Hepatoprotective and Anti-Hepatitis C Viral Activity of *Platycodon grandiflorum* Extract on Carbon Tetrachloride-Induced Acute Hepatic Injury in Mice

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Summary The present study aims to evaluate the anti-HCV activity of hotwater extract from *Platycodon grandiflorum* (BC703) with HCV genotype 1b subgenomic replicon system and investigate its hepatoprotective activity on carbon tetrachloride (CCl₄)-induced acute liver damage in mice. BC703 produced significant hepatoprotective effects against CCl₄-induced acute hepatic injury by decreasing the activities of serum enzymes, nitric oxide and lipid peroxidation. Histopathological studies further substantiated the protective effect of BC703. Furthermore, BC703 inhibited the HCV RNA replication with an EC₅₀ value and selective index (CC₅₀/EC₅₀) of 2.82 µg/mL and above 35.46, respectively. However, digested BC703 using a simulated gastric juice showed poor protective effect against CCl₄-induced hepatotoxicity in mice and decreased anti-HCV activity as compared to the intact BC703. Although further studies are necessary, BC703 may be a beneficial agent for the management of acute hepatic injury and chronic HCV infection.

Key Words *Platycodon grandiflorum*, carbon tetrachloride, hepatotoxicity, hepatitis C virus

Herbal medicines that have been used in China for thousands of years are now being manufactured in Korea as drugs with standardized quality and quantities of ingredients. The roots of *Platycodon grandiflorum* have been consumed as a foodstuff and as a folk remedy for diseases such as bronchitis, asthma, pulmonary tuberculosis and inflammation (1). It was reported to contain a lot of carbohydrates (at least 90%), protein (2.4%), lipids (0.1%) and ash (1.5%) (1). Recently, it has been reported that the polysaccharides isolated from the root of *Platycodon grandiflorum* prevent obesity, hypercholesterolemia, hypertension, diabetes, immune modulation and hyperlipidemia (2–5). In addition, triterpenoid saponins in the roots of the *Platycodon grandiflorum* are believed to prevent chemical-induced hepatotoxicity (6, 7). In our previous study, the standardized aqueous extract of *Platycodon grandiflorum* was shown to attenuate thioacetamide-induced fulminant hepatic failure in mice (5). Although the beneficial effect of *Platycodon grandiflorum* on acute hepatic failure using various chemical induced hepatic injury models has been widely studied, little is known about the preventa-

tive effect of *Platycodon grandiflorum* decoction on CCl₄-induced acute hepatic injury in mice. In addition, information on its stability in the stomach has not been investigated and this information may be important to contribute to the development of *Platycodon grandiflorum* extract, which can later be directed to consumers with an increased knowledge of the content and biological activity of this medicinal herb.

The liver plays a central role in transforming and clearing xenobiotics, and is susceptible to their toxicity (8). Carbon tetrachloride (CCl₄) has been one of the most intensively studied hepatotoxicants and provides a relevant model for liver damage (8–10). CCl₄ is a potent hepatotoxicant and a single exposure to it can rapidly lead to severe centrilobular necrosis and steatosis (9–12). During its metabolism, an unstable trichloromethyl free radical (CCl₃) is formed and rapidly converted to trichloromethyl peroxide (9–11). These free radical result in the peroxidation of fatty acids found in the phospholipids making up the cell membranes. Lipid peroxide radicals, lipid hydroperoxides and lipid breakdown products develop in this process and each constitutes an active oxidizing agent. Consequently, cell membrane structures and intracellular organelle membrane structures are completely broken down (9, 10, 12).

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Hepatitis C virus (HCV) infection is a major health-care problem around the world (13). HCV infection usually runs a chronic infection in an estimated 70% of HCV patients. Approximately 170 million subjects are chronically infected with HCV (13–15). A chronic HCV infection leads to cirrhosis and hepatocellular carcinoma and causes 300,000 deaths per year (13). Current treatment is based on a combination of pegylated interferon (IFN)- α and ribavirin, which directly inhibits HCV replication and causes progressive infected cell clearance through intricate and only partly understood mechanisms (13–15). Among natural antiviral agents, recent investigations have reconsidered the interest of phyto-polysaccharides, which act as potent inhibitors of different viruses (16–18).

In the present study, the standardized hot water extract from *Platycodon grandiflorum* was tested for its antiviral properties using a cell-based HCV subgenomic replicon system. We also investigated its protective effect on CCl₄-induced acute hepatic injury in mice.

MATERIALS AND METHODS

Chemicals and reagents. Thiobarbituric acid reactive substances (TBARS), glutathione (GSH), and nitric oxide (NO) assay kits were purchased from BioAssay Systems (Hayward, CA, USA). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) test kits were obtained from IDEXX Laboratories (Westbrook, ME, USA). The superoxide dismutase (SOD) assay kit was obtained from Cayman (Ann Arbor, MI, USA). Carbon tetrachloride, Folin-Ciocalteu phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and other chemicals were purchased from Sigma Chemicals (St. Louis, MO, USA). Acetonitrile and methanol as HPLC-grade were obtained from Mallinckrodt Baker (Phillipsburg, NJ, USA).

Preparation of BC703. The standardized *Platycodon grandiflorum* hot water extract (BC703) was manufactured by SkyHerb Pharmaceuticals (Zhejiang, China) according to full good manufacturing practice (GMP). Briefly, the dried roots of *Platycodon grandiflorum* were cut into slices and extracted in distilled water with occasional shaking at 60–90°C for 6–10 h. The aqueous extract was then filtered, concentrated under reduced pressure in a rotary evaporator and spray-dried into powder.

Simulated gastric juices were prepared by suspending pepsin (1 : 10,000, ICN) in sterile saline (0.5% w/v) to a final concentration of 3 g/L and adjusting to pH 2, and BC703 was incubated for 2 h at 37°C for preparation of digested BC703.

HPLC-ELSD analysis. HPLC analysis of BC703 was carried out as previously described (5). In brief, HPLC analysis of BC703 was performed on a Younglin ACME 9000 (Seoul, Korea) equipped with a Sedex 55 evaporative light scattering detector (ELSD; SEDERE, Alfortville, France). Sample separation was achieved in a Gemini C₁₈ column (100 mm×4.6 mm, 3 μ m particle size; Phenomenex, Torrance, CA, USA) with a precolumn (C₁₈, 3.5 μ m, 2×20 mm) at room temperature. The

mobile phase consisted of 0.1% formic acid/methanol/acetonitrile (75 : 5 : 20, v/v/v; A) and 0.1% formic acid/methanol/acetonitrile (70 : 5 : 25, v/v/v; B) and gradient runs programed as follows: 0–10 min (0% B), 10–17 min (0–50% B), 25–34 min (50–80% B), 42–52 min (100% B) and then equilibration with 0% B for 10 min at a flow rate of 1 mL/min. The injection volume was 20 μ L. The ELSD was set to a probe temperature of 70°C, a gain of 7, and the nebulizer gas nitrogen adjusted to 2.5 bar.

Anti-HCV activity in the HCV replicon model of BC703. The plasmid pNNeo/3-5B replicon of Ikeda et al. (19) contains the sequence of a similar HCV replicon in which almost all of the NS3-NS5B sequence comprising the 3'cistron is derived from an infectious molecular clone of the genotype 1b virus, HCV-N (GenBank accession no. AF139594). It was kindly provided by Prof. Soon-bong Hwang (Ilseong Institute of Life Science, Hanlym University, Anyang, Korea). An expression vector harboring HCV replicon proceeded to in vitro transcription and the obtained HCV replicon was transfected into the Huh7 cells by electroporation. To select only those cells having the HCV replicon, Huh7 cells were cultured with Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% of fetal bovine serum, 1% nonessential amino acids, and 500 μ g/mL of geneticin.

HCV replicon-harboring cells were seeded at a low density of 1×10^4 cells per well in a 96-well plate and maintained at 37°C and 5% CO₂. The next day, the culture medium was replaced with medium containing serially diluted compounds in the presence of 2% FBS and 0.1% DMSO. After the cells were treated for 72 h, total RNA was extracted using a CellAmp Direct RNA Prep Kit (Korea Biomedical, Seoul, Korea). The HCV RNA levels were quantified by a quantitative real-time polymerase chain reaction assay using an IQ5 real time PCR detection system (Bio-Rad, Hercules, CA, USA) with HCV-specific primers (5'-GACACTCCACCATAGATCACTC-3' and 5'-CCCAACACTACTCGGCTAG-3') and probe (5'-FAM-CCCAAATCTCCAGGCATTGAGCGG-3' BHQ-1). Results were normalized to glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH). Each data point represents the average of at least 3 replicates in cell culture. Relative HCV RNA reduction was assessed by comparing the level of HCV RNA in compound treated cells to control cells treated with 0.1% DMSO.

Cytotoxicity of BC703. The cytotoxicity of BC703 on Huh7 cells was determined. Cell viability was then assessed with a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide colorimetric assay. The 50% cytotoxic concentration (CC₅₀), defined as the compound concentration that inhibited proliferation of exponentially growing cells by 50%, was calculated.

Polyphenolic content and DPPH radical scavenging activity. The total phenolic content was determined according to the method described by Slinkard and Singleton (20). The sample was accurately weighed and dissolved in a 100 mL brown volumetric flask with distilled water. The standard solution of gallic acid (0–

Fig. 1. Representative HPLC chromatograms of triterpenoids from extract of *Platycodon grandiflorum* and BC703. The numbers indicate each platycoside: 1, deapioplatycoside E; 2, platycoside E; 3, platycodin D₃; 4, platycodin D₂; 5, platycodin D; 6, platycodin A; 7, polygalacin D; 8, 2''-O-acetyl platycodin D.

0.06 mg/mL) was prepared. The standard and the sample (0.40 mL each) were mixed with the Folin-Ciocalteu phenol reagent (0.5 mL) and 20% of Na₂CO₃ (1.5 mL). The absorbance at 760 nm was measured 20 min after incubation at 30°C using an ultraviolet-visible spectrophotometer.

The free radical scavenging activity of BC703 was measured using the stable radical DPPH (21). A solution of DPPH (0.1 mM) in ethanol was prepared, and 900 μ L of this solution was added to 100 μ L of BC703 solution at different concentrations (0.05 to 5 mg/mL). The resulting solution was thoroughly mixed by vortexing and left in the dark, and then the absorbance was measured at 517 nm at different times until the reaction reached a steady state. The scavenging activity was determined by comparing the absorbance with that of the blank. When the reaction reached a plateau, the IC₅₀ value of BC703 was calculated from the results.

Animals. Male ICR mice, weighing between 32 and 36 g at the age of 5–6 wk, were used for this study. They were obtained from OrinetBio (Sungnam, Korea) and acclimated for 1 wk before experiments. Healthy ICR mice were randomly allocated into 5 groups of 6 male mice each. One group served as a control to orally receive distilled water and the others were administered BC703 (0, 1 and 5 mg/kg bodyweight) and digested BC703 with simulated gastric juice (10 mg as BC703/kg bodyweight) for 3 consecutive days. At 3 h after the last treatment, mice were intraperitoneally injected with olive oil or 50% of CCl₄ (1 mL/kg bodyweight in olive oil). After 24 h of CCl₄ administration, surviving mice were killed by cardiac puncture after being anesthetized lightly with carbon dioxide. Collected blood samples were separated by centrifuging at 800 $\times g$ for 15 min and the serum samples were subjected to biochemical investigations. Liver samples from each mouse were removed for histopathological and biochemical examination. The experimental protocols were approved by the Institutional Animal Care and Use Committee of Chungnam National University (Daejeon, Korea).

Serum and hepatic biochemical examination. The serum ALT and AST activities were determined on a dry chemistry system, the Vetest 8008 blood chemistry analyzer (IDEXX Laboratories). The content of malondi-

aldehyd (MDA), a terminal product of lipid peroxidation, was measured with the thiobarbituric acid reduction method using a commercially available kit (Quatichrom TBARS assay kit, Bioassay Systems). The hepatic GSH levels were determined using the improved DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)) method (QuantiChrom GSH Assay kit; BioAssay Systems). The hepatic SOD levels were also evaluated using the superoxide dismutase assay kit (Cayman).

Histopathological examination. The liver slices were made from a part of the left lobe and fixed immediately in a 10% buffered formalin phosphate solution, embedded in paraffin, and sectioned at 5 μ m. The serial sections were stained with hematoxylin and eosin (H&E) to evaluate the portal inflammation, hepatocellular necrosis and Kupffer cell hyperplasia. To quantify the degree (%) of hepatic necrosis, liver H&E sections were digitally photographed, and the percentage of necrotic areas was quantified as the mean of 10 randomly selected fields within each slide.

Statistical analysis. Results were expressed as mean $\pm$ standard error (SE). The significances of differences among experimental groups were determined using one-way analysis of variance (ANOVA) or the corresponding non-parametric Kruskal-Wallis test, as required. Where significant effects were found, post-hoc analysis using Turkey's multiple comparison test or the Mann-Whitney *U*-test was performed and $p < 0.05$ was considered to be statistically significant.

RESULTS

HPLC-ELSD fingerprint-analysis

On the basis of fingerprint analysis of BC703, it contained a large group of oleanane-type triterpenoid saponins, such as deapioplatycoside E, platycoside E, platycodin D₃, platycodin D₂, platycodin D, platycodin A, polygalacin D, 3''-O-acetyl polygalacin D, 2''-O-acetyl platycodin D (Fig. 1). Among these triterpenoid saponins, platycodin D is the major and potent constituent of triterpenoid saponin found in the root of *Platycodon grandiflorum*. Thus, BC703 was standardized in reference to platycodin D (at least 0.8%) using a validated HPLC assay method as previously described (5). A number of other non-saponin chemical constituents have been identified from *Platycodon grandiflorum*, including

Fig. 2. Total phenolic content and DPPH free radical scavenging activity of BC703. (A) total phenolic content of BC703 before and after gastric digestion with pepsin-HCl (pH 2.0) for 2 h at 37°C. The total phenolic contents were expressed as mg of gallic acid equivalent (GAE) per g of BC703. (B) changes in DPPH free radical scavenging activity of BC703 (500 µg/mL) before and after gastric digestion with pepsin-HCl (pH 2.0) for 2 h at 37°C. Vitamin C (10 µg/mL) served as a positive control for DPPH free radical scavenging activity. Values are expressed as means±SE. * $p<0.05$, a significant difference in comparison with gastric digestion of BC703 (10 µg/mL) with pepsin-HCl (pH 2.0) for 2 h.

Fig. 3. Effect of BC703 on liver function in CCl₄-induced acute hepatic injury. Mice were given orally BC703 (0, 1 and 5 mg/kg bodyweight) once daily for 3 consecutive days prior to intraperitoneal injection of 50% CCl₄ (1 mL/kg bodyweight in olive oil) or olive oil. Mice in the digested BC703 with a stimulated gastric juice treated group were given orally digested BC703 (10 mg/kg bodyweight) once daily for 3 consecutive days prior to intraperitoneal injection of 50% CCl₄ (1 mL/kg in olive oil). Values are expressed as means±SE (in surviving animals). ^a $p<0.05$, a significant difference in comparison with the control group. ^b $p<0.05$, a significant difference in comparison with the CCl₄ alone treated group.

α -spinasterol, α -spinasteryl- β -glucoside, stigmasterol, inulin-type polyfructose, and pectin (22, 23).

Total phenol content and DPPH radical scavenging activity

The DPPH free radical scavenging activity of BC703 is shown in Fig. 2 and the calculated EC₅₀ of BC703 was 0.91 mg/mL. Simulated gastric digestion of BC703 with pepsin-HCl (pH 2.0) for 2 h did not show any difference of phenolic content, whereas DPPH radical scavenging activity was significantly decreased in gastric digestion of BC703 ($p<0.05$, Fig. 2). Triterpenoids in BC703 after 2 h of digestion with pepsin under acidic conditions were also slightly decreased to approximately $92.24 \pm 4.32\%$ of intact BC703, based on the relative amount of platygodin D.

Hepatoprotective activity of BC703

BC703 increased the survival rate of mice challenged with a lethal dose of CCl₄. After 24 h, viability in the CCl₄ alone treated and digested BC703 (10 mg/kg bodyweight) treated group was 50%, while that in the BC703 (1 mg/kg and 5 mg/kg bodyweight) treated group was 66% and 83%, respectively. In the CCl₄ alone treated group, serum ALT and AST were increased as compared to the negative control group ($p<0.05$, Fig. 3). The elevated levels of serum ALT and AST were reduced in the group treated with 5 mg/kg bodyweight

of BC703 ($p<0.05$). Treatment with BC703 decreased ALT and AST levels in CCl₄ treated mice in a dose-dependent manner (Fig. 3). Similarly, the liver homogenate showed significant reduced activities of GSH and SOD, whereas increased levels of NO and lipid peroxidation were shown in the CCl₄ alone treated group (Fig. 4). The administration of BC703 showed an increase in GSH and SOD levels with decreased levels of NO and lipid peroxidation (Fig. 4). BC703 showed hepatoprotective activity in mice with CCl₄-induced hepatic injury. However, for digested BC703 with stimulated gastric juice under acidic conditions for 2 h, the hepatoprotective activity was decreased in the CCl₄-induced hepatic injury group as compared to BC703 treated groups.

Histopathological observations

The liver from animals in the negative control group had normal hepatic cells with well-preserved cytoplasm, a prominent nucleus, nucleolus and visible central veins, whereas the liver collected from CCl₄-treated mice showed extensive, mainly pericentral necrosis with loss of hepatic architecture, vacuolar fatty change and mild inflammatory cell infiltration comprised predominantly of mononuclear cells and macrophages (Fig. 5). The lower dose of BC703 (1 mg/kg bodyweight) did not prevent the toxic effect of CCl₄ with large

Fig. 4. Effect of BC703 on TBARS (A), NO (B), GSH (C) and SOD (D) content on CCl₄-induced acute hepatic injury. Mice were given orally BC703 (0, 1 and 5 mg/kg bodyweight) once daily for 3 consecutive days prior to intraperitoneal injection of 50% CCl₄ (1 mL/kg bodyweight in olive oil) or olive oil. Mice in the digested BC703 with a stimulated gastric juice treated group were given orally digested BC703 (10 mg/kg bodyweight) once daily for 3 consecutive days prior to intraperitoneal injection of 50% CCl₄ (1 mL/kg bodyweight in olive oil). Values are expressed as means $\pm$ SE (in survival animals). ^a p < 0.05, a significant difference in comparison with the control group. ^b p < 0.05, a significant difference in comparison with the CCl₄ alone treated group.

Fig. 5. The histopathological changes in liver stained with hematoxyline & eosin in CCl₄-induced hepatotoxicity ($\times 200$). Mice were given orally BC703 (0, 1 and 5 mg/kg bodyweight) once daily for 3 consecutive days prior to intraperitoneal injection of 50% CCl₄ (1 mL/kg bodyweight in olive oil) or olive oil. Mice in the digested BC703 with a stimulated gastric juice treated group were given orally digested BC703 (10 mg/kg bodyweight) once daily for 3 consecutive days prior to intraperitoneal injection of 50% CCl₄ (1 mL/kg in olive oil). A, non-treated negative control; B, CCl₄ alone treated group; C, CCl₄ + 1 mg/kg of BC703 treated group; D, CCl₄ + 5 mg/kg of BC703 treated group; E, CCl₄ + 10 mg/kg of digested BC703 treated group. ^a p < 0.05, a significant difference in comparison with the control group. ^b p < 0.05, a significant difference in comparison with the CCl₄ alone treated group.

Fig. 6. Suppressive effect of BC703 on HCV RNA replication. Huh7 cells harboring the HCV genotype 1b replicon were treated with different concentrations (1, 2, 5, 10 and 20 $\mu\text{g/mL}$) of BC703 (A) before and after simulated gastric digestion of BC703 (10 $\mu\text{g/mL}$) with pepsin-HCl (pH 2.0) for 2 h (B). Treatment with 0.1% DMSO served as a mock control for anti-HCV activity. Total RNA of BC703 treated Huh7 cells was extracted to quantify HCV RNA levels by RT-qPCR. Cytotoxicity was simultaneously evaluated by MTT assay. Values are expressed as means $\pm$ SE for triplicate experiments. * $p < 0.05$, a significant difference in comparison with gastric digestion of BC703 (10 $\mu\text{g/mL}$) with pepsin-HCl (pH 2.0) for 2 h.

necrotic areas still present. However, the high dose of BC703 (5 mg/kg bodyweight) showed a more or less normal lobular pattern with mild degree of fatty degenerations and necrosis (Fig. 5).

Anti-HCV activity of BC703

The anti-HCV activity of BC703 was evaluated in Huh7 cells harboring the HCV genotype 1b replicon. BC703 caused a dose-dependent inhibition of HCV RNA replication without affecting cell viability or cell growth. The calculated EC_{50} of BC703 was 2.82 $\mu\text{g/mL}$ (Fig. 6). However, the anti-HCV activity of digested BC703 with simulated gastric juice significantly decreased ($p < 0.05$). Cytotoxicity of BC703 on Huh7 Cells was also studied. The CC_{50} value of BC703 was above 100 $\mu\text{g/mL}$ and the selective index ($\text{CC}_{50}/\text{EC}_{50}$) of BC703 in vitro was more than 35.46.

DISCUSSION

CCl_4 is a well-known model compound for inducing liver injury (6, 7). Its biotransformation by the hepatic microsomal cytochrome P450 produces hepatotoxic metabolites (8, 9). The covalent binding of the trichloromethyl free radical to the cell proteins is considered to be the initial step in a chain of events that eventually lead to cell necrosis (10). The results of the present study showed that CCl_4 administration caused severe acute liver damage in mice, demonstrated by remarkable elevation of serum AST and ALT levels. The increased serum levels of AST and ALT are attributed to the damaged structural integrity of the liver. Administration of BC703 prevented CCl_4 -induced lethality, elevation of ALT, AST and lipid peroxidation in a dose-dependent manner, indicating its hepatoprotective effect. These findings were also confirmed by histological observation. The changes mostly included hepatocellular necrosis or apoptosis, fatty accumulation, inflammatory cells in filtration and other histological manifestations, which were also consistent with the findings of other studies (10, 11, 24).

GSH is the major non-enzymatic antioxidant and regulator of intracellular redox homeostasis, ubiquitously

present in all cell types (25). The detoxification mechanism in CCl_4 -induced hepatotoxicity involves GSH conjugation of the trichloromethyl radical, a cytochrome P450 2E1-mediated CCl_4 metabolite (8, 9). CCl_4 administration leads to a significant decrease in the glutathione level, which can be an important factor in CCl_4 toxicity (25). In addition, lipid peroxidation has been implicated in the pathogenesis of hepatic injury by the free radical derivatives of CCl_4 and is responsible for cell membrane damage and the consequent release of marker enzymes of hepatotoxicity (10, 11). In this study, pretreatment with BC703 prior to CCl_4 -induced hepatic injury inhibited lipid peroxidation and reduced CCl_4 -induced hepatic GSH depletion, as well as restoring hepatic Cu/Zn SOD activities in the liver. A sufficient regenerative response of BC703 by reducing the intensity of liver damage could be also expected.

The current standard care for patients with chronic hepatitis C is combination therapy with pegylated IFN- α and ribavirin (13–15). It is effective in approximately 80% of patients with HCV genotype 2 or 3 infection, but less than 50% of patients with HCV genotype 1, by far the most frequent HCV genotype worldwide (14, 15). In addition, the combination therapy is prolonged, costly, and may be associated with adverse effects that are difficult for many patients to tolerate (15). Thus, more effective, more tolerable, and/or more tailored therapies are required.

Numerous studies recently showed that plant extracts exhibit an inhibitory effect on HCV enzyme and replication (4, 16–18, 26). They have demonstrated their utility in eradicating chronic hepatitis C through a multitude of hepatoprotective functions in initial trials. Therefore, plant extracts have been considered as potential sources of new bioactive compounds against HCV infection (4, 16–18, 26). Ma et al. (27) reported that 27 of 44 medicinal herbs showed potent or moderate antiviral activities against respiratory syncytial virus (RSV). Among them, *Platycodon grandiflorum* extract exhibited an inhibitory effect against RSV with an IC_{50} value of 44.1 mg/mL and an SI index of 8.0 (27). In

the present study, we showed that BC703 strongly inhibited HCV genotype 1b replicon replication in Huh7 cells with an EC₅₀ of 2.82 µg/mL and an SI index of >35.46, although the detailed mechanism has not been clearly defined. BC703 may be considered as a potential source of new bioactive compounds against HCV infection.

There is still debate about the stability and absorption of plant-derived compounds comprising a wide range of bioactive constituents under gastrointestinal conditions. Inulin is a polysaccharide with β (2-1) linkages by which D-fructose is polymerized. Inulin is widely distributed throughout the plant kingdom and exists as a reserve substance in the tuber or tuberous roots of plants including *Platycodon grandiflorum* (22, 23). Plant-origin inulin is resistant to hydrolysis by pancreatic amylase and saccharidase in the upper gastrointestinal tract and it reaches the large intestine unabsorbed and is utilized as a carbohydrate substrate for the growth of indigenous bifidobacteria (22, 23). However, from many other studies on the degradation and metabolism of β-glycosides of plant origin, it is known that the metabolism proceeds mainly via degradation processes already occurring in the gastrointestinal tract (27, 28). Moreover, Shimizu et al. reported that saikosaponins in *Bupleurum* species converted into their corresponding structural isomers of diene derivatives with acidic treatment or in gastric juice with low pH (29). Under acidic conditions, ginsenoside Re was mainly hydrolyzed to yield Rg2 and then ginsenoside Rg2 was transformed to 20(R)-Rg2 by epimerization, or changed to ginsenoside F4 and Rg6 via a dehydration reaction (30). In the present study, the treatment group with digested BC703 using a simulated gastric juice showed poor protective activity against CCl₄-induced hepatotoxicity in mice and anti-HCV activity in HCV genotype 1b subgenomic replicon RNA containing Huh7 cells as compared to the BC703. On the basis of these results, active ingredients of BC703 having the anti-HCV and hepatoprotective activity may be not stable in human gastric juice. Thus, BC703 for clinical use in humans may require a specific drug delivery system such as an enteric coated tablet formulation to minimize the degradation of its active ingredient by low gastric pH and to improve its bioavailability.

In conclusion, BC703 attenuates the CCl₄-induced acute hepatotoxicity in mice and inhibits HCV RNA replication in Huh7 cells harboring the HCV genotype 1b replicon. Although further studies are necessary, BC703 may be a beneficial agent for the management of acute hepatic injury and chronic HCV infection.

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