bar graph (a-c) are significantly different ($P$<0.05) according to Tukey's test. Vc, vitamin C.

increase in MDA content in the model group (0.10±0.01 μmol/L) compared with the control group (0.08±0.01 μmol/L), but the difference was not significant ($P$>0.05). However, after being treated with 200 μg/mL of Vc and LCWE, the intracellular MDA content was significantly ($P$<0.05) attenuated by 0.07±0.01 and 0.04±0.01 μmol/L, respectively. Moreover, the MDA content after LCEE treatment was 0.09±0.01 μmol/L. The inhibitory effect was not significant and similar to that of the control group. As shown in Fig. 4, the content of NO secreted by RAW 264.7 cells treated with $H_2O_2$ (0.5 mmol/L) for 4 h increased significantly (10.20±0.22 mmol/L, $P$<0.05) compared with the control group. Moreover, 200 μg/mL of Vc, LCEE, and LCWE significantly decreased NO secretion ($P$<0.05) compared with the model group. Of note, no significant difference in NO secretion was observed after Vc (9.13±0.07 mmol/L), LCEE (9.32±0.08 mmol/L), and LCWE (9.48±0.10 mmol/L) treatment. These values were similar to that observed in the control group (9.40±0.13 mmol/L).

### Effects of LCEE and LCWE on SOD and CAT enzyme activities in RAW 264.7 cells induced by $H_2O_2$

As shown in Fig. 5, the relative enzyme activity of SOD in RAW 264.7 cells treated with $H_2O_2$ (0.5 mmol/L) for 4 h significantly decreased ($P$<0.05) compared with that in the control group. However, the relative enzyme activity of SOD in $H_2O_2$-induced cells was increased after treatment with 200 μg/mL of LCWE and LCEE. In particular, the relative enzyme activity of SOD in the LCWE treatment group (16.24%±0.43%) was higher than that of the LCEE group (12.87%±1.38%) ($P$<0.05), which was close to that of the Vc group (15.21%±1.12%).

Compared with the control group (0.04±0.01 $\mu \cdot mL^{-1}$ $min^{-1}$), the CAT enzyme activity of RAW 264.7 cells treated with $H_2O_2$ (0.5 mmol/L) for 4 h was significantly decreased (0.02±0.01 $\mu \cdot mL^{-1}$ $min^{-1}$, $P$<0.05; Fig. 5). Compared with the model group, the CAT enzyme activity of RAW 264.7 cells treated with Vc, LCEE, and LCWE significantly increased ($P$<0.05; 0.10±0.01, 0.04±0.01, and 0.07±0.01 $\mu \cdot mL^{-1}$ $min^{-1}$, respectively). Moreover, the CAT enzyme activity of the LCEE treatment group was similar to that of the control group, and no significant difference was observed ($P$>0.05).

### Effects of LCEE and LCWE on LPS-induced IL-1β, IL-6, and TNF-α levels in RAW 264.7 cells

We evaluated the ameliorative effect of 200 μg/mL of LCEE (or LCWE) on LPS-induced inflammation in RAW264.7 cells (Fig. 6). Compared with the control group, 1 μg/mL of LPS increased the secretion of IL-1β, IL-6, and TNF-α in RAW 264.7 cells (15.53±0.31, 150.61 ±21.54, and 83.72±8.23 pg/mL, respectively). Compared with the model group, IL-1β, IL-6, and TNF-α levels significantly decreased after treatment with Vc, LCEE, and LCWE ($P$<0.05). In particular, IL-6 and TNF-α were remarkably downregulated compared with IL-1β. No significant differences in IL-6 downregulation were observed in the Vc, LCEE, and LCWE groups ($P$>0.05), and the levels were between that of the control group and model groups. No significant differences in TNF-α downregulation were observed in the Vc, LCEE, and LCWE groups. However, LCWE exhibited the strongest effect. In particular, TNF-α levels were lower in the Vc, LCEE, and LCWE compared with those in the control group. In the downregulation of IL-1β, the effect of Vc was close to that of the control group ($P$>0.05), and the effect of LCEE and LCWE was between that of Vc and the model group. However, LCWE exhibited a stronger downregulation effect than LCEE ($P$>0.05).

## DISCUSSION

Several studies have evaluated the antioxidant capacity of *L. caerulea* L., especially its total phenolic content and free radical scavenging activity (Li et al., 2019; Zhao et al., 2012). These studies found that different varieties or

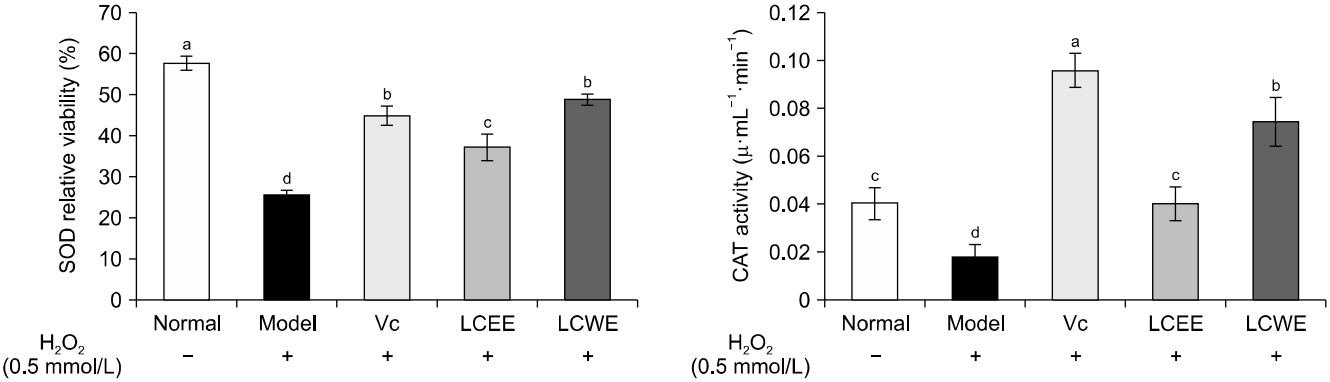



**Fig. 5.** Effects of *Lonicera caerulea* L. ethanol extract (LCEE) and *L. caerulea* L. water extract (LCWE) on the catalase (CAT) and superoxide dismutase (SOD) activity of hydrogen peroxide ($H_2O_2$)-induced RAW 264.7 cells. Mean values with different letters in the same bar graph (a–d) are significantly different ($P$<0.05) according to Tukey's test. Vc, vitamin C.

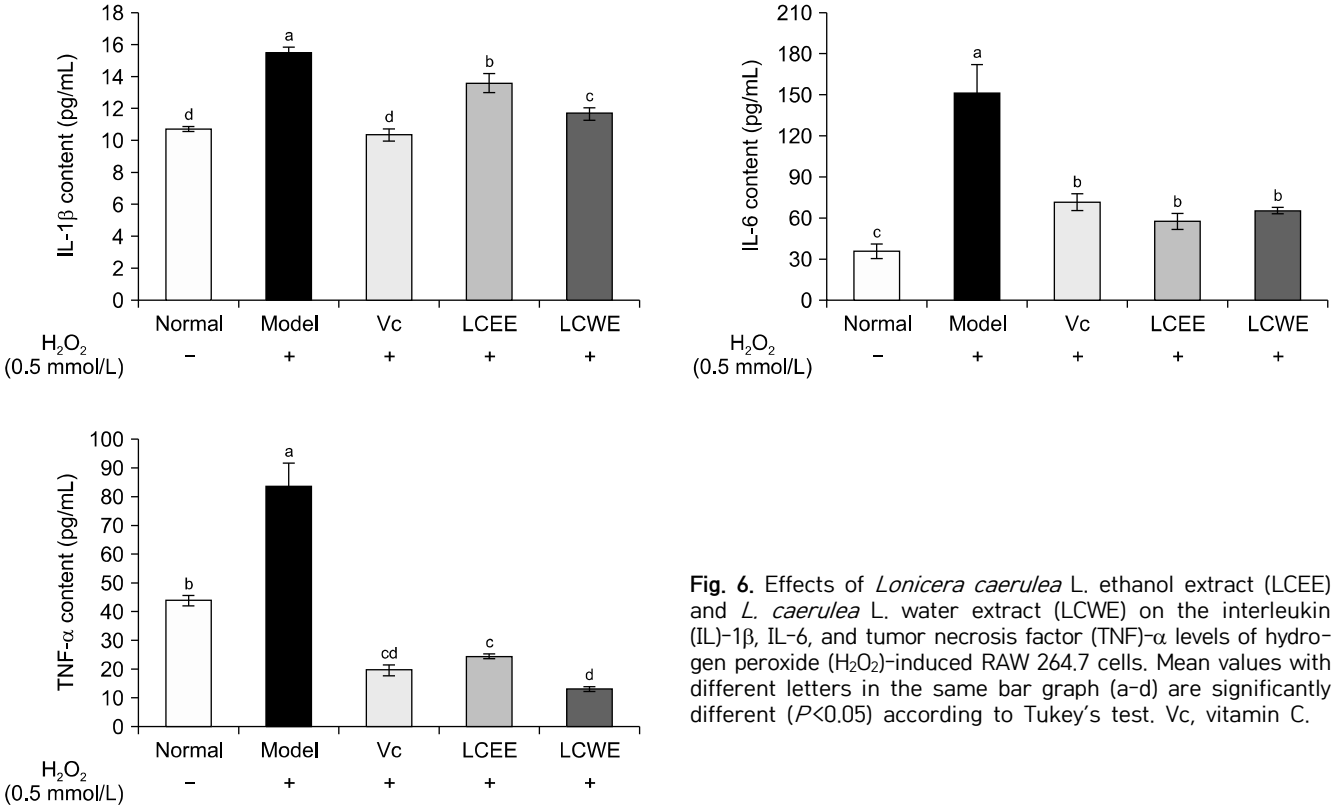



**Fig. 6.** Effects of *Lonicera caerulea* L. ethanol extract (LCEE) and *L. caerulea* L. water extract (LCWE) on the interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α levels of hydrogen peroxide ($H_2O_2$)-induced RAW 264.7 cells. Mean values with different letters in the same bar graph (a–d) are significantly different ($P$<0.05) according to Tukey's test. Vc, vitamin C.

origins of *L. caerulea* L. have different antioxidants and that low temperatures and radiation help the accumulation of organic acids (Vc, citric acid) and polyphenols (anthocyanins, phenolic acids, and flavonols) (Ochmian et al., 2012; Senica et al., 2018). Other health functions of *L. caerulea* L., including its anti-inflammatory, hepatoprotective and radioprotective, and blood sugar and neuromodulatory properties, are also mostly related to its phenolic compounds (Wu et al., 2018; De Silva and Vasantha Rupasinghe, 2020; Gołba et al., 2020; Dayar et al., 2021). Although the antioxidative and hypoglycemic functions of *L. caerulea* L. have been demonstrated, few studies have investigated the polysaccharides of *L. caerulea* L. compared with its phenolic content (Pei et al., 2022). Therefore, we prepared ethanolic (LCEE) and water extracts (LCWE) of *L. caerulea* L. and compared their antioxidant and anti-inflammatory properties. Macrophages are major sources of oxidative stress. Activated macrophages are the main source of reactive oxygen species, reactive nitrogen species, and peroxynitrite produced during respiratory bursts. The constitutive release of proinflammatory cytokines, especially TNF-α and NF-κB, leads to the overproduction of reactive oxygen and nitrogen species in macrophages (Castaneda et al., 2017). Oxidants such as $H_2O_2$ can activate macrophages, leading to protein and lipid peroxidation and cellular oxidative damage (Gamaley et al., 1994). Therefore, RAW264.7 cells were exposed to $H_2O_2$ in the present study to investigate the *in vitro* antioxidant effects of LCEE and LCWE. In macrophages, NO is synthesized by inducible NO synthase, and superoxide is mainly produced by NADPH oxidase. The reaction of superoxide with NO leads to peroxynitrite formation *in vivo*, which not only causes cytotoxicity in macrophages but also directly leads to peroxidation, thereby mediating lipid, DNA, and protein interactions (Xia and Zweier, 1997). As a lipid peroxidation marker, MDA is one of the final products of intracellular polyunsaturated fatty acid peroxidation and is used to reflect organisms' oxidative stress state (Gaweł et al., 2004). In the presence of a large amount of reactive oxygen species, NO bioavailability is reduced, which in turn reflects the oxidative stress state (Pierini and Bryan, 2015). In the present study, we found that LCEE and LCWE could reverse the $H_2O_2$-mediated antiproliferative effect and the production of peroxidation products (e.g., MDA and NO) in RAW264.7 cells. On the other hand, SOD and CAT are endogenous antioxidant enzymes, and their effectiveness is indispensable in the body's entire antioxidant system, especially in terms of superoxide anion free radical scavenging (Ighodaro and Akinloye, 2018). Our experiments showed that LCEE and LCWE antagonized the peroxidative state by increasing SOD and CAT levels in RAW264.7 cells. Macrophages play key roles in LPS-induced, inflammation-related pathogenesis. LPS activates macrophages/monocytes by binding to the TLR4 receptor on macrophages' surface, followed by polymorphonuclear leukocytes and T cells, which are inflammatory cells that play a role in diseases by synthe-

sizing IL-1β, IL-6, and TNF-α mediators (Lu et al., 2008). Therefore, we used LPS to induce inflammatory phenotype in RAW264.7 cells, which in turn increased IL-1β, IL-6, and TNF-α levels. We found that LCEE and LCWE treatment could be used to inhibit the secretion of these inflammatory factors and exert anti-inflammatory effects *in vitro*. Of note, LCWE exhibited more active antioxidant and anti-inflammatory activities than LCEE. Previous studies have shown that hot water extraction can obtain more total phenolic components compared with petroleum ether, benzene, chloroform, ethyl acetate, and methanol solvent extraction (Anusuya and Manian, 2013; Caprioli et al., 2016). Moreover, hot water extraction can obtain 7.20% of the total sugar (mainly glucose and fructose, followed by binding sugars) and 12% of organic acids (mainly ascorbic acid, citric acid, and malic acid) of the fruit biomass (Svarcova et al., 2007). The plant components of *L. caerulea* L. vary depending on the geographical location, variety, harvest date, and extraction method (De Silva and Vasantha Rupasinghe, 2020). Therefore, we speculated that LCWE contains more complex components such as total phenols, polysaccharides, and organic acids, thus exhibiting stronger antioxidant and anti-inflammatory activities. In summary, we reported the *in vitro* antioxidant and anti-inflammatory effects of LCWE and LCEE. LCWE and LCEE upregulated the secretion of SOD and CAT and downregulated the production of MDA and NO and the release of inflammatory factors IL-1β, IL-6, and TNF-α, thereby inhibiting $H_2O_2$-induced oxidative damage and LPS-induced inflammation in RAW 264.7 cells. Of note, LCWE showed better effects than LCEE, both in terms of antioxidant and anti-inflammatory properties, which may be related to its chemical composition. These findings are promising and warrant further in-depth studies.

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## AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

## AUTHOR CONTRIBUTIONS

Concept and design: CL, MHP. Analysis and interpretation: CL, KIJ. Data collection: JHK. Writing the article: CL. Critical revision of the article: KIJ, MHP. Final approval of the article: all authors. Statistical analysis: CL. Obtained funding: MK, JHK. Overall responsibility: MK.

## REFERENCES

Anusuya N, Manian S. Antioxidant and free radical scavenging potential of different solvent extracts of *Indigofera tinctoria* L. leaves. Int J Pharm Pharm Sci. 2013. 5:142-147.

Caprioli G, Iannarelli R, Innocenti M, Bellumori M, Fiorini D, Sagratini G, et al. Blue honeysuckle fruit (*Lonicera caerulea* L.) from eastern Russia: phenolic composition, nutritional value and biological activities of its polar extracts. Food Funct. 2016. 7:1892-1903.

Castaneda OA, Lee SC, Ho CT, Huang TC. Macrophages in oxidative stress and models to evaluate the antioxidant function of dietary natural compounds. J Food Drug Anal. 2017. 25:111-118.

Celli GB, Ghanem A, Brooks MSL. Haskap berries (*Lonicera caerulea* L.)—a critical review of antioxidant capacity and health-related studies for potential value-added products. Food Bioprocess Technol. 2014. 7:1541-1554.

Dayar E, Cebova M, Lietava J, Panghyova E, Pechanova O. Antioxidant effect of *Lonicera caerulea* L. in the cardiovascular system of obese zucker rats. Antioxidants. 2021. 10:1199. https://doi.org/10.3390/antiox10081199

De Silva ABKH, Vasantha Rupasinghe HP. Polyphenols composition and anti-diabetic properties *in vitro* of haskap (*Lonicera caerulea* L.) berries in relation to cultivar and harvesting date. J Food Compos Anal. 2020. 88:103402. https://doi.org/10.1016/j.jfca.2019.103402

Elsayed EA, El Enshasy H, Wadaan MA, Aziz R. Mushrooms: a potential natural source of anti-inflammatory compounds for medical applications. Mediators Inflamm. 2014. 2014:805841. https://doi.org/10.1155/2014/805841

Gamaley IA, Kirpichnikova KM, Klyubin IV. Activation of murine macrophages by hydrogen peroxide. Cell Signal. 1994. 6:949-957.

Gaweł S, Wardas M, Niedworok E, Wardas P. Malondialdehyde (MDA) as a lipid peroxidation marker. Wiad Lek. 2004. 57:453-455.

Gołba M, Sokół-Łętowska A, Kucharska AZ. Health properties and composition of honeysuckle berry *Lonicera caerulea* L. An update on recent studies. Molecules. 2020. 25:749. https://doi.org/10.3390/molecules25030749

Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alex J Med. 2018. 54:287-293.

Janero DR. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic Biol Med. 1990. 9:515-540.

Khattab R, Celli GB, Ghanem A, Brooks MSL. Effect of frozen storage on polyphenol content and antioxidant activity of haskap berries (*Lonicera caerulea* L.). J Berry Res. 2015. 5:231-242.

Li C, He Y, Yang Y, Gou Y, Li S, Wang R, et al. Antioxidant and inflammatory effects of *Nelumbo nucifera* Gaertn. leaves. Oxid Med Cell Longev. 2021. 2021:8375961. https://doi.org/10.1155/2021/8375961

Li F, Zhao H, Xu R, Zhang X, Zhang W, Du M, et al. Simultaneous optimization of the acidified water extraction for total anthocyanin content, total phenolic content, and antioxidant activity of blue honeysuckle berries (*Lonicera caerulea* L.) using response surface methodology. Food Sci Nutr. 2019. 7:2968-2976.

Lu YC, Yeh WC, Ohashi PS. LPS/TLR4 signal transduction pathway. Cytokine. 2008. 42:145-151.

Ochmian ID, Skupien K, Grajkowski J, Smolik M, Ostrowska K.

Chemical composition and physical characteristics of fruits of two cultivars of blue honeysuckle (*Lonicera caerulea* L.) in relation to their degree of maturity and harvest date. Not Bot Horti Agrobot Cluj Napoca. 2012. 40:155-162.

Pei F, Lv Y, Cao X, Wang X, Ren Y, Ge J. Structural characteristics and the antioxidant and hypoglycemic activities of a polysaccharide from *Lonicera caerulea* L. pomace. Fermentation. 2022. 8:422. https://doi.org/10.3390/fermentation8090422

Pierini D, Bryan NS. Nitric oxide availability as a marker of oxidative stress. Methods Mol Biol. 2015. 1208:63-71.

Rupasinghe HPV, Yu LJ, Bhullar KS, Bors B. Short communication: haskap (*Lonicera caerulea*): A new berry crop with high antioxidant capacity. Can J Plant Sci. 2012. 92:1311-1317.

Safaeian L, Javanmard SH, Mollanoori Y, Dana N. Cytoprotective and antioxidant effects of human lactoferrin against $H_2O_2$-induced oxidative stress in human umbilical vein endothelial cells. Adv Biomed Res. 2015. 4:188. https://doi.org/10.4103/2277-9175.164010

Senica M, Stampar F, Mikulic-Petkovsek M. Blue honeysuckle (*Lonicera cearulea* L. subs. *edulis*) berry; A rich source of some nutrients and their differences among four different cultivars. Sci Hortic. 2018. 238:215-221.

Sun J, Zhang X, Broderick M, Fein H. Measurement of nitric oxide production in biological systems by using Griess reaction assay. Sensors. 2003. 3:276-284.

Svarcova I, Heinrich J, Valentova K. Berry fruits as a source of biologically active compounds: the case of *Lonicera caerulea*. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2007. 151: 163-174.

Tang GY, Meng X, Gan RY, Zhao CN, Liu Q, Feng YB, et al. Health functions and related molecular mechanisms of tea components: an update review. Int J Mol Sci. 2019. 20:6196. https://doi.org/10.3390/ijms20246196

Vallianou NG, Gounari P, Skourtis A, Panagos J, Kazazis C. Honey and its anti-inflammatory, anti-bacterial and anti-oxidant properties. Gen Med. 2014. 2:2. https://doi.org/10.4172/2327-5146.1000132

Vasantha Rupasinghe HP, Arumuggam N, Amararathna M, De Silva ABKH. The potential health benefits of haskap (*Lonicera caerulea* L.): Role of cyanidin-3-O-glucoside. J Funct Foods. 2018. 44:24-39.

Wu S, Hu R, Nakano H, Chen K, Liu M, He X, et al. Modulation of gut microbiota by *Lonicera caerulea* L. berry polyphenols in a mouse model of fatty liver induced by high fat diet. Molecules. 2018. 23:3213. https://doi.org/10.3390/molecules23123213

Xia Y, Zweier JL. Superoxide and peroxynitrite generation from inducible nitric oxide synthase in macrophages. Proc Natl Acad Sci U S A. 1997. 94:6954-6958.

Xu J, Zhao Y, Aisa HA. Anti-inflammatory effect of pomegranate flower in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages. Pharm Biol. 2017. 55:2095-2101.

Zhang S, Zhang H, Qin R, Jiang W, Liu D. Cadmium induction of lipid peroxidation and effects on root tip cells and antioxidant enzyme activities in *Vicia faba* L.. Ecotoxicology. 2009. 18:814-823.

Zhao H, Wang Z, Cheng C, Yao L, Wang L, Lu W, et al. *In-vitro* free radical scavenging activities of anthocyanins from three berries. J Med Plants Res. 2012. 6:101-107.