Nutrition Research and Practice 2018;12(6):486-493
ⓒ2018 The Korean Nutrition Society and the Korean Society of Community Nutrition
http://e-nrp.org

Evaluation of in vitro anti-oxidant and anti-inflammatory activities of Korean and Chinese *Lonicera caerulea*

You-Suk Lee[1], Il Je Cho[2], Joo Wan Kim[3], Sun-Kyoung Lee[4], Sae Kwang Ku[5§] and Hae-Jeung Lee[1§]

[1]Department of Food and Nutrition, College of BioNano Technology, Gachon University, 1342, Seongnam-daero, Sujeong-gu, Seongnam, Gyeonggi 13120, Korea
[2]The Medical Research Center for Globalization of Herbal Formulation and Department of Herbal Formulation, College of Oriental Medicine, Daegu Haany University, Gyeongbuk 38610, Korea
[3]Department of Internal Medicine, College of Veterinary Medicine, Kyungpook National University, Daegu 41566, Korea
[4]Department of Life Physical Education, Myongji University, Seoul 03674, Korea
[5]Department of Anatomy and Histology, College of Oriental Medicine, Daegu Haany University, 1, Hanuidae-ro, Gyeongsan, Gyeongbuk 38610, Korea

BACKGROUND/OBJECTIVE: The honeysuckle berry (HB) contains ascorbic acid and phenolic components, especially anthocyanins, flavonoids, and low-molecular-weight phenolic acids. In order to examine the potential of HB as a hepatoprotective medicinal food, we evaluated the *in vitro* anti-oxidant and anti-inflammatory activities of Korean HB (HBK) and Chinese HB (HBC).
MATERIALS/METHODS: Antioxidant and anti-inflammatory effects of the extracts were examined in HepG2 and RAW 264.7 cells, respectively. The anti-oxidant capacity was determined by DPPH, SOD, CAT, and ARE luciferase activities. The production of nitric oxide (NO) as an inflammatory marker was also evaluated. The *Nrf2*-mediated mRNA levels of heme oxygenase-1 *(HO-1)*, NAD(P)H dehydrogenase [quinone] 1 (*Nqo1*), and glutamate-cysteine ligase catalytic subunit (*Gclc*) were measured. The concentrations of HB extracts used were 3, 10, 30, 100, and 300 μg/mL.
RESULTS: The radical scavenging activity of all HB extracts increased in a concentration-dependent manner ($P < 0.01$ or $P < 0.05$). SOD ($P < 0.05$) and CAT ($P < 0.01$) activities were increased by treatment with 300 μg/mL of each HB extract, when compared to those in the control. NO production was observed in cells pretreated with 100 or 300 μg/mL of HBC and HBK ($P < 0.01$). Treatment with 300 μg/mL of HBC significantly increased *Nqo1* ($P < 0.01$) and *Gclc* ($P < 0.05$) mRNA levels compared to those in the control. Treatment with 300 μg/mL of HBK ($P < 0.05$) and HBC ($P < 0.01$) also significantly increased the *HO-1* mRNA level compared to that in the control.
CONCLUSIONS: Thus, the Korean and Chinese HBs were found to possess favorable *in vitro* anti-oxidant and anti-inflammatory activities. *Nrf2* and its related anti-oxidant genes were associated with both anti-oxidant and anti-inflammatory activities in HB-treated cells. Further studies are needed to confirm these *in vivo* effects.

Nutrition Research and Practice 2018;12(6):486-493; https://doi.org/10.4162/nrp.2018.12.6.486; pISSN 1976-1457 eISSN 2005-6168

Keywords: Honeysuckle berry, hepatoprotective effect

INTRODUCTION

The liver plays vital roles in several processes, including protein synthesis, glucose homeostasis, detoxification, and cycling of various nutrients [1,2]. Liver damage causes metabolic dysfunctions. If liver injury persists, transient elevation of liver enzymes, hepatic fibrosis, cirrhosis, and hepatocellular carcinoma occur sequentially or simultaneously [3]. Oxidative stress and inflammation are closely related to the etiology of liver damage [4]. Although treatments are continuously being developed to protect the liver from damage, reliable hepatoprotective drugs remain insufficient. Therefore, it is necessary to develop functional foods that can help prevent and treat liver damage.

Oxidative stress is induced by reactive oxygen species (ROS) that can damage cells and result in diseases such as diabetes, cardiovascular ailments, cancer, and liver damage [5]. Anti-oxidant vitamins and enzymes, such as superoxide dismutase, glutathione peroxidase, and glutathione reductase, can reduce excessive ROS production [6]. Phytochemicals with distinct flavors, odors, and colors can also exhibit anti-oxidant activities, and it has been reported that berries contain many phytochemicals such as a flavonoids, anthocyanins, and phenolic acids [7].

The honeysuckle berry (HB) *Lonicera caerulae* has been used

This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through High Value-added Food Technology Development Program funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (grant 116019-3) and Korea Food Research Institute (grant E0164500-03).
§ Corresponding Authors: Hae-Jeung Lee, Tel. 82-31-750-5968, Fax. 82-31-750-5974, Email. skysea@gachon.ac.kr; skysea1010@gmail.com
Sae Kwang Ku, Tel. 82-53-819-1549, Fax. 82-53-819-1570, Email. gucci200@hanmail.net
Received: May 28, 2018, Revised: June 22, 2018, Accepted: August 13, 2018
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

in folk medicine in China, Japan, and northern Russia. However, the HB is not well-known as an edible berry in North America, Europe, or Korea. HB contains ascorbic acid and a number of phenolic components, especially anthocyanins, flavonoids, and low molecular-weight phenolic acids. It has been reported that HB extract exerts strong anti-oxidant effects [8], radioprotective effects against ionizing radiation in mice [5], hepatoprotective effects [9], anti-inflammatory effects [10,11], improvement of lipid and glucose metabolism [12], and therapeutic effects on hyperthyroidism [13]. Especially, HB extract has been shown to have the strongest anti-oxidant activity among 12 types of colored berries [14]. Abundant phenols in HB extract have been shown to possess anti-inflammatory and wound-healing properties both *in vitro* and *in vivo* [10]. Furthermore, they have shown skin-protective effects against ultraviolet-induced damages [15,16].

Nuclear factor-erythroid 2-related factor-2 (*Nrf2*) induces the expression of anti-oxidant-related genes such as *HO-1*, *Nqo1*, and *Gclc*, and these genes were reported to reduce proinflammatory cytokine levels [17,18]. Furthermore, it has been indicated that proinflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, affect lipopolysaccharide (LPS)-stimulated production of nitric oxide (NO) [19,20]. Although a number of studies with respect to anti-oxidant and anti-inflammatory effects of the HB have been reported, studies regarding the expression of *Nrf2*-related anti-oxidant genes by HB extracts have been scarce.

Recently, HB cultivation in Korea has been increasing, although HB used in the Korean industry is mainly imported from China. HB has attracted attention as a health food, but has not been developed as a functional food for improving liver function. Therefore, in order to examine the possibility of using HB as a hepatoprotective medicinal food, we evaluated the *in vitro* anti-oxidant and anti-inflammatory efficacy, luciferase reporter activity, and *Nrf2*-related gene expression of Korean and Chinese HB (HBK and HBC, respectively).

MATERIALS and METHODS

Cell culture

The human hepatocyte cell line (HepG2) and murine macrophage cell line (RAW 264.7) were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). These cells were seeded in 6-well plates (1×10^5 per well) at 70-80% confluence. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco®, MA, USA) containing 10% fetal bovine serum (Hyclone™, Victoria, Australia), 50 units/mL penicillin (Sigma-Aldrich, St. Louis, MO, USA), and 50 µg/mL streptomycin (Sigma-Aldrich) in a 5% CO_2 humidified incubator at 37°C.

Test materials

HBK was prepared and supplied by Aribio Co. Ltd. (Seongnam, Korea). HBC was imported from China and supplied by H&K Bioscience Co., Ltd. (Seoul, Korea). Briefly, HBK was prepared as follows: HBK samples were squeezed, decompressed, and condensed at 55-65°C (EYELA, N-N series, Tokyo, Japan) and completely lyophilized in a freeze dryer (Operon FDB-5503, Kimpo, Korea). For HBC preparation, concentrated Chinese HB juice (63 brix) was decompressed and condensed and then lyophilized as specified previously for HBK. The prepared samples were stored at -20°C until further use. The concentrations of HB extracts used were 3, 10, 30, 100, and 300 µg/mL.

Radical scavenging activity assay

Radical scavenging activity was determined using 2,2-Diphenyl -1-Picrylhydrazyl (DPPH) assay [21]. Briefly, DPPH (Sigma-Aldrich) in ethanol solution (150 µM) was incubated with HBK (10-300 µg/mL), HBC (10-300 µg/mL), or Trolox (100 µM), an anti-oxidant derived from α-tocopherol. Samples were incubated at room temperature for 30 min under light protection. The absorbance was measured at a wavelength of 517 nm using a microplate reader (Infinite 200 PRO, Tecan, Männedorf, Switzerland).

Cell viability assay

HepG2 or RAW 264.7 cells were cultured in 24-well plates (5×10^4 cells/well). To examine the cytoprotective effect of each HB extract in HepG2 cells, 3-300 µg/mL of each HB extract was pre-incubated with HepG2 cells at 1 h prior to the addition of 150 µM tert-butyl hydroperoxide (*t*-BHP) for 12 h. Likewise, in RAW 264.7 cells, 3-300 µg/mL of each HB extract was pre-incubated with cells at 1 h prior to the addition of 1 µg/mL LPS for 18 h. After incubation, viable cells were incubated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (0.1 µg/mL) for 4 h.

Reporter gene assay

To determine luciferase activity, stably transfected cells with pGL4.37 were seeded onto 12-well cell culture plates at a density of 5×10^5 cells/well followed by serum starvation for 12 h. Cells were then exposed to 10-300 µg/ml of each HB extract for 24 h. After adding a luciferase assay reagent (Promega), luciferase activities in cell lysates were measured using a microplate reader (Tecan).

Total RNA isolation and Real-time PCR

Total RNA was isolated from treated cells using Trizol reagent (Invitrogen, Carlsbad, CA, USA). RNA (2 µg each) was then reverse-transcribed to cDNA using oligo-d(T)16 primers on a thermal cycler (Mastercyler Nexus Gradient Thermal Cycler, Eppendorf AG, Hamburg, Germany). PCR was performed using gene-specific primers targeting human heme oxygenase-1 (*HO-1*) (sense, CAGGAGCTGCTGACCCATGA; antisense, AGCAACTGTCG CCACCAGAA; PCR product size, 195 bp), glutamate-cysteine ligase catalytic subunit (*Gclc*) (sense, GAAGTGGATGTGGACA CCAG; antisense, TTGTAGTCAGGATGGTTTGCGA; PCR product size, 128 bp), NAD(P)H dehydrogenase quinone 1 (*Nqo1*) (sense, GGATTGGACCGAGCTGGAA; antisense, TGCAGTGAAGATGAAGG CAAC; PCR product size, 137 bp). Primers were synthesized by Bioneer (Daejeon, Korea).

Superoxide dismutase (SOD) and catalase (CAT) activity assay

SOD and CAT activities were determined by using HepG2 cell lysates after cells were treated with 300 µg/mL of each HB extract for 24 h. Catalase activity in cell homogenate was measured by using a catalase assay kit (Cayman Chemical, Ann Arbor, MI, USA). The activity of SOD in cell homogenate was

determined using a superoxide dismutase assay kit (Cayman Chemical). A tetrazolium salt was provided in the kit to detect superoxide radicals generated by xanthine oxidase and hypoxanthine at a wavelength of 550 nm.

Determination of NO production

To quantify NO in the conditioned medium, RAW 264.7 cells were pretreated with 10-300 μg/mL of each HB extract for 1 h and subsequently exposed to 1 μg/mL LPS for 18 h. After treatment, 100 μL of collected culture medium was incubated with an equal volume of Griess reagent. The absorbance was measured at a wavelength of 550 nm by using an automated microplate reader (Tecan). Recorded values are presented as fold versus control.

Statistical analysis

All data are expressed as Mean±SD from three experiments. Multiple comparison tests were performed for different groups according to dose. An equilibrium test was performed using the Levene test. Least-significant differences (LSD) multiple comparisons were used for group comparisons. In cases of unequal distribution, the Kruskal-Wallis H test was performed with Levene test and nonparametric test.

When the Kruskal-Wallis H test showed significant differences, Dunnett's tests were performed to determine specific pairs between groups. Differences were considered significant at $P < 0.05$ level. All statistical analyses were performed using SPSS for Windows (14.0K, SPSS Inc., Chicago, IL, USA).

RESULTS

Radical scavenging activity of HB extract

To compare the *in vitro* anti-oxidant capacity of HB extract of different origins, radical scavenging activity was examined using a DPPH assay. The radical scavenging activity of all HB extracts increased in a concentration-dependent manner (Fig. 1). There were significant differences in radical scavenging activity for both HBK and HBC compared to the control ($P < 0.01$). At a concentration of 100 μg/mL, and HBK and HBC had 33.9% and 42.75% activity, respectively, whereas at 300 μg/mL, they exhibited 86.1% and 92.96% activity, respectively.

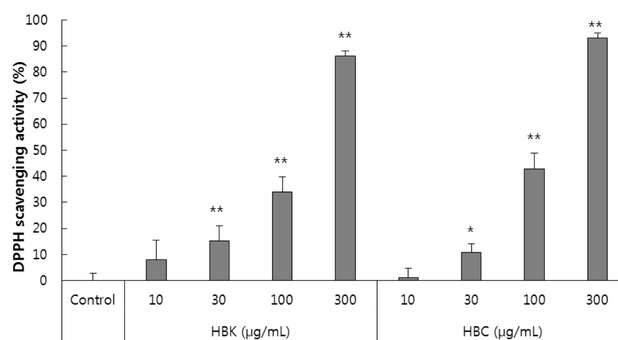

Fig. 1. DPPH scavenging activities of honeysuckle berry extracts. DPPH, 2, 2-Diphenyl-1-Picrylhydrazyl; LSD, least-significant differences; HBK, Korean honeysuckle berry; HBC, Chinese honeysuckle berry. Values are expressed as Mean±SD. Significant compared to the control (100 μM Trolox-treated) by LSD test. ** $P < 0.01$; * $P < 0.05$.

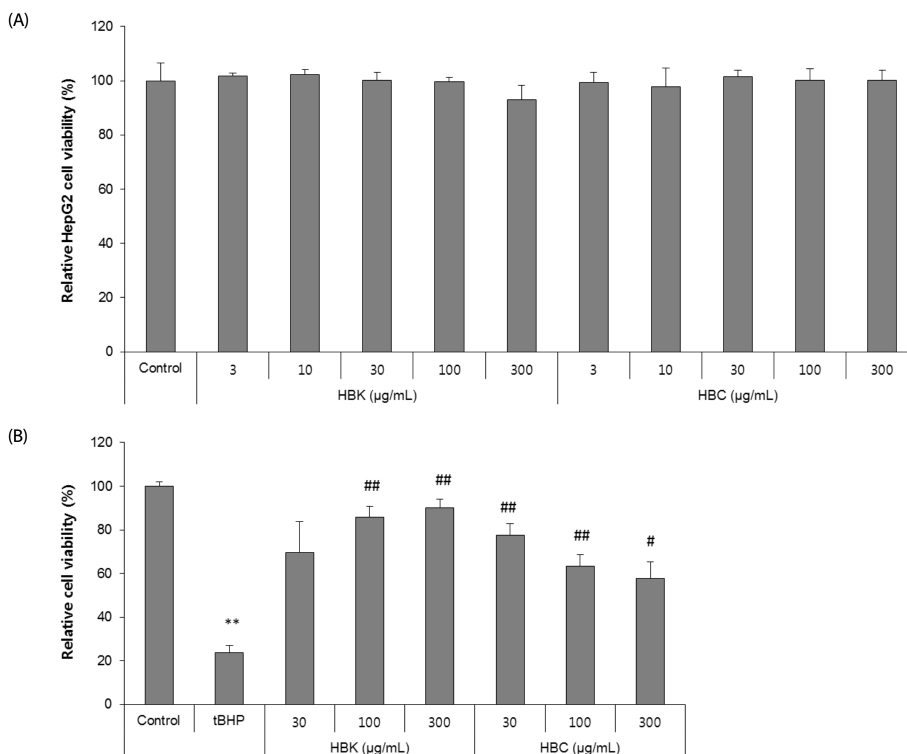

Fig. 2. Effect of honeysuckle berry extract on the viability of HepG2 cells. (A) Cell viability by MTT assay. (B) Cell viability induced by t-BHP. MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; t-BHP, *tert*-butyl hydroperoxides; HBK, Korean honeysuckle berry; HBC, Chinese honeysuckle berry. Values were expressed as Mean±SD. Significant compared to the control by Dunnett's test, ** $P < 0.01$ compared to *t*-BHP treated cells by Dunnett's test; ## $P < 0.01$; # $P < 0.05$.

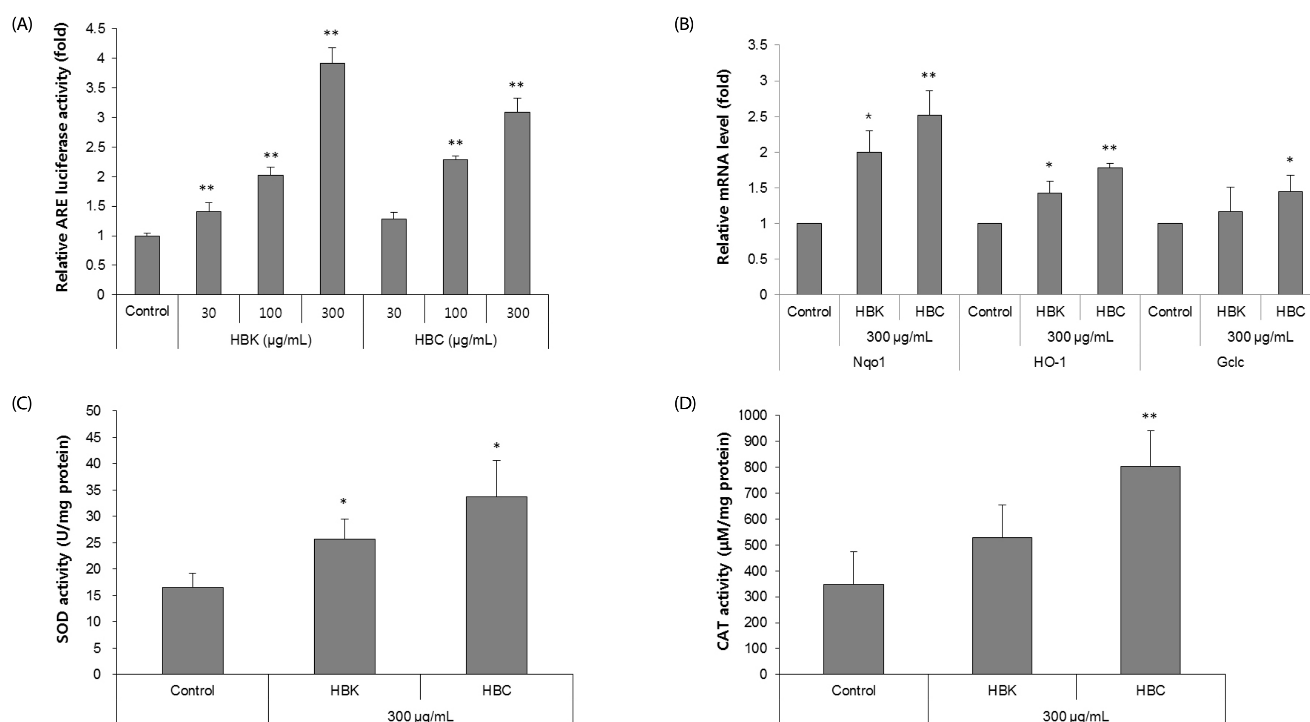

Fig. 3. Effect of honeysuckle berry extract in HepG2 cells. (A) *Nrf2* transactivation. (B) *Nrf2*-dependent anti-oxidant gene expressions. (C) SOD activity. (D) CAT activity. HBK, Korean honeysuckle berry; HBC, Chinese honeysuckle berry; *Nrf2*, nuclear factor (erythroid-derived 2)-like 2; SOD, superoxide dismutase; CAT, catalase. Values are expressed as Mean±SD. Significant compared to the control by least-significant differences (LSD) test. ** $P<0.01$; * $P<0.05$.

Effect of HB extract on HepG2 cell viability

Cell viability was investigated in HepG2 cells after treatment with 3-300 μg/mL of each HB extract for 24 h. The results of the MTT assays indicated that treatment with each HB extract up to 300 μg/mL did not affect the viabilities of HepG2 cells (Fig. 2A). Because 300 μg/mL of each HB extract did not show any cytotoxicity, this concentration was chosen as the maximal concentration for each HB extract for subsequent experiments using HepG2 cells.

Effect of HB extract on t-BHP-induced cytotoxicity

To examine the effect of each HB extract against oxidative stress-mediated cytotoxicity, HepG2 cells were pretreated with 30-300 μg/mL of each HB extract for 1 h and subsequently exposed to 150 μM of *t*-BHP for 12 h. Cell viability was then determined by the MTT assay. Exposure to *t*-BHP for 12 h significantly decreased cell viabilities. However, pretreatment with each HB extract increased cell viabilities. Statistically significant differences in cell viabilities were observed between HB pretreated cells and cells treated with *t*-BHP alone (23.6%) compared to the control (Fig. 2B). When an equal concentration of each HB extract used for the pretreatment was compared, HBK at 300 μg/mL showed the most potent cytoprotective activity (89.99%) against *t*-BHP-induced cytotoxicity. However, differences in cell viabilities were not statistically significant between HBK and HBC-treated cells.

Effect of HB extract on Nrf2 transactivation

To examine whether *Nrf2* was involved in HB-mediated cytoprotection, HepG2 cells stably transfected with pGL4.37 containing an anti-oxidant response element (ARE)-driven reporter gene were treated with 30-300 μg/mL of each HB extract for 24 h, and luciferase activities were then monitored. The treatment with each HB extract significantly increased ARE-driven luciferase activities in a dose-dependent manner. Statistically significant differences in ARE-driven luciferase activities were observed for all HB pretreated cells (except cells treated with 30 μg/mL of HBC) when compared to those of controls (Fig. 3A). When an equal concentration of each HB extract was compared, HBK (3.9 fold) at 300 μg/mL showed the most potent induction of luciferase activity.

Effect of HB extract on the expression of Nrf2-dependent anti-oxidant genes

To examine whether *Nrf2* activation induced by the HB extract might affect the transcription of anti-oxidant genes, HepG2 cells were treated with 300 μg/mL of each HB extract for 24 h, and the mRNA levels of anti-oxidant genes were then monitored by real-time PCR. The results of real-time PCR indicated that treatment with each HB extract tended to increase the mRNA levels of *Nqo1*, *HO-1*, and *Gclc*. Treatment with both 300 μg/mL of HBK and HBC increased mRNA expression, i.e. 1.4-fold and 1.8-fold, respectively, for *HO-1*, and 2.0-fold and 2.5-fold for *Nqo1*, respectively, compared to the control. As for *Gclc*, HBC showed higher mRNA expression than the control (Fig. 3B).

Effect of HB extract on SOD and CAT activities

SOD activities were significantly increased by treatment with

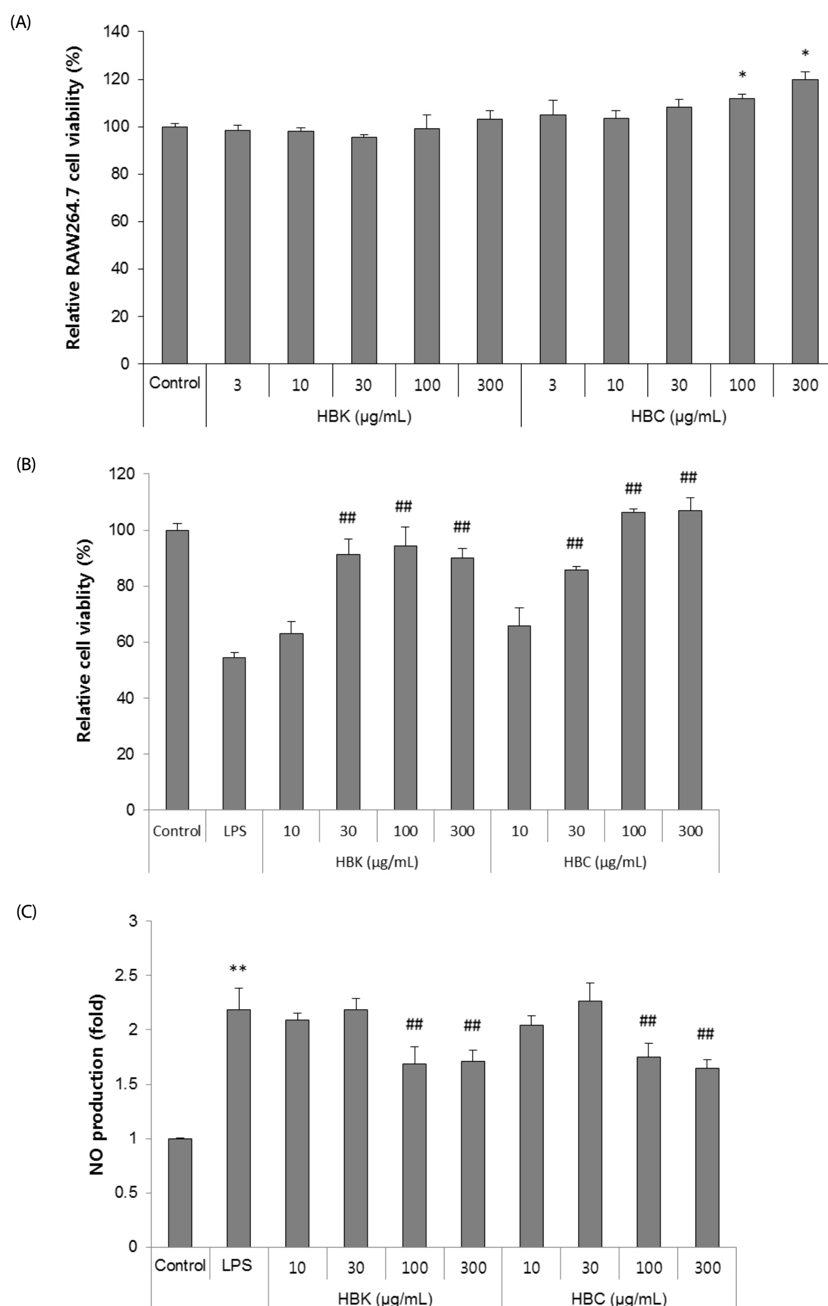

Fig. 4. **Effect of honeysuckle berry extract in RAW 264.7 cells.** (A) Cell viability by MTT assay. (B) Lipopolysaccharides (LPS)-mediated cell viability. (C) LPS-mediated NO production. HBK, Korean honeysuckle berry; HBC, Chinese honeysuckle berry; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NO, nitric oxide. Values are expressed as Mean±SD. Significant compared to the control by Dunnett's test. * $P < 0.05$; ** $P < 0.01$ compared to LPS-treated cells by Dunnett's test; ## $P < 0.01$.

300 μg/mL of each HB extract (HBK, 25.7 U/mg protein; HBC, 33.7 U/mg protein) as compared to those of the control (Fig. 3C). CAT activities also tended to be increased by treatment with 300 μg/mL of each HB extract (HBK, 506.5 μm/mg protein; HBC, 802.2 μm/mg protein) as compared to those of the control (Fig. 3D). However, statistically significant differences in CAT activities were only observed in 300 μg/mL of HBC-treated cells.

Effect of HB extract on RAW 264.7 cell viability, LPS-induced NO production, and cytotoxicity

Treatment with HBK up to 300 μg/mL did not affect the viabilities of RAW 264.7 cells. However, treatment with 100 or 300 μg/mL HBC significantly increased cell viabilities (Fig. 4A). Statistically significant differences in cell viabilities were observed between HBC and HBK pretreated cells and cells treated with LPS alone (Fig. 4B). When different concentrations of each HB used for the pretreatment were compared, HBC at

300 μg/mL showed the most potent cytoprotective activity against LPS in RAW 264.7 cells. Statistically significant differences in NO production were observed in cells pretreated with 100 and 300 μg/mL, respectively, of HBK and HBC compared to that seen with LPS treatment alone (Fig. 4C).

DISCUSSION

The present study revealed that HB extracts from both origins increased radical scavenging activity in a concentration-dependent manner. Our results are similar to those of a previous study showing that the HB extracts possess the highest anti-oxidant effects among 12 types of colored berries [14]. In our study, HBC at 300 μg/mL showed the most potent radical scavenging activity among all concentrations tested. It was higher than the radical scavenging activity of HBK at the same concentration. Moreover, the results of the MTT assays suggested that each HB extract up to 300 μg/mL had no negative effect on the viabilities of HepG2 or RAW 264.7 cells. Thus, HB extracts of both origins seem to have potent radical scavenging activity without any cytotoxicity. However, further studies are required to evaluate their *in vivo* toxicities.

To explore whether the radical scavenging activity of HB extract contributed to cell protection against oxidative stress, cell viability was monitored after treatment of t-BHP and LPS in HepG2 and RAW 264.7 cells, respectively. As expected, treatment with 150 μM of t-BHP in HepG2 cells and 1 μg/mL of LPS in RAW 264.7 cells significantly decreased cell viabilities. Nevertheless, the pretreatment with each HB extract in HepG2 cells increased cell viabilities. Significant differences in cell viabilities were observed across all HB-pretreated cells (except cells pretreated with 30 μg/mL of HBK) and those treated by t-BHP alone. These results indicated that polyphenol components of the HB extracts have an effect on the cell viability in HepG2 cells induced by t-BHP. This finding is supported by another study where phenolic compounds displayed protective effects against oxidative stress, leading to the maintenance of a normal redox state of the cell [22]. HBK and HBC showed increased cell viabilities in a dose-dependent manner, although 30 μg/mL of HBK did not show a significant increase in cell viabilities. The differences between HBK and HBC cell viabilities may have been due to differences between the geographical location and soil environments of the HBs. The plant's phytochemical content may vary in the composition depending on the environment [23,24]. Also, the HBs used in this study were not representative of their respective sources. Thus, further studies are needed with multiple samples from China and Korea. Both HBK and HBC provided protection by increasing cell viability against LPS-induced cytotoxicity. Statistically significant differences in such protection were observed in all HB-pretreated cells when compared to cells treated with LPS alone. Collectively, these results suggest that HBK and HBC have a potent protective effect on hepatocytes against oxidative stress and inflammation in RAW 264.7 cells in this study.

Phenolic compounds and flavonoids are well-known *Nrf2* inducers [25], and the presence of these compounds in HB extract is expected to activate *Nrf2*. In fact, the highest activity of *Nrf2* was observed at the highest dose of HB. The activation of *Nrf2* was linked to various cellular signaling pathways such as p38 MAPK, extracellular-regulated protein kinase, phosphatidylinositol 3 kinase, protein kinase C, and casein kinase [26-30]. Therefore, further studies are needed to elucidate the essential components of upstream signaling molecules that contribute to *Nrf2* activation in HB.

Similar to other studies [17,31], treatment with HB extract tended to increase mRNA levels of *Nqo1*, *HO-1*, and *Gclc*. It has been well known that anti-oxidant genes such as *HO-1*, *Nqo1*, and *Gclc* contain AREs in their promoter region, which can be induced by *Nrf2* activation [26,32,33]. Increasing evidence suggests that induction of *HO-1* can protect tissues against a variety of chronic diseases by preventing oxidative stress-mediated apoptosis and inflammation [34]. This is also strongly supported by the observation that the cytotoxic effects of oxidative stress are exacerbated in cells lacking *HO-1* [35,36]. *Gclc* is a heterodimeric enzyme involved in the first rate-limiting regulatory step of glutathione biogenesis. Thus, *Gclc* plays an essential role in maintaining cellular redox homeostasis and reducing oxidative damage by glutathione synthesis [37]. *Nqo1* is a member of the NAD(P)H dehydrogenase family. It involves two-electron reduction of quinones to hydroquinones. Therefore, *Nqo1* could play an important role in preventing the production of ROS [38]. Our study suggests that the compounds of HB extracts have a potential to be developed as hepatoprotective agents since pretreatment with HBK and HBC increased *Nrf2*-mediated anti-oxidant genes.

Anti-oxidant enzymes such as CAT and SOD were also essential in both scavenging ROS and maintaining cellular integrity [39]. From parallel results of real-time PCR, at 300 μg/mL of HBK and HBC, significant increases in SOD and CAT activities were observed. These results were similar to other studies using phenolic-rich plants with elevated SOD and CAT activities [40]. Taken together, HBC and HBK might have a potent effect in inducing anti-oxidant genes, because all HB extracts were capable of transactivating *Nrf2*. An inflammatory response is mainly regulated by NO, eicosanoids, and cytokines, all of which are released by injured cells. They play important roles in the progression of inflammatory states such as edema, intra/intercellular stress, and tissue necrosis. Although NO production under physiological conditions by inflammatory mediators contribute to the elimination of ingested pathogens in macrophages, excessive and prolonged production of NO may attack neighboring cells through reactive nitrogen species, which can accelerate inflammation-mediated tissue injuries.

Because common cellular signaling pathways are involved in the production of proinflammatory mediators in macrophages, monitoring changes in NO production in macrophages is a representative method to examine the anti-inflammatory potential of drug candidates. Therefore, instead of HepG2 cells, we measured NO production using RAW 264.7 cells, which is a kind of macrophage. Pretreatment with HBK and HBC significantly decreased NO production at a high dose compared to that at a low dose. Previous studies reported that the enhanced expression of ROS scavengers, such as *Nqo1* and *Gclc*, has beneficial effects on improving immunity [17-20]. The present study revealed elevated expressions of *Nrf2*-mediated anti-oxidant genes and reduced NO production at 300 μg/mL

of HB. Further studies are needed to explore the effects of HB on other pro-inflammatory mediators and to elucidate the signaling pathways involved.

In conclusion, this study showed that HB extract has favorable *in vitro* anti-oxidant and anti-inflammation activities, and *Nrf2* and its related anti-oxidant genes were associated with both anti-oxidant and anti-inflammatory effects in HB-treated cells. Our results suggest that both HBK and HBC are potentially suitable as preventive and therapeutic foods.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interests.

ORCID

You-Suk Lee: https://orcid.org/0000-0001-8360-1976
Il Je Cho: https://orcid.org/0000-0001-6295-0608
Joo Wan Kim: https://orcid.org/0000-0003-1150-2295
Sun-Kyoung Lee: https://orcid.org/0000-0002-6270-5183
Sae Kwang Ku: https://orcid.org/0000-0003-1269-3804
Hae-Jeung Lee: https://orcid.org/0000-0001-8353-3619

REFERENCES

1. Yang BY, Zhang XY, Guan SW, Hua ZC. Protective effect of procyanidin B2 against CCl4-induced acute liver injury in mice. Molecules 2015;20:12250-65.
2. Lu Y, Hu D, Ma S, Zhao X, Wang S, Wei G, Wang X, Wen A, Wang J. Protective effect of wedelolactone against CCl4-induced acute liver injury in mice. Int Immunopharmacol 2016;34:44-52.
3. Sun H, Chen L, Zhou W, Hu L, Li L, Tu Q, Chang Y, Liu Q, Sun X, Wu M, Wang H. The protective role of hydrogen-rich saline in experimental liver injury in mice. J Hepatol 2011;54:471-80.
4. Berasain C, Castillo J, Perugorria MJ, Latasa MU, Prieto J, Avila MA. Inflammation and liver cancer: new molecular links. Ann N Y Acad Sci 2009;1155:206-21.
5. Zhao H, Wang Z, Ma F, Yang X, Cheng C, Yao L. Protective effect of anthocyanin from *Lonicera caerulea* var. edulis on radiation-induced damage in mice. Int J Mol Sci 2012;13:11773-82.
6. Petersen F, Rodrigo R, Richter M, Kostin S. The effects of polyunsaturated fatty acids and antioxidant vitamins on atrial oxidative stress, nitrotyrosine residues, and connexins following extracorporeal circulation in patients undergoing cardiac surgery. Mol Cell Biochem 2017;433:27-40.
7. Szajdek A, Borowska EJ. Bioactive compounds and health-promoting properties of berry fruits: a review. Plant Foods Hum Nutr 2008;63:147-56.
8. 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;151:163-74.
9. Palíková I, Valentová K, Oborná I, Ulrichová J. Protectivity of blue honeysuckle extract against oxidative human endothelial cells and rat hepatocyte damage. J Agric Food Chem 2009;57:6584-9.
10. Jin XH, Ohgami K, Shiratori K, Suzuki Y, Koyama Y, Yoshida K, Ilieva I, Tanaka T, Onoe K, Ohno S. Effects of blue honeysuckle (*Lonicera caerulea* L.) extract on lipopolysaccharide-induced inflammation *in vitro* and *in vivo*. Exp Eye Res 2006;82:860-7.
11. Zdarilová A, Rajnochová Svobodová A, Chytilová K, Simánek V, Ulrichová J. Polyphenolic fraction of *Lonicera caerulea* L. fruits reduces oxidative stress and inflammatory markers induced by lipopolysaccharide in gingival fibroblasts. Food Chem Toxicol 2010;48:1555-61.
12. Jurgoński A, Juśkiewicz J, Zduńczyk Z. An anthocyanin-rich extract from Kamchatka honeysuckle increases enzymatic activity within the gut and ameliorates abnormal lipid and glucose metabolism in rats. Nutrition 2013;29:898-902.
13. Park SI, Lee YJ, Choi SH, Park SJ, Song CH, Ku SK. Therapeutic effects of blue honeysuckle on lesions of hyperthyroidism in rats. Am J Chin Med 2016;44:1441-56.
14. Chen L, Xin X, Yuan Q, Su D, Liu W. Phytochemical properties and antioxidant capacities of various colored berries. J Sci Food Agric 2014;94:180-8.
15. Svobodová A, Rambousková J, Walterová D, Vostálová J. Protective effects of phenolic fraction of blue honeysuckle fruits against UVA-induced damage to human keratinocytes. Arch Dermatol Res 2008;300:225-33.
16. Vostálová J, Galandáková A, Palíková I, Ulrichová J, Doležal D, Lichnovská R, Vrbková J, Rajnochová Svobodová A. *Lonicera caerulea* fruits reduce UVA-induced damage in hairless mice. J Photochem Photobiol B 2013;128:1-11.
17. Choi JY, Kwun MJ, Kim KH, Lyu JH, Han CW, Jeong HS, Ha KT, Jung HJ, Lee BJ, Sadikot RT, Christman JW, Jung SK, Joo M. Protective effect of the fruit hull of *Gleditsia sinensis* on LPS-induced acute lung injury is associated with *Nrf2* activation. Evid Based Complement Alternat Med 2012;2012:974713.
18. Kim J, Cha YN, Surh YJ. A protective role of nuclear factor-erythroid 2-related factor-2 (*Nrf2*) in inflammatory disorders. Mutat Res 2010;690:12-23.
19. Wu PS, Ding HY, Yen JH, Chen SF, Lee KH, Wu MJ. Anti-inflammatory activity of 8-hydroxydaidzein in LPS-stimulated BV2 microglial cells via activation of *Nrf2*-antioxidant and attenuation of Akt/NF-κB-inflammatory signaling pathways, as well as inhibition of COX-2 activity. J Agric Food Chem 2018;66:5790-801.
20. Bueno-Silva B, Kawamoto D, Ando-Suguimoto ES, Casarin RC, Alencar SM, Rosalen PL, Mayer MP. Brazilian red propolis effects on peritoneal macrophage activity: nitric oxide, cell viability, pro-inflammatory cytokines and gene expression. J Ethnopharmacol 2017;207:100-7.
21. Kim DO, Chun OK, Kim YJ, Moon HY, Lee CY. Quantification of polyphenolics and their antioxidant capacity in fresh plums. J Agric Food Chem 2003;51:6509-15.
22. Abdallah H, Farag M, Osman S, Kim DH, Kang K, Pan CH, Abdel-Sattar E. Isolation of major phenolics from *Launaea spinosa* and their protective effect on HepG2 cells damaged with t-BHP. Pharm Biol 2016;54:536-41.
23. Choi KH, Oh HJ, Jeong YJ, Lim EJ, Han SJ, Kim JH, Kim OY, Lee HS. Physico-chemical analysis and antioxidant activities of Korea *Aronia melanocarpa*. J Korean Soc Food Sci Nutr 2015;44:1165-71.
24. Hong SB, Lee SJ, Kim YH, Hwang YS, Yoon KH, Lee SI, Nam MY, Song LS, Choung MG. Variation of anthocyanin, and isoflavone contents in Korean black soybeans grown at different latitudinal locations. Korean J Environ Agric 2010;29:129-37.
25. Surh YJ. Cancer chemoprevention with dietary phytochemicals. Nat Rev Cancer 2003;3:768-80.
26. Kensler TW, Wakabayashi N, Biswal S. Cell survival responses to

environmental stresses via the Keap1-*Nrf2*-ARE pathway. Annu Rev Pharmacol Toxicol 2007;47:89-116.
27. Zhang H, Forman HJ. Acrolein induces heme oxygenase-1 through PKC-δ and PI3K in human bronchial epithelial cells. Am J Respir Cell Mol Biol 2008;38:483-90.
28. Apopa PL, He X, Ma Q. Phosphorylation of *Nrf2* in the transcription activation domain by casein kinase 2 (CK2) is critical for the nuclear translocation and transcription activation function of *Nrf2* in IMR-32 neuroblastoma cells. J Biochem Mol Toxicol 2008;22:63-76.
29. Rushworth SA, Ogborne RM, Charalambos CA, O'Connell MA. Role of protein kinase C δ in curcumin-induced antioxidant response element-mediated gene expression in human monocytes. Biochem Biophys Res Commun 2006;341:1007-16.
30. Zimmermann K, Baldinger J, Mayerhofer B, Atanasov AG, Dirsch VM, Heiss EH. Activated AMPK boosts the *Nrf2/HO-1* signaling axis--A role for the unfolded protein response. Free Radic Biol Med 2015;88:417-26.
31. Wang X, Liu J, Zhang X, Zhao S, Zou K, Xie J, Wang X, Liu C, Wang J, Wang Y. Seabuckthorn berry polysaccharide extracts protect against acetaminophen induced hepatotoxicity in mice via activating the Nrf-2/*HO-1*-SOD-2 signaling pathway. Phytomedicine 2018;38:90-7.
32. Dinkova-Kostova AT, Holtzclaw WD, Cole RN, Itoh K, Wakabayashi N, Katoh Y, Yamamoto M, Talalay P. Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. Proc Natl Acad Sci U S A 2002;99:11908-13.
33. Lu SC. Regulation of glutathione synthesis. Mol Aspects Med 2009;30:42-59.
34. Gozzelino R, Jeney V, Soares MP. Mechanisms of cell protection by heme oxygenase-1. Annu Rev Pharmacol Toxicol 2010;50:323-54.
35. Poss KD, Tonegawa S. Reduced stress defense in heme oxygenase 1-deficient cells. Proc Natl Acad Sci U S A 1997;94:10925-30.
36. Yet SF, Layne MD, Liu X, Chen YH, Ith B, Sibinga NE, Perrella MA. Absence of heme oxygenase-1 exacerbates atherosclerotic lesion formation and vascular remodeling. FASEB J 2003;17:1759-61.
37. Franklin CC, Backos DS, Mohar I, White CC, Forman HJ, Kavanagh TJ. Structure, function, and post-translational regulation of the catalytic and modifier subunits of glutamate cysteine ligase. Mol Aspects Med 2009;30:86-98.
38. Ross D, Kepa JK, Winski SL, Beall HD, Anwar A, Siegel D. NAD(P)H:quinone oxidoreductase 1 (*NQO1*): chemoprotection, bioactivation, gene regulation and genetic polymorphisms. Chem Biol Interact 2000;129:77-97.
39. Somani SM, Husain K, Diaz-Phillips L, Lanzotti DJ, Kareti KR, Trammell GL. Interaction of exercise and ethanol on antioxidant enzymes in brain regions of the rat. Alcohol 1996;13:603-10.
40. Su D, Zhang R, Zhang C, Huang F, Xiao J, Deng Y, Wei Z, Zhang Y, Chi J, Zhang M. Phenolic-rich lychee (*Litchi chinensis* Sonn.) pulp extracts offer hepatoprotection against restraint stress-induced liver injury in mice by modulating mitochondrial dysfunction. Food Funct 2016;7:508-15.