Introduction

Hepatocellular carcinoma (HCC) is one of the most prevalent malignant diseases in Korea. It shows an incidence of 22.6 per 100,000, similar to other Asian countries\(^1\). The major cause of widespread HCC in Korea is known to stem from hepatitis B virus (HBV) infection\(^2\). Most patients diagnosed with HCC have low recovery rates and survival. Unfortunately, conventional western therapies currently available are rarely successful\(^3\). Therefore, it is necessary to search for more effective novel agents with fewer side effects for HCC treatment.

*Coptis chinensis* has been used in traditional medicine for clearing heat and depriving dampness for treatment of dysentery and jaundice, and purging the sthenic fire and clearing away toxic material for the cases of seasonal febrile diseases, carbuncle and sore throat\(^4\). This herbal medicine possesses broad-spectrum antibacterial and anti-protozoal effects. Also, it has been applied to clinical anti-tumor use for a long time. Its anti-tumor effect as a potent anti-angiogenic agent has recently been noted. Nevertheless, research about the mechanism of such anti-tumor activities is much lacking. Although a recent study has shown that *Coptis chinensis* extract (CCE) inhibits
tumor growth and angiogenesis, information on its anti-tumor properties and cellular mechanism remains limited\(^5\).

This study aimed to elucidate anti-hepatoma activity of CCE and evaluate its effect on proliferation of human hepatoma Hep G2 cells. To identify CCE and control the quality, we performed fingerprinting by high-performance thin layer chromatography (HPTLC). To investigate effects of CCE on anti-hepatoma activity, we measured cytotoxicity against Hep G2 cells compared to treatment with paclitaxel and 5-fluorouracil (5-FU). To examine the mechanism of inhibitory effect of CCE on Hep G2 cell proliferation, cell cycle distribution was evaluated using Fluorescent Activated Cell Sorter (FACS).

**Materials and Methods**

1. Materials

*Coptis chinensis* was obtained from Daejeon Oriental Medical Hospital. Dried *Coptis chinensis* rhizoma was powdered with a grinder. Ten grams of the powder were mixed with 50% ethanol at room temperature, and the whole mixture was agitated for 24 h at 50°C. The mixture was centrifuged for 30 min at 2,000×g and supernatant was concentrated with vacuum evaporator (BUCHI, Switzerland) and then lyophilized. The yield CCE (Coptis chinensis extract) was 21% (w/w) in terms of the dried medicinal herbs. Berberine sulfate was obtained from Wako (Japan) and cell counting kit-8 (CCK-8) was purchased from Dojindo (Kumamoto, Japan). Other chemicals were purchased from Sigma (St Louis, MO).

2. Fingerprinting of CCE by HPTLC

High-performance thin layer chromatography (HPTLC)-based fingerprint was produced by CAMAG application system (Switzerland) as presented in Fig. 1. For the HPTLC analysis, CCE were dissolved in HPLC-grade methanol and applied to the pre-washed silica gel 60 F254 HPTLC plates (size 10 × 10 cm; thickness of the silica gel 0.2 mm; Merck, Germany) with an automated applicator (Linomat IV, CAMAG, Merck KGaA, Germany). Then the samples were separated (migration distance 60 mm) using HPLC-grade butanol/acetic acid/water (7:1:2). The migrated components were visualized at 366 nm with a digital camera (CAMAG, Germany).
3. Cell culture

Hep G2 cells were obtained from Korean Cell Line Bank (Seoul, Korea). The cells were cultured in DMEM (Welgene, Korea) supplemented with 10% fetal bovine serum (FBS, Welgene, Korea).

4. Measurement of cytotoxicity

Hep G2 \((3 \times 10^5)\) were seeded into 96-well plates and cultured for 24 h at 37°C in 5% CO2 incubator. The cells were treated with CCE (0, 25, 50, 100, 200 \(\mu\text{g/mL}\)), berberine (0, 25, 50, 100, 200 \(\mu\text{g/mL}\)), 5-FU (0, 1, 5, 10, 25 \(\mu\text{g/mL}\)) and paclitaxel (0, 0.001, 0.005, 0.01, 0.05, 0.1 \(\mu\text{g/mL}\)). CCK-8 (20 \(\mu\text{L}\)) was added to each well after 48 h of CCE treatment. Two hours later, 200 \(\mu\text{L}\) of each culture medium was transferred to a new plate to determine optical density.

5. Combination therapy with anticancer drug

Hep G2 \((3 \times 10^5)\) were seeded into 96-well plates and cultured for 24 h at 37°C in 5% CO2 incubator. The cells were treated with CCE (0, 25, 50, 100 \(\mu\text{g/mL}\)) and paclitaxel (0.001, 0.005 \(\mu\text{g/mL}\)) or 5-FU (0.5, 1, 2 \(\mu\text{g/mL}\)). CCK-8 (20 \(\mu\text{L}\)) was added to each well after 48 h of CCE and anti-cancer drug treatment. Two hours later, 200 \(\mu\text{L}\) of each culture medium was transferred to a new plate to determine optical density.
6. Cell cycle analysis
The cells were seeded into 60 mm culture dish and cultured for 24 h at 37°C in 5% CO2 incubator. CCE were treated at concentrations of 50 and 100 μg/ml for 24 h and 48 h. Berberine were treated at concentrations of 10, 25 and 50 μg/ml for 24 h. The cells were trypsinized, harvested, fixed with 70% cold ethanol and stocked at 4°C until analysis. The fixed cells were washed with PBS containing 0.1% FBS and treated with RNase A (1 mg/ml). After propidium iodide (PI) (1 μg/ml) staining for 30 min, cell cycle analysis was performed with FACS (BD, NJ, USA).

7. Statistical analysis
Statistical analysis of the data was carried out by Student’s t-test, and results were expressed as mean ± SD. Difference from the respective control data at the levels of p < 0.05, p < 0.01 and p < 0.001 was regarded statistically significant.

Results
1. Fingerprinting of CCE
To identify CCE and control the quality, we performed fingerprinting by HPTLC. As shown in Fig. 1, three components in CCE were observed clearly at UV 366 nm without derivertization. The color of berberine (BB) turned to green.

Fig. 4. Cytotoxicity of 5-FU on Hep G2 cells. Hep G2 cells (3×10⁵) were seeded into 96-well plates and cultured for 24 h. The cells were treated with 5-FU (0, 1, 5, 10, 25 μg/ml). OD450-590 was determined at 2 h after CCK-8 addition. Data represented as mean ± SD. Statistically significant value compared with control was measured by t-test (*: p<0.05, **: p<0.01, ***: p<0.001).

Fig. 5. Cytotoxicity of paclitaxel on Hep G2 cells. Hep G2 cells (3×10⁵) were seeded into 96-well plates and cultured for 24 h. The cells were treated with paclitaxel (0, 0.001, 0.005, 0.01, 0.05, 0.1 μg/ml). OD450-590 was determined at 2 h after CCK-8 addition. Data represented as mean ± SD. Statistically significant value compared with control was measured by t-test (*: p<0.05, **: p<0.01***: p<0.001).
at UV 366 nm. CCE has a component that also changed to the same color and achieved the same Rf value.

2. Cell proliferation

Hep G2 (3×10^5) were seeded into 96-well plates, and were treated with various concentrations of CCE (0, 25, 50, 100 μg/ml) and 5-FU (0, 0.5, 1.0, 2.0 μg/ml). OD450-590 was determined at 2 h after CCK-8 addition. Data represented as mean ± SD.

Anti-cancer drugs 5-FU and paclitaxel showed cytotoxicity at low concentrations in a dose-dependent manner.

3. Effect of CCE combined with 5-FU or paclitaxel on cell proliferation

As shown in Fig. 6, 7, 8, 9, CCE and its main component, berberine, have no synergistic effect on cell growth combined with 5-FU. However, CCE inhibited synergistically cell growth treated together with paclitaxel at the concentration of 0.005 μg/ml.

---

**Fig. 6. Cytotoxicity of CCE combined with 5-FU on Hep G2 cells.** Hep G2 cells (3×10^5) were seeded into 96-well plates and cultured for 24 h. The cells were treated with CCE (0, 25, 50, 100 μg/ml) and 5-FU (0, 0.5, 1.0, 2.0 μg/ml). OD450-590 was determined at 2 h after CCK-8 addition. Data represented as mean ± SD.

**Fig. 7. Cytotoxicity of berberine combined with 5-FU on Hep G2 cells.** Hep G2 cells (3×10^5) were seeded into 96-well plates and cultured for 24 h. The cells were treated with berberine (0, 10, 25, 50 μg/ml) and 5-FU (0, 0.5, 1.0, 2.0 μg/ml). OD450-590 was determined at 2 h after CCK-8 addition. Data represented as mean ± SD.
4. Cell cycle analysis

To learn how CCE inhibits cell growth, the cell cycle was analyzed by using FACS after PI staining. After 24 h treatment of CCE (50, 100 μg/ml), the G2/M stage increased by 26.5% and 42% respectively whereas only 21.3% in control (Fig. 10). The G0/G1 stage decreased by 57.4% and 40.8%, whereas 63.1% in control. After 48 h treatment of CCE (50, 100μg/ml), the G2/M stage was much increased (31.5%, 49.7% respectively) over that of 24 h (Fig. 11). 5-FU and paclitaxel increased G0/G1 stage and G2/M stage respectively. Berberine also showed similar effect as CCE (Fig. 12). CCE (25 μg/ml) and berberine (10 μg/ml) treatment combined with paclitaxel (0.0025 μg/ml) increased sub-G1 phase by 43.4% and 32.7% respectively, whereas only 27.8% in the paclitaxel-only treatment group (Fig. 13).

Discussion

Recently, research on herbal medicine for anti-cancer effect has focused on activity capable
been shown to possess anti-cancer effects by inhibiting cell cycle progression and/or causing apoptosis. *Coptis chinensis*, one of the most common anti-inflammatory agents in herbal medicine, has been shown to possess anti-cancer effects by anti-angiogenic activity. In this study, we further examined the anti-proliferative effect of CCE on the Hep G2 cell, which is one of the human hepatoma cell lines.

As with other chemotherapeutic agents (5-FU and paclitaxel), CCE significantly inhibited cell growth of Hep G2 cells. Furthermore, treatment with CCE combined with paclitaxel inhibited synergistically cell growth of Hep G2 cells. However, treatment with CCE combined with 5-FU had no synergistic effect on proliferation of Hep G2 cells. These results imply that CCE possesses a growth-inhibitory effect on hepatoma cells. Also, it is inferred from synergistic effect that CCE and paclitaxel have action mechanisms of similar pathways.

Next, to investigate the mechanism of anti-proliferative effect of CCE on Hep G2 cells, we analyzed cell cycle distribution using FACS. In

---

**Fig. 10. Effects of CCE on cell cycle.** Hep G2 cells (3×10⁵) were seeded into 60 mm culture dishes and cultured for 24 h. The cells were treated with CCE for 24 h. Cell cycle was analyzed by FACS.

---

**Fig. 11. Effects of CCE on cell cycle.** Hep G2 cells (3×10⁵) were seeded into 60 mm culture dishes and cultured for 24 h. The cells were treated with CCE for 48 h. Cell cycle was analyzed with FACS.
general, paclitaxel and 5-FU are two of the most widely used chemotherapeutic agents for various cancer treatments. Paclitaxel is one of the best anti-cancer drugs found in recent decades. It is a naturally occurring antimitotic agent that has been shown to stabilize microtubules, induce mitotic arrest, and ultimately induce apoptotic cell death. 5-FU is one of the oldest chemotherapeutic drugs, and is commonly used against many cancers. These chemotherapeutic agents exhibit cytotoxic action at different phases of the cell cycle. Paclitaxel prevents the majority of cells from progressing to the G2/M phase, while 5-FU is one of the G1-S arresting agents.

As in previous studies, 5-FU and paclitaxel increased G0/G1 phase and G2/M phase of Hep G2 cells respectively in our study. On the other hand, CCE showed a significant effect to arrest Hep G2 cells at the G2/M phase of the cell cycle. Through these results, it is presumed that CCE acts as an antimitotic agent like paclitaxel in Hep G2 cells. Interestingly, previous studies have demonstrated 5-FU, G1-S arresting agents, in combination with paclitaxel significantly
represses paclitaxel-induced mitotic arrest and apoptosis. Moreover, from the extended studies, several antimitotic agents (vinblastine, colchicine, and nocodazole) in various combinations with the G1-S arresting agents 5-FU and hydroxyurea (HU) could significantly interfere with the overall cytotoxicity as compared with antimitotic agents alone. These results suggest that careful consideration or experimental evaluation is required when combining anti-neoplastic drugs that exert their cytotoxic activity as compared with the treatment of a single drug. As for this point, we should clinically consider the use of CCE in combination with chemotherapy like paclitaxel in HCC.

From this discussion, we can conclude that CCE may present anticancer effects through inhibition of HCC cell proliferation via G2/M arrest. It can be a useful anticancer agent for HCC and more needs to be learned about their mechanisms of action in various cancer cell lines, and more clinical trials should be expected.

**Conclusion**

This experimental study was carried out to evaluate anti-proliferative effect of CCE in Hep G2 Cells. The results were obtained as follows:

1. CCE significantly inhibited cell growth of Hep G2 cells.
2. CCE combined with paclitaxel inhibited synergistically cell growth of Hep G2 cells.
3. CCE showed a significant effect that arrests Hep G2 cells at the G2/M phase of the cell cycle.

From these results, it can be concluded that CCE is an effective herbal agent for inhibition of HCC cell proliferation.

**References**