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Effect of hop mixture containing xanthohumol on sleep enhancement in a mouse model and ROS scavenging effect in oxidative stress-induced HT22 cells

Byungjick MIN[1,2#], Chun Woong PARK[1,3#], Yeljin AHN[1], Ki-Bae HONG[4], Hyeok-Jun CHO[2], Jang Hyun LEE[2], Kyungae JO[1*], Hyung Joo SUH[1,3*]

Abstract

Hops (*Humulus lupulus* L. [Cannabaceae]), used to increase storage and palatability of food, exert various pharmacological effects, including sleep-promoting and antioxidant effects. Here, we evaluated the effects of Saaz, Saphir, Simcoe, and their mixtures on pentobarbital-induced sleep in mice and reactive oxygen species (ROS) production in H_2O_2-treated HT22 cells. Compared to administration of Saaz, Simcoe, and Saphir alone, the administration of Saaz/Saphir (75:25) and Simcoe/Saphir (50:50) mixtures resulted in slightly longer sleep durations in pentobarbital-administered mice. The Saaz/Saphir (75:25) group showed a longer sleep duration in the caffeine-induced insomnia model and slightly lower IC_{50} values for radicals than the Simcoe/Saphir (50:50) group. Xanthohumol present in hop mixtures suppressed ROS production and increased the expression of superoxide dismutase-1, catalase, and glutathione peroxidase-1 in H_2O_2-treated HT22 cells. Collectively, Simcoe/Saphir (50:50) and Saaz/Saphir (75:25) mixtures were more effective in removing ROS in brain cells and promoting sleep than hops alone.

Keywords: hop mixture; sleep; ROS; HT22 cell; mouse.

Practical Application: Consider the ROS scavenging effect of the hop mixture and its functional quality on sleep enhancement.

1 Introduction

Sleep is an important factor in maintaining homeostasis in the body and regulating the central and autonomic nervous systems. Sleep disorders, such as sleep deprivation, act as stressors and affect physical activities and brain functions (Grimaldi et al., 2021). Active metabolic activity during waking requires high oxygen consumption, which generates a large amount of free radicals (Maniaci et al., 2021; Prabhakar et al., 2020); these free radicals are removed by sleep. As nerve cells are extremely sensitive to the environment, waste products from nerve metabolism must be quickly and efficiently removed from the brain's intercellular space. Levels of certain oxidative stress biomarkers are increased in subjects with insomnia (Belcaro et al., 2018; Gulec et al., 2012) and sleep deprivation (Trivedi et al., 2017).

Hops (*Humulus lupulus* L. [Cannabaceae]) have been used to treat insomnia since the 19th century and as a medicinal plant for over 2000 years (Koetter & Blendl, 2010). The combination of hops and valerian (*Valeriana officinalis*) is the most frequently administered form of herbal-based hypnotics and sedatives (Dimpfel & Suter, 2008). We previously demonstrated that a mixture of valerian and Cascade hops increases non-rapid eye movement sleep due to an increase in delta waves via $GABA_A$ receptors (Choi et al., 2018). Additionally, hops are used as anti-inflammatory, antiseptic, antidiuretic, sedative, and topical skin ulcer treatments (Olšovská et al., 2016; Zanoli & Zavatti, 2008). Hops exhibiting these physiological activities are rich in phenolic compounds, such as phenolic acids, flavonoids, proanthocyanidins, prenylated chalcones, and catechins (Taylor et al., 2003).

Depending on the variety, hops have a unique taste and flavour, because of which they are used for brewing, and different components, such as flavonoids, humulones (α-acids), and lupulones (β-acids), which can remove ROS (Van Cleemput et al., 2009).

To treat sleep disorders, a compound with sleep-promoting and ROS-scavenging activities is desirable. Hops, which have been used to treat sleep disorders, contain various flavonoids; thus, they may be effective in removing ROS generated during sleep disorders. In this study, the optimal mixing ratios of Simcoe/Saphir and Saaz/Saphir mixtures for sleep enhancement and ROS-scavenging ability were determined.

2 Materials and methods

2.1 Animals

ICR male mice (20 ± 2 g; Orient Bio Inc., Seongnam, Korea) were housed at 22 ± 2 °C, relative humidity of 55 ± 5%, ventilation frequency of 10 to 12 h, and a 12 h light-dark cycle. All animal procedures were approved by the Korean University Institutional Animal Care and Use Committee (KUIACUC-2021-0020).

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[1] *Department of Integrated Biomedical and Life Science, Graduate School, Korea University, Seoul, Republic of Korea*
[2] *Lotte R&D Center, Seoul, Korea*
[3] *BK21FOUR R&E Center for Learning Health Systems, Korea University, Seoul, Republic of Korea*
[4] *Department of Food Science and Nutrition, Jeju National University, Jeju, Republic of Korea*
*Corresponding author: kyungae11@korea.ac.kr; suh1960@korea.ac.kr
#These authors contributed equally to this work

2.2 Hop extraction

For ethanolic hop extraction, 25 times 70% ethanol was added to 20 g of hops, and reflux extraction was performed twice at 95 °C for 1 h. The extract was filtered (Whatman No. 1), concentrated under reduced pressure, and lyophilised for further experiments.

2.3 Pentobarbital-induced sleep test

Mice were fasted for 24 h and hop extract (100 or 150 mg/kg) was orally administered. After 40 min, pentobarbital (42 mg/kg) was injected intraperitoneally, and sleep induction time and sleep duration were measured. The sleep induction time was defined as the time from pentobarbital administration to the disappearance of the directional reflex, and sleep duration was defined as the recovery time from the loss of the directional reflex. Animals that did not sleep within 15 min after pentobarbital administration were excluded from the experiment. There were seven mice per group.

To evaluate the sleep-enhancing effects of hops mixtures, we established a model of insomnia mice with oral administration of caffeine. The caffeine control group was orally administered with caffeine (40 mg/kg) only, and the hop mixture treated group was orally administered with the sample and caffeine. After that, a pentobarbital-induced sleep test was performed in the same manner as described above.

2.4 ABTS and DPPH radical-scavenging activities

The 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, St Louis, MO, USA) radical-scavenging ability of hop extracts was analysed as described previously Kim et al. (2002) with some modifications. The extract (100 μL) was added to a 0.1 mM DPPH solution dissolved in ethanol and stored for 30 min in the dark. Absorbance at 520 nm was measured using a microplate reader.

The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, Sigma-Aldrich Co.) radical-scavenging ability of the extracts was determined as described previously Re et al. (1999). The radical-scavenging activity was expressed as the IC_{50} value of the sample, which shows a 50% decrease in absorbance compared to that of the control.

2.5 Mouse hippocampal HT22 cell culture

Mouse hippocampus-derived HT22 cells (Korea Cell Line Bank, Seoul, Korea) were cultured in Dulbecco's modified Eagle's medium (Welgene, Daegu, Korea) containing 10% foetal bovine serum (Welgene), penicillin (100 IU/mL, Welgene), and streptomycin (100 μg/mL, Welgene) at 37 °C in a 5% CO_2 incubator.

2.6 HT22 cell viability and mRNA expression of antioxidant enzymes

HT22 cells were cultured in 96-well plates (5×10^4 cells/well) for 24 h at 37 °C in a 5% CO_2 incubator and then treated with hop extracts and hydrogen peroxide (H_2O_2; 300 μM) for 12 h. Cytotoxicity was assessed using a WST-8 cell viability assay kit according to the manufacturer's protocol (Biomax, Seoul, Korea).

The effect of hop extracts on the mRNA expression of superoxide dismutase-1 (SOD-1; NM_011434.1), catalase (CAT; NM_009804.2), and glutathione peroxidase-1 (GPx-1; NM_008160.6) was analysed by quantitative real-time polymerase chain reaction (qRT-PCR). cDNA was prepared using Superscript III reverse transcriptase (Invitrogen). mRNA expression was analysed using the double-stranded DNA dye (SYBR® green, Applied Biosystems, Foster City, CA, USA) and StepOne Plus Real-time PCR system (Applied Biosystems) as previously described (Jo et al., 2020). GAPDH (NM_008084.3) was used as the housekeeping gene.

2.7 Statistical analysis

Data are expressed as mean ± standard deviation. Statistical analysis was performed using SPSS v21.0 (SPSS Inc., Chicago, IL, USA). The significance of data was compared by one-way analysis of variance followed by the Tukey's multiple comparison test. P values < 0.05 were considered statistically significant.

3 Results and discussion

3.1 Effect of different mixing ratios of Simcoe/Saphir and Saaz/Saphir mixtures on sleep latency and duration

Hops are perennial plants used for extending shelf life and imparting bitterness in the brewing industry and for medicinal purposes. Hops are known to act on the central nervous system and relieve anxiety, excitement, and insomnia (Kyrou et al., 2017; Zanoli et al., 2005). Moreover, flavonoids contained in hops promote sleep (Min et al., 2021). Flavonoids obtained from natural products are exogenous antioxidants; they are considered neuroprotective agents because of their involvement in neurogenesis and nerve regeneration (Dias et al., 2012). The derivatives of the flavonoid xanthohumol present in hops have a prenylated chalcone structure and exist in large amounts, accounting for 0.1 to 1% of hops (Lee et al., 2010). Xanthohumol derivatives exert oestrogen-like and antibacterial effects (Gerhauser et al., 2002; Piersen, 2003), reduce oxidative damage, and protect the nervous system (Dostálek et al., 2017; Yao et al., 2015). Xanthohumol alleviates acute ethanol-induced brain oxidative damage by reducing ROS levels in the brain (Pinto et al., 2014). Saaz, Saphir, and Simcoe used in this study contained 1.94–4.23 μg/mg of xanthohumol (Table 1). Hop flavonoids exhibit a sedative effect by increasing the activity of γ-aminobutyric acid (GABA), a neurotransmitter that suppresses the central nervous system (Franco et al., 2012), and xanthohumol acts on GABA receptors to promote sleep (Min et al., 2021). The xanthohumol content varies depending on the hop variety, explaining the difference in antioxidant and sleep-promoting effects. Among the hop varieties available, Saaz, Saphir, and Simcoe, which are estimated to exhibit excellent sleep-promoting activity through a preliminary experiment, were mixed to evaluate their sleep-promoting activity. Among the groups treated with Saaz, Saphir, and Simcoe alone, sleep latency decreased and sleep duration time increased when Saphir was treated compared to the normal group. Therefore, the sleep promoting activity of each ratio of hop mixture including Saphir was confirmed (Figure 1).

Table 1. α-acid, β-acid, xanthohumol, polyphenol, and flavonoid contents and yield of hops and hop mixtures.

Variety	α-acid (μg/mg)		β-acid (μg/mg)		Xanthohumol	Polyphenols	Flavonoids
	Cohumulone	Adhumulone	Colupulone	Adlupulone	(μg/mg)	(μg/mg)	(μg/mg)
Saaz	5.41 ± 0.01	17.91 ± 0.05	20.68 ± 0.06	28.83 ± 0.53	2.51 ± 0.13	99.70 ± 2.78	13.08 ± 0.12
Saphir	3.14 ± 0.09	27.67 ± 0.24	27.13 ± 0.07	39.24 ± 0.10	4.23 ± 0.14	117.19 ± 4.39	15.88 ± 0.28
Simcoe	21.24 ± 0.23	96.96 ± 0.99	12.64 ± 0.08	19.13 ± 0.11	1.94 ± 0.06	83.09 ± 3.42	5.94 ± 0.04
Saz-Sap	4.82 ± 0.03	20.35 ± 0.098	22.30 ± 0.063	31.43 ± 0.42	2.94 ± 0.13	104.07 ± 3.18	13.78 ± 0.16
Sim-Sap	12.19 ± 0.16	62.19 ± 0.62	19.89 ± 0.075	29.19 ± 0.11	3.09 ± 0.10	100.14 ± 3.91	10.91 ± 0.163.92

Data are presented as the mean ± standard deviation (SD). Saz/Sap, Saaz/Saphir (75:25) mixture; Sim/Sap, Simcoe/Saphir (50:50) mixture.

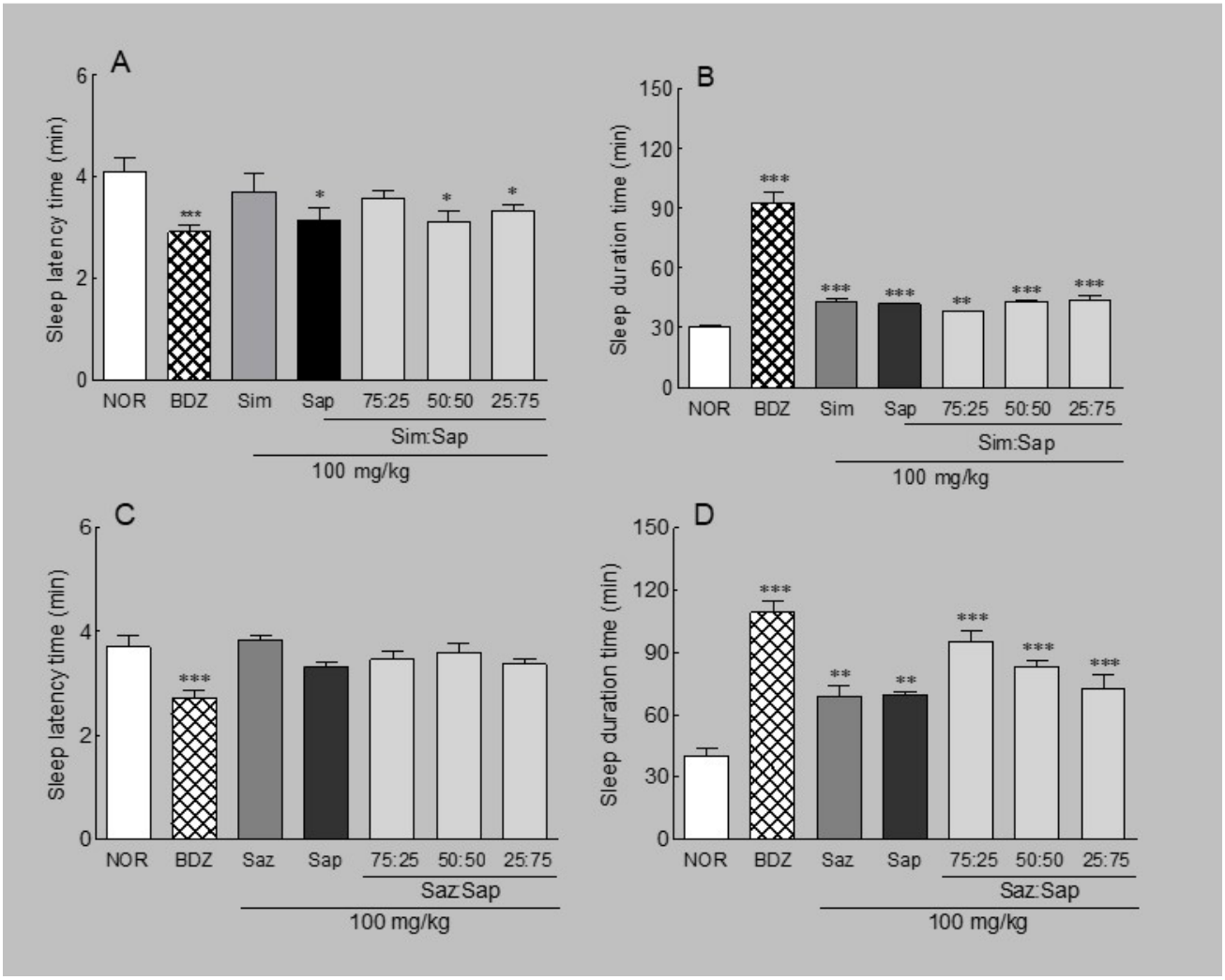

Figure 1. Effect of Sim/Sap and Saz/Sap mixtures on (A, B) sleep latency time and (C, D) sleep duration time in mice administered a hypnotic dose of pentobarbital (42 mg/kg, intraperitoneally). NOR, 0.9% NaCl (physiological saline)-administered group (normal control); BDZ, benzodiazepine-administered group (positive control, 200 μg/kg). Hops (Simcoe, Saphir, and Saaz) and their mixtures (Simcoe/Saphir and Saaz/Saphir) were orally administered at 100 mg/kg. Data are presented as the mean ± standard error of the mean (SEM) for each group (n=7). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs. normal group by Tukey's multiple comparison test. Sim, Simcoe; Sap, Saphir; Saz, Saaz; Sim/Sap, Simcoe/Saphir (50:50) mixture; Saz/Sap, Saaz/Saphir (75:25) mixture.

Saphir alone and Simcoe/Saphir mixtures (50:50 and 25:75) significantly reduced sleep latency compared to that in the normal group (Figure 1A; $p < 0.05$). Contrarily, Simcoe/Saphir mixtures (50:50 and 25:75) significantly increased sleep duration compared to that in the normal group (Figure 1B; $p < 0.001$). However, sleep duration was not significantly different between hop-alone and hop mixture treatments.

All Saaz/Saphir mixtures significantly increased sleep duration compared to that in the normal group (Figure 1D; $p < 0.001$). Additionally, Saaz/Saphir mixtures considerably increased sleep

duration compared to Saaz or Saphir alone. Collectively, Simcoe/Saphir (50:50) and Saaz/Saphir (75:25) mixtures showed better sleep-promoting activity than hops alone or other mixing ratios.

3.2 Effect of different concentrations of Simcoe/Saphir and Saaz/Saphir mixtures on sleep latency and duration

Sleep latency was not significantly different between Saaz/Saphir (100 and 150 mg/kg) and Simcoe/Saphir (150 mg/kg) mixture groups and the normal group (Figure 2A). Sleep duration was significantly longer in 100 and 150 mg/kg hop mixture groups ($p < 0.05$ and $p < 0.001$, respectively) than in the normal group (Figure 2B). The sleep duration increased in a dose-dependent manner. Taken together, the Saaz/Saphir mixture was more effective in increasing sleep duration than the Simcoe/Saphir mixture.

3.3 Effect of the Saaz/Saphir mixture on sleep latency and duration in the caffeine-induced insomnia model

The sleep latency and duration in the caffeine-induced insomnia model treated with the Saaz/Saphir mixture were measured (Figure 3). Sleep latency and duration were significantly different between the normal group (administered physiological saline) and control group (administered caffeine) ($p < 0.01$ and $p < 0.05$, respectively), indicating successful development of the caffeine-induced insomnia model. In the caffeine-induced insomnia model, sleep latency of the 150 mg/kg Saaz/Saphir and Simcoe/Saphir mixture groups was similar. However, the Simcoe/Saphir mixture group had a shorter sleep duration than the Saaz/Saphir mixture group. Thus, the Saaz/Saphir mixture may exert greater sleep-promoting effect than the Simcoe/Saphir mixture.

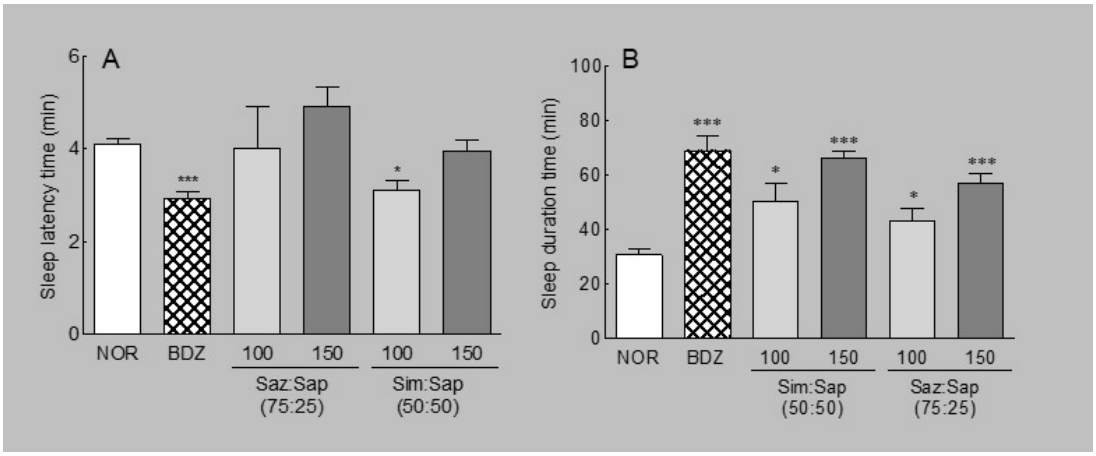

Figure 2. Effect of Sim/Sap and Saz/Sap mixtures on (A) sleep latency time and (B) sleep duration time in mice administered a hypnotic dose of pentobarbital (42 mg/kg, intraperitoneally). NOR, 0.9% NaCl (physiological saline)-administered group (normal control); BDZ, benzodiazepine-administered group (positive control, 200 μg/kg); Sim/Sap, Simcoe/Saphir (50:50; 100 and 150 mg/kg) mixture; Saz/Sap, Saaz/Saphir (75:25; 100 and 150 mg/kg) mixture. Data are presented as the mean ± standard error of the mean (SEM) for each group (n=7). $^{*}p < 0.05$ and $^{***}p < 0.001$ vs. normal group.

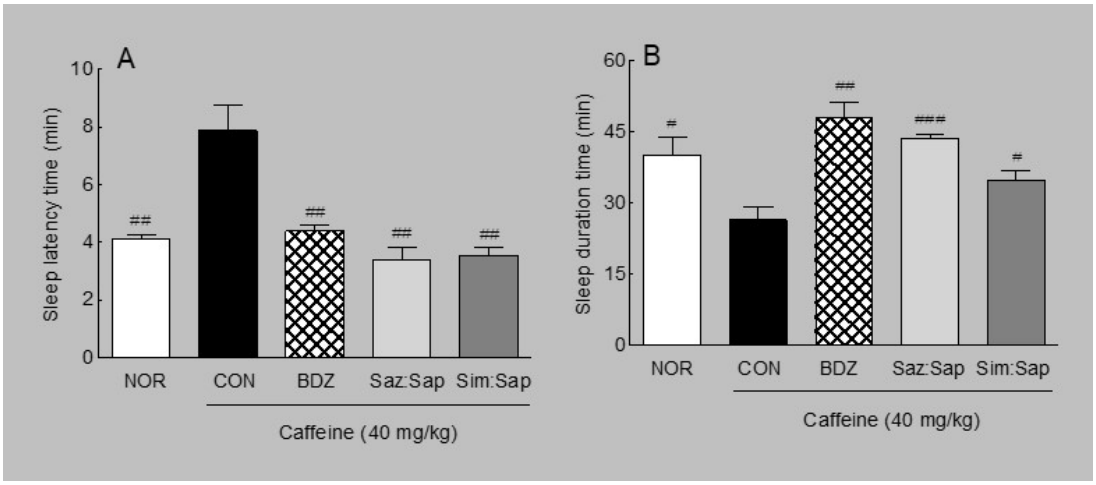

Figure 3. Effect of the Saz/Sap and Sim/Sap mixtures on (A) sleep latency time and (B) sleep duration time in the caffeine-induced insomnia mice model. NOR, 0.9% NaCl (physiological saline)-administered group (normal control); CON, 40 mg/kg caffeine-administered group; BDZ, 200 μg/kg benzodiazepine and 40 mg/kg caffeine-administered group (positive control); Saz/Sap, oral administration of the Saaz/Saphir (75:25; 150 mg/kg) mixture and 40 mg/kg caffeine; Sim/Sap, oral administration of the Simcoe/Saphir (50:50; 150 mg/kg) mixture and 40 mg/kg caffeine. Data are presented as the mean ± standard error of the mean (SEM) for each group (n = 7). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ vs. control group.

The Saaz/Saphir mixture exerted greater sleep-promoting effects than the Simcoe/Saphir mixture in the caffeine-induced insomnia model (Figure 3); therefore, the Saaz/Saphir mixtures seems to be more suitable for promoting sleep. In a previous study, xanthohumol contained in hops was identified as an active substance exhibiting sleep-promoting activity (Choi et al., 2017; Min et al., 2021). Many studies also report xanthohumol as a sleep-promoting substance in hops (Benkherouf et al., 2019; Meissner & Häberlein, 2006). The focus has been on the action of prenylflavonoids (xanthohumol and its derivatives), which were considered important active substances. Although there are few reports of sleep-promoting activity of humulone, further studies are needed because of conflicting reports on sleep-promoting activity of alpha acids and beta acids (humulone and lupulone) (Benkherouf et al., 2020; Zanoli et al., 2005; Zanoli et al., 2007).

3.4 ABTS and DPPH radical-scavenging effects of hop mixtures

IC_{50} values for DPPH and ABTS radical scavenging in Saaz/Saphir and Simcoe/Saphir mixture groups were evaluated (Figure 4). The ABTS radical-scavenging effect of the Saaz/Saphir mixture was significantly different from that of Simcoe alone ($p < 0.001$), but not other hops or mixtures. The IC_{50} values were significantly higher in the Simcoe/Saphir mixture group than in Saaz, Saphir, and Saaz/Saphir mixture groups. The IC_{50} value for DPPH radical was significantly lower in the Saaz/Saphir mixture group than in the Saaz group, but not the Saphir group. IC_{50} values were significantly higher in the Simcoe/Saphir mixture group than in the Saaz/Saphir mixture group, but not Saaz and Saphir groups. Collectively, the Saaz/Saphir mixture exhibited greater radical-scavenging effects than the Simcoe/Saphir mixture, and Saphir seemed to play a role in enhancing radical-scavenging effects of the hop mixture. Alpha-acids and beta-acids, known as other active substances of hop, have been reported to have relatively lower ROS activity than xanthohumol. Although there was a difference in the content of active ingredients contained in the extract, beta-acids reported higher ROS scavenging activity than alpha-acids (van Hoyweghen et al., 2010). The ROS scavenging ability of Simcoe, which had a slightly higher alpha acid content compared to the other two varieties, was lower than that of the other varieties. As such, xanthohumol seems to play a major role not only in sleep promoting activity but also in ROS scavenging activity.

Natural materials for sleep promotion can increase sleep duration and remove ROS generated during insomnia. During a person's daily life, ROS accumulate in the brain, and sleep increases the antioxidant activity to facilitate ROS removal (Villafuerte et al., 2015). However, animal studies revealed that sleep deprivation owing to sleep disturbance suppresses ROS removal (Besedovsky et al., 2019). In a previous study, it was confirmed that the non-rapid eye movement time, which is deep sleep, decreased and H_2O_2 and malondialdehyde in the brain increased in a rat model that caused sleep deprivation through physical stress. The sleep disorder induced by oxidative stress was improved by treating ethanol extract of green lettuce leaves, one of the natural materials with antioxidant activity (Jo et al., 2021). Ohayon & Partinen (2002) and Doghramji (2006) reported that 30-35% of the total population has transient sleep disturbance, suggesting that sleep disturbance is involved in neurodegeneration and neurological progression. ROS accumulated in the brain are considered a major risk factor for exacerbating the damage caused by cerebral ischaemic disease (Olmez & Ozyurt, 2012). Among ROS, H_2O_2 is a free radical containing superoxide (O_2^-) and hydroxyl radical (OH^-) and a risk factor for various diseases related to the cardiovascular system and aging (Phaniendra et al., 2015). Oxidative damage caused by free radicals in cranial nerve cells causes neurological diseases, such as senile dementia and stroke (Uttara et al., 2009). Therefore, the ROS-scavenging activity of hops is as important as its sleep-promoting activity. The ROS-scavenging activity of hop extracts might be owing to the presence of phenolic substances. Isoquercitrin and quercetin contained in hops are major flavonoids with high antioxidant activity (Karabin et al., 2015; Schroeter et al., 2002). Xanthohumol inhibits the formation of superoxide anion radicals and nitric oxide, protects against oxidative damage in PC12 cells (Pinto et al., 2014), and exhibits ROS-scavenging activity (Zhang et al., 2014).

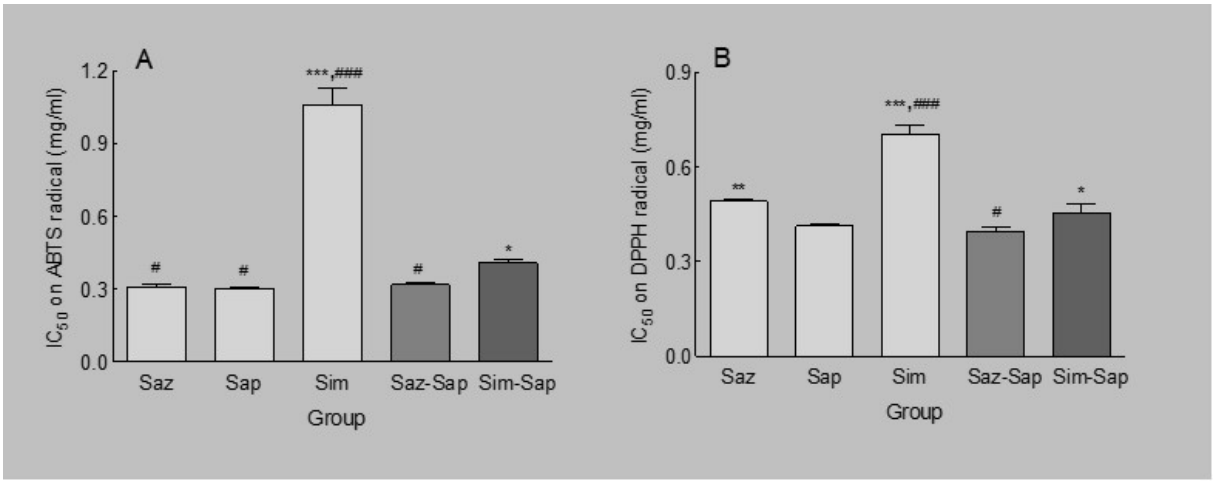

Figure 4. Scavenging effects of Sim/Sap (75:25) and Saz/Sap (50:50) mixtures on (A) ABTS and (B) DPPH radicals. Data are presented as the mean ± standard deviation (SD). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs. Saz/Sap and $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ vs. Sim/Sap. Saz, Saaz; Sap, Saphir; Sim, Simcoe; Saz/Sap, Saaz/Saphir (75:25) mixture; Sim/Sap, Simcoe/Saphir (50:50) mixture.

In this study, the Saaz/Saphir mixture showed greater ABTS and DPPH scavenging effects than the Simcoe/Saphir mixture owing to the addition of Saphir; Saphir had the highest content of flavonoids, including xanthohumol (Figure 4 and Table 1). The slightly lower DPPH scavenging activity than ABTS scavenging activity was probably because of the xanthohumol in the hops. Xanthohumol inhibits low-density lipoprotein oxidation (Dostálek et al., 2017), scavenges hydroxyl and peroxyl radicals, and inhibits superoxide anion and nitric oxide production (Zhang et al., 2014). However, xanthohumol has a low DPPH scavenging activity (Zhang et al., 2014).

3.5 Effect of hop mixtures on ROS production in H_2O_2-treated HT22 cells

The viability of hop-treated HT22 cells was examined (Figure S1); Saaz and Simcoe at 600 μg/mL were not cytotoxic, whereas Saphir at a concentration of 200 μg/mL and higher was cytotoxic (data not shown). The viability of 300 μM H_2O_2-treated HT22 cells was 58.9%. Treatment with 100 μg/mL of hops increased the cell viability to approximately 90%, whereas hop mixtures increased it to 96.53% (Saaz/Saphir) and 97.61% (Simcoe/Saphir). Hops inhibited H_2O_2-mediated cell damage; however, hop mixtures exerted a greater inhibitory effect on cell damage than hops. Furthermore, viability of 0.6 μg/mL xanthohumol-treated cells was 93.50%, suggesting that xanthohumol had an inhibitory effect on H_2O_2-induced cell damage.

Furthermore, ROS production was increased in H_2O_2-treated HT22 cells, but hops significantly reduced it when compared to that in the control group (Figure 5A; $p < 0.001$). Saaz/Saphir (75:25) and Saphir/Simcoe (50:50) mixtures also significantly inhibited H_2O_2-mediated ROS production in HT22 cells compared to that in the control group ($p < 0.001$); ROS levels in the mixture groups were similar to those in the normal group (without H_2O_2 treatment; Figure 5A). Similarly, xanthohumol, the active compound in hops, significantly inhibited H_2O_2-mediated ROS production in HT22 cells ($p < 0.001$); ROS levels were similar to those in the normal group (Figure 5B). Taken together, Saaz, Saphir, Simcoe, Saaz/Saphir, Saphir/Simcoe, and xanthohumol inhibited H_2O_2-mediated ROS generation.

Here, hops exerted inhibitory effects on ROS production and neuroprotective effects in HT22 cells with H_2O_2-induced oxidative damage (Figure 5 and Figure S1). Under normal physiological conditions, there is a balance between ROS generation and endogenous antioxidant activity to protect tissues from oxidative damage. The endogenous antioxidant mechanisms that suppress ROS generation or remove free radicals include antioxidant enzymes (SOD, CAT, GPx, glutathione reductase [GR]) and non-enzymatic antioxidants (glutathione, ascorbic acid, sulfhydryl groups, vitamin A, vitamin E, etc.) (Irato & Santovito, 2021).

3.6 Effect of hop mixtures on the expression of antioxidant enzymes in H_2O_2-treated HT22 cells

The expression levels of SOD-1, GPx-1, and CAT, enzymes that inhibit H_2O_2-induced ROS production, were analysed (Figure 6). SOD-1, GPx-1, and CAT expression was significantly lower in H_2O_2-treated HT22 cells than in normal cells ($p < 0.001$) but higher in Saphir and Simcoe groups than in the control group ($p < 0.01$ and $p < 0.001$, respectively). There was a significant difference in GPx-1 and CAT expression ($p < 0.001$), but not SOD1 expression, between Saaz and control groups. SOD-1, GPx-1, and CAT expression was significantly higher in Saaz/Saphir and Simcoe/Saphir mixture groups than in the control group. These mixtures increased GPx-1 and CAT expression compared to Saaz, Saphir, and Simcoe. When H_2O_2-treated HT22 cells were treated with 0.6 and 0.8 μg/mL of xanthohumol, the expression of these enzymes significantly increased compared to that in the control group (Figure 6; $p < 0.001$).

As Saaz/Saphir and Saphir/Simcoe mixtures significantly increased GPX1 and CAT expression compared to hop extracts,

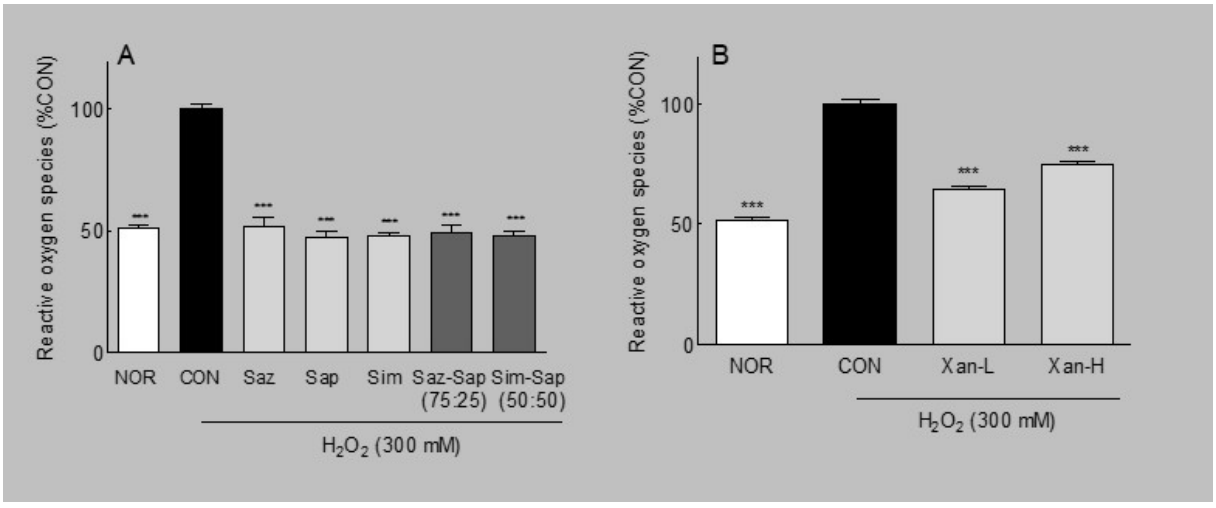

Figure 5. Effect of (A) hops and hop mixtures and (B) xanthohumol on ROS production in H_2O_2-treated HT22 cells. HT22 cells were treated with 100 μg/mL of hops (Saaz, Saphir, and Simcoe), their mixtures (Saaz/Saphir and Simcoe/Saphir), and 0.6 (Xan-L) and 0.8 μg/mL (Xan-H) of xanthohumol. Data are presented as the mean ± standard deviation (SD) for each group. $^{***}p < 0.001$ vs. control group. NOR, normal; CON, control; Saz, Saaz; Sap, Saphir; Sim, Simcoe; Saz/Sap, Saaz/Saphir (75:25) mixture; Sim/Sap, Simcoe/Saphir (50:50) mixture.

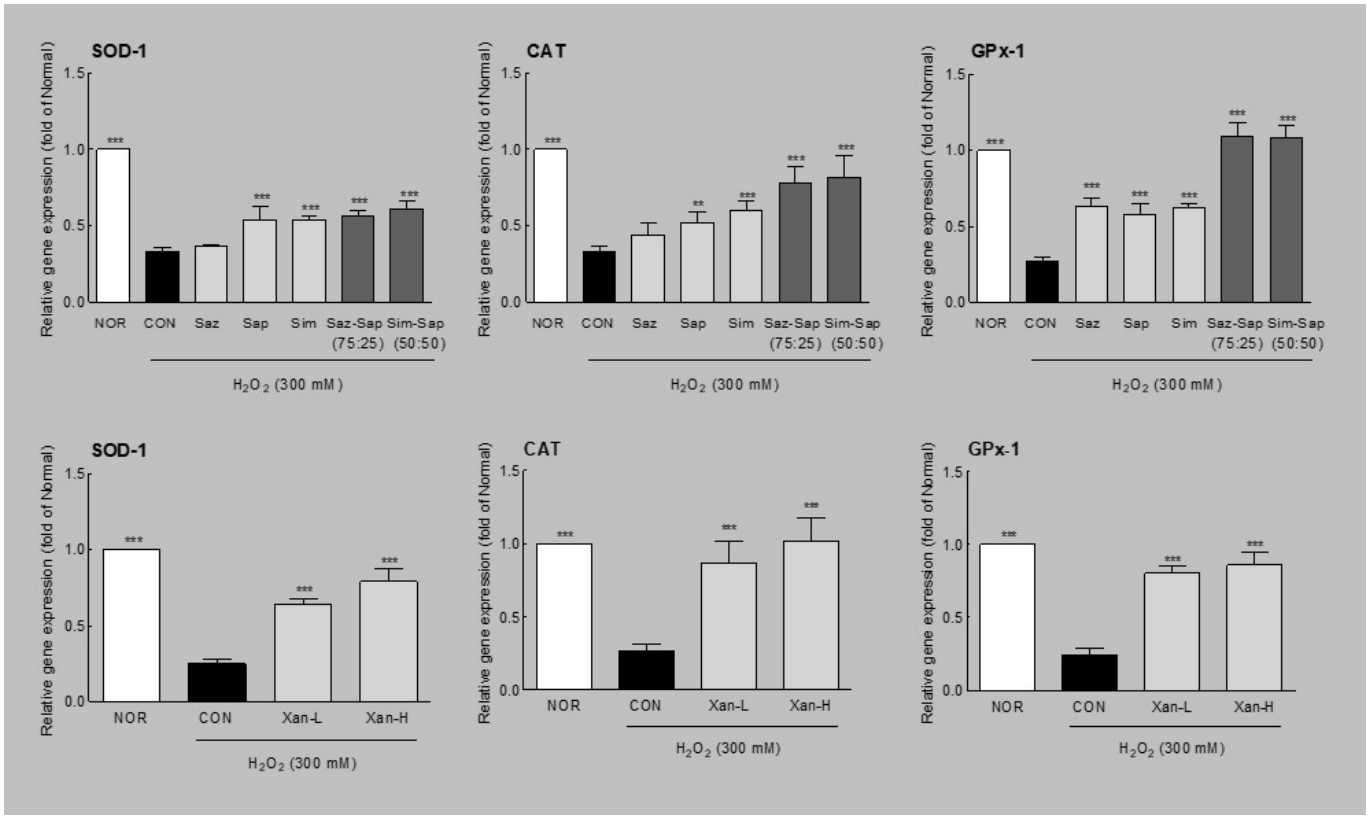

Figure 6. Effect of hop mixtures on the expression of antioxidant enzymes in H2O2-treated HT22 cells. HT22 cells were treated with 100 μg/mL of hops (Saaz, Saphir, and Simcoe), hop mixtures (Saaz/Saphir and Simcoe/Saphir), and 0.6 (Xan-L) and 0.8 μg/mL (Xan-H) of xanthohumol. Data are presented as the mean ± standard deviation (SD) for each group. $^{**}p < 0.01$ and $^{***}p < 0.001$ vs. control group. NOR, normal; CON, control; Xan, xanthohumol; SOD-1, superoxide dismutase-1; CAT, catalase; GPx-1, glutathione peroxidase-1; Saz, Saaz; Sap, Saphir; Sim, Simcoe; Saz/Sap, Saaz/Saphir (75:25) mixture; Sim/Sap, Simcoe/Saphir (50:50) mixture.

hop mixtures would be more effective in protecting HT22 cells from ROS than the hops alone. This effect is likely because of xanthohumol present in the hops.

Antioxidant enzymes act as antioxidants by inactivating or removing ROS, which can cause lipid peroxidation-mediated damage to the cell membranes, inactivation of sulfhydryl-containing enzymes, and cross-linking of constituent proteins (Juan et al., 2021). The enzymatic antioxidant system plays a major role in ROS removal in cells. The neuroprotective effect and the inhibitory effect on ROS production may be via an increase in SOD1, GPx1, and CAT expression in HT22 cells and hop flavonoids, including xanthohumol (Figure 6).

4 Conclusions

In conclusion, the sleep-promoting activity of hop mixtures was similar, but the sleep duration was increased when Saaz and Saphir were mixed at 75:25 and Simcoe and Saphir at 50:50 ratios. Among the three hop varieties, the radical-scavenging effect of Saphir was slightly better, and this contributed to enhancing the radical-scavenging effect of the mixture. As Saaz/Saphir and Simcoe/Saphir mixtures exert neuroprotective and radical-scavenging effects on neuronal HT22 cells, they may promote sleep and scavenge radicals during insomnia. In particular, the Saaz/Saphir mixture was more effective in promoting sleep and preventing neuronal damage caused by ROS.

Ethical approval

Animal procedures were approved by the Korean University Institutional Animal Care and Use Committee (KUIACUC-2021-0020).

Conflict of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Availability of data and material

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

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Supplementary material

Supplementary material accompanies this paper.

Figure S1. Viability of HT22 cells treated with or without H2O2 and Saaz, Saphir, Simcoe, Simcoe/Saphir (75:25) mixture, and Saaz/Saphir (50:50) mixture. HT22 cells were treated with 300 μM H2O2, 100 μg/mL hops, and/or 0.6 μg/mL xanthohumol. Data are presented as the mean ± standard deviation (SD) for each group. ***$p < 0.001$ vs. control group (with H2O2) and ###$p < 0.001$ vs. control group (without H2O2). CON, control; Saz, Saaz; Sap, Saphir; Sim, Simcoe; XN, xanthohumol.

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