**Cytotoxic Effect of *Capparis spinosa* L. on *PLC/PRF/5* Human Hepatocellular Carcinoma Cell Line**

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**ABSTRACT**

**Background and Objectives:** *Capparis spinosa* has been used in traditional medicine for various applications including treatment of liver disorders and cancer. We studied the effects of this plant on cell proliferation and morphological characteristics of *PLC/PRF/5* liver cancer cell line.

**Methods:** After preparing ethanolic extract of the plant, the inhibitory effect of the extract was assessed using MTT assay, and morphological changes were assessed by an inverted microscope.

**Results:** *C. spinosa* ethanolic extract exhibited anti-cancer effects in a concentration-dependent manner. Half-maximal inhibitory concentration of the extract was 1051±4.21 mg/mL. Morphological changes including cell shrinkage, reduction of cell volume and nuclear condensation confirmed the inhibitory effect of *C. spinosa* on *PLC/PRF/5* cells.

**Conclusion:** According to the results of this study, extract of *C. spinosa* seems to be suitable for prevention and treatment of liver cancer. Further studies on animal models could verify the efficiency of the extract against cancer cells.

**KEYWORDS:** Plants, Medicinal, *Capparis spinosa*, Liver Neoplasms.
INTRODUCTION
Liver cancer is the third leading cause of cancer death worldwide (1, 2). According to the world health organization (WHO), the incidence of liver cancer will increase dramatically by 2030, making it the second leading cause of death (3, 4). High mortality rates caused by cancer, limited number of effective drugs and toxic effects of the drugs currently used highlight the need for identification of safe alternatives for treatment of cancer. Medicinal plants have been used for treatment of various diseases. In fact, about 50% of all licensed drugs in developing countries have a natural origin (5). Several studies have shown that many plants that grow naturally in Iran can affect the function of liver and could be used for treatment of liver disease such as liver cancer. Capparis spinosa is a native medicinal plant found in the north, center and west of Iran. Different parts of the plant including root, fruit, leaves and buds have been used in traditional medicine for treatment of liver diseases (6, 7). The plant has different chemical constituents including glucosinolates, flavonoids (rutin, kaempferol and quercetin), alkaloids (capparisine), phenolic acids, vitamins and fatty acids (8). It has been well demonstrated that C. spinosa has antioxidant, anticancer, liver-protecting, anti-hyperglycemic, antiviral, and antibacterial properties (9, 10). Considering the protective effect of this plant on liver, we aimed to evaluate the effect of C. spinosa on PLC/PRF/5 liver cancer cell line.

MATERIAL AND METHODS
RPMI-1640 medium, L-glutamine, fetal bovine serum (FBS), penicillin-streptomycin and trypsin-EDTA were obtained from Gibco BRL (Grand Island, NY, USA). Dimethyl sulfoxide (DMSO) and 3-(4, 5 Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) were purchased from Sigma (St. Louis, MO, USA). C. spinosa that was free from fungal, bacterial and any other plant diseases was obtained from Gonbad Qabus (northeast of Iran) in July 2014. The plant was dried at room temperature for 5 days. The extract was obtained by mixing the powdered parts of the plant with 80% ethanol (1:10, w/v). The mixture was filtered through Whatman filter paper (No. 4), concentrated, lyophilized and stored at -20 °C. The lyophilized powder was reconstituted with 0.4% methanol when required. PLC/PRF/5 human liver cancer cell line was obtained from the Pasteur Institute Collection of Cell Cultures, Tehran, Iran. The cells were cultured in RPMI-1640 medium containing 4.5 g/l glucose, L-glutamine, 10% FBS, 100 U/ml penicillin and 0.1 g/l streptomycin. The cells were incubated in 5% CO2 and 95% humidity. The culture medium was replaced at least every two days for all experiments. Cell proliferation inhibition was assessed by MTT assay. PLC/PRF/5 cells were transferred to 96-well plates at density of 5.6×103 cells/well, and then incubated for 24 h. The cells were treated with different concentrations (150, 310, 620, 1250, 2500 and 5000 µg/mL) of C. spinosa ethanolic extract (CSE) for 48 h. Then, cancer cells were incubated with fresh medium containing 1 g/L MTT at 37 °C for 4 h. Old medium containing MTT was gently replaced by 200 µL of DMSO and then mixed gently by pipetting to dissolve any formed Formazan crystals. Finally, the microplates were incubated at room temperature for 30 min. Formation of Formazan crystals was assessed at 570 nm using a microplate reader. Morphological study was done using an inverted microscope. Untreated cells were used as negative controls. Data were reported as mean ± standard deviation (SD) of three independent experiments, and analyzed by T-test. P-values < 0.05 were considered statistically significant.

RESULTS
Table 1 shows the cytotoxic effect of different concentrations of CSE on PLC/PRF/5 cell line using MTT assay. The growth of PLC/PRF/5 cells was significantly inhibited in a concentration dependent manner (Figure 1). However, the addition of 620- 5000 µg/mL of the extract caused a significant increase in the cytotoxicity. The growth inhibitory effect of CSE was highest at 5000 µg/mL. Concentration of 150 µg/mL of the extract did not have any significant cytotoxic effect on the cells. Half-maximal inhibitory concentration (IC50) of CSE was determined as 1051±4.21 mg/mL. Morphology of the cells treated with IC50 of CSE for 48 h changed dramatically, exhibiting hallmark features of apoptosis including cell shrinkage with irregular shape, reduction of cell volume, plasma membrane blebbing, nucleus pigmentation and apoptotic bodies (Figure 2).
**DISCUSSION**

According to reports of the world health organization, liver cancer is one of the most important and deadliest diseases in the world. About 746000 individuals in 2012 died because of this disease (4). The disease is asymptomatic in the early stages and in advanced and terminal stages at the time of diagnosis, resulting in a poor prognosis. In Europe, five-year survival rate of patients with liver cancer is less than 10 percent (11, 12).

In this study, we examined the potential inhibitory effect of *C. spinosa* on cancer cells. Several studies have reported the cytotoxic effect of *C. spinosa* on different tumor cells in both in vivo and in vitro conditions. According to studies on the inhibitory effect of *C. spinosa* and its derivatives on HepG2 (liver), HT-29 (colon), MCF-7 (breast), and Hela (cervix) cells, *C. spinosa* has the highest inhibitory effects on liver cancer cell lines (8, 13). Aghel et al. reported that injection of carbon tetrachloride in rats increases liver enzyme levels and causes liver damage. However, the level of these enzymes was reduced in mice treated with *C. spinosa* root bark (14). Moradi et al. demonstrated that CSE inhibits the proliferation of HepG2 hepatocellular carcinoma cells. CSE also induces cell apoptosis by up regulation of caspase8/9 activity and down regulation of Bcl-2, which are involved in extrinsic and intrinsic pathway of apoptosis, respectively. Furthermore, CSE induces cell cycle arrest by expression of Cip1/p21(15). According to our results, CSE significantly reduces cell proliferation rate in a concentration-dependent manner. However, higher efficiency against the cancer cells can be achieved by using the pure compounds.
obtained from the plant. Apoptotic signs such as irregular shape, reduction of cell volume, plasma membrane blebbing and nuclear condensation were observed in the morphological assessment of the cells, which confirmed the inhibitory effect of CSE IC50 on the PLC/PRF/5 cells.

**CONCLUSION**

According to the results of this study, extract of *C. spinosa* seems to be suitable for prevention and treatment of liver cancer.

**REFERENCES**


Further studies using animal models could verify the efficiency of the extract against cancer cells.

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**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.