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.

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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% CO_2 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 5.6×10^{-3} 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).

Figure 1- Effect of CSE on growth and viability of *PLC/PRF/5* cells in MTT assay

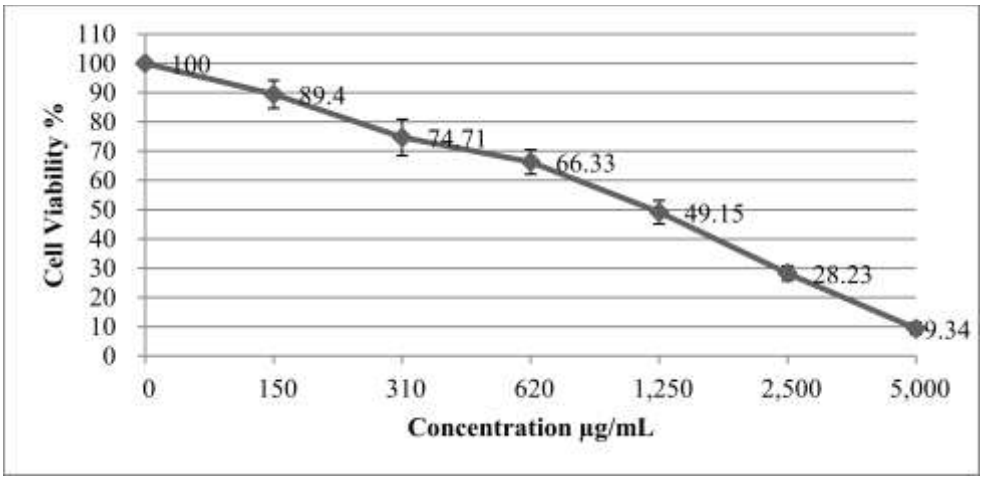
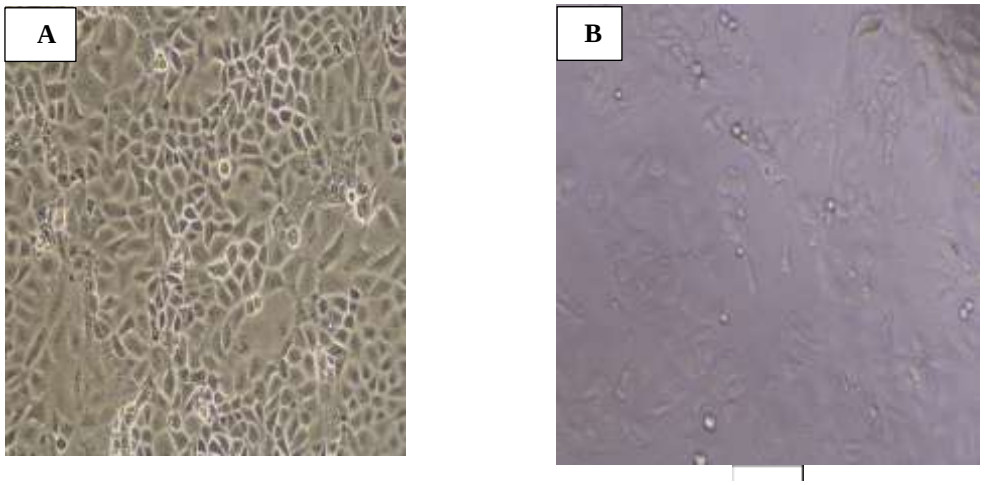


Figure 2- A) Untreated *PLC/PRF/5* cells after 48 h, B) *PLC/PRF/5* cells treated with IC50 of CSE for 48 h.



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. 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.

REFERENCES

1. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. *Sorafenib in advanced hepatocellular carcinoma.* The New England journal of medicine. 2008; 359(4): 378-90. DOI: 10.1056/NEJMoa0708857.

2. El-Serag HB. *Hepatocellular carcinoma.* The New England journal of medicine. 2011; 365(12): 1118-27. DOI: 10.1056/NEJMra1001683.

3. Marrero JA, Feng Z, Wang Y, Nguyen MH, Befeler AS, Roberts LR, et al. *Alpha-fetoprotein, des-gamma carboxyprothrombin, and lectin-bound alpha-fetoprotein in early hepatocellular carcinoma.* Gastroenterology. 2009; 137(1): 110-8. doi: 10.1053/j.gastro.2009.04.005.

4. McMichael AJ. *Food, nutrition, physical activity and cancer prevention.* Authoritative report from World Cancer Research Fund provides global update. Public health nutrition. 2008; 11(7): 762-3.

5. Newman DJ, Cragg GM. *Natural products as sources of new drugs over the 30 years from 1981 to 2010.* Journal of natural products. 2012; 75(3): 311-35. doi: 10.1021/np200906s.

6. Eddouks M, Lemhadri A, Michel JB. *Caraway and caper: potential anti-hyperglycaemic plants in diabetic rats.* Journal of ethnopharmacology. 2004; 94(1): 143-8. DOI: 10.1016/j.jep.2004.05.006.

7. Jiang HE, Li X, Ferguson DK, Wang YF, Liu CJ, Li CS. *The discovery of Capparis spinosa L. (Capparidaceae) in the Yanghai Tombs (2800 years b.p.), NW China, and its medicinal implications.* Journal of ethnopharmacology. 2007; 113(3): 409-20.

8. Kulisic-Bilusic T, Schmoller I, Schnabele K, Siracusa L, Ruberto G. *The anticarcinogenic potential of essential oil and aqueous infusion from caper (Capparis spinosa L.).* Food chemistry. 2012; 132(1): 261-7. doi: 10.1016/j.foodchem.2011.10.074.

9. Eddouks M, Lemhadri A, Michel JB. *Hypolipidemic activity of aqueous extract of Capparis spinosa L. in normal and diabetic rats.* Journal of ethnopharmacology. 2005; 98(3): 345-50.

10. Panico AM, Cardile V, Garufi F, Puglia C, Bonina F, Ronsisvalle G. *Protective effect of Capparis spinosa on chondrocytes.* Life sciences. 2005; 77(20): 2479-88.

12. Srivatanakul P, Sriplung H, Deerasamee S. *Epidemiology of liver cancer: an overview. Asian Pacific journal of cancer prevention*. APJCP. 2004; 5(2): 118-25.

11. Tabor E. Hepatocellular carcinoma: global epidemiology. *Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver*. 2001; 33(2): 115-7.

13. Lam SK, Ng TB. *A protein with antiproliferative, antifungal and HIV-1 reverse transcriptase inhibitory activities from caper (Capparis spinosa) seeds. Phytomedicine.* international journal of phytotherapy and phytopharmacology. 2009; 16(5): 444-50. doi: 10.1016/j.phymed.2008.09.006.

14. Aghel N, Mombeini A. *Hepatoprotective activity of Capparis spinosa root bark against ccl4 induced hepatic damage in mice.* Iranian journal of pharmaceutical research : IJPR. 2007; 6(4): 285-290.

15. Sheikh M, Eshraghi HR, Khoshnia M, Mazandarani M, Moradi A. *Cytotoxicity effect of Capparis spinosa L. on the HepG2 human hepatocellular carcinoma cell line.* Indian J Tradit Knowle. 2017; 6(1): 230-8.