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Apoptosis Induction in Esophageal Squamous Cell Carcinoma Cells by Hydroalcoholic Extract of *Cuscuta epithymum*

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ABSTRACT

Introduction: Esophageal cancer is the eighth most common cancer and the sixth most common cause of cancer death in the world. Apoptosis facilitates the removal of aged, damaged, infected, or dangerous cells. Therefore, the induction of apoptosis by different drugs or herbal extracts is a therapeutic strategy to control and treat various cancers. *Cuscuta epithymum* is the most common species of Cuscuta, which is widely used in traditional medicine, and its antitumor properties have been reported in recent studies. Therefore, this study aimed to investigate the in vitro induction of apoptosis in the esophageal cancer cell line by *C. epithelium*.

Methods and Materials: In the present study, the hydroalcoholic extract of *C. epithymum* (Aphthymon) was prepared using the Soxhlet method. Then, the Kyse 30 esophageal cancer cell line was treated with concentrations of 100, 200, 400, and 600 µg/ml of the extract for 24 hours. Then, the induction of apoptosis in the treated cells was checked using Annexin V-FITC/PI staining and flow cytometry method. In addition, the specific morphology of apoptotic and necrotic cells was assayed by Annexin V-FITC/PI staining and fluorescence microscopy and compared with the morphology of untreated cells.

Results: The results showed that treating cells with hydroalcoholic extract of *C. epithelium* in a dose-dependent manner causes a significant increase in the percentage of primary and secondary apoptotic cells compared to untreated cells. Analysis with the flow cytometry displayed that at the 100 µg/ml concentration, about 8% of the cells had undergone primary apoptosis. At 200, 400, and 600 µg/ml concentrations, the primary apoptotic cells were 11, 23, and 39%, respectively, which was significant compared to the untreated group ($p < 0.01$). The percentage of secondary apoptotic and necrotic cells also increased with increasing concentration. Staining the cells using Annexin V-FITC/PI and observing them under the fluorescence microscope revealed the apoptotic and necrotic cell morphology of treated cells.

Keywords: Apoptosis, Cuscuta, Esophageal neoplasms, Herbal medicine

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