

Anticancer activity of EA1 extracted from *Equisetum arvense*

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Abstract: Plants and their byproducts have been used for the treatment of various diseases from the time immemorial. The current study was carried out to investigate the anticancer activity of ethanol extract of arial parts of *Equisetum arvense* (EA1). To check the anticancer potential of EA1, A549 lung carcinoma cells were treated with various concentrations of EA1 (100µg/mL, and 150µg/mL). The cell viability was checked using MTT assay, and apoptosis (programmed cell death) was assessed by acridine orange staining.. The results depicted that EA1 manifested cytotoxicity and decreased the cell viability of A549 cells in a concentration dependent manner. Moreover, EER induced apoptotic cell death as monitored using acridine orange staining. The results obtained suggest EA1 extracted from *Equisetum arvense* as a potential biological resource with pharmacological significance.

Keywords: Apoptosis, cancer; cytotoxicity, *Equisetum arvense*.

INTRODUCTION

Human life has suffered from diseases since its existence. Cancer is a fatal disease and one of the leading causes of deaths worldwide. Lung cancer, also known as lung carcinoma, is the most common type of cancer with over 1.4 million deaths; followed by stomach cancer with 740,000 deaths; liver cancer with 700,000 deaths; colorectal with 610,000 deaths and breast cancer with 460,000 deaths yearly (Ferlay *et al.*, 2008; Jemal *et al.*, 2011). It is believed that around 85% of lung cancer cases are related to smoking. Cancer starts when the cells start to grow out of control. At present, there are different treatments for cancer; however, the most accepted modality for cancer treatment involves surgery, radiation therapy, and medical prescription single or in a combination. A successful treatment should kill or incapacitate cancer cells without harming the normal cells. This is achieved by inducing apoptosis, a highly programmed cell death to eliminate abnormal cells.

Plants and plant-derived compounds have been used since ages as a major source for treating diseases in human (Ramawat and Merillon, 2008). Considering their high efficacy and low side effects, they have become the first priority of pharmaceutical industries. Therefore, there is a growing demand for screening plant-derived compounds against many complicated diseases, including cancer, diabetes, and obesity and so on. Hence, for the treatment of disease states, wherein drug therapy is a rational approach, plant materials represent legitimate starting materials for the discovery of new agents. In the case of human cancers, thus far, nine plant-derived compounds

have been approved for clinical use as anticancer drugs in the United States (Wang *et al.*, 1997; Lee, 1999a; Lee, 1999). However, still, most of the patients treated for cancer die from its treatment. Therefore, there is need of development of new and effective anticancer drugs. Nevertheless, there are many plant-derived anticancer drugs currently used for the treatment of cancer (da Rocha *et al.*, 2001).

Equisetum arvense (horsetail) is widely used in Saudi Arabia for generations as a traditional medicine for kidney related disorders, diuretics, gastroenteritis and urinary infections. The plant belongs to family Equisetaceae and has been used throughout the world, particularly, in the Middle East, Canada, Europe, and some Asian countries (Jinous A and Elnaz R 2012). In this study, the Anticancer, antidiabetic and antibacterial activities ethanolic extract of *Equisetum arvense* was evaluated.

MATERIAL AND METHODS

Preparation of plant material and extraction

Equisetum arvense was received as a gift sample in summer 2016 from department of botany and microbiology, King Saud University, Saudi Arabia. The sample was identified by the help of a botanist, and a voucher specimen number (KS-EA 00712) was deposited in the library of department of botany and microbiology, King Saud University. The collected plant was shade-dried for a period of one week (aerial part). The material was coarse powdered by grinding using a blender. Approximately, 50g of powdered leaves was extracted with 500ml of 70% ethanol for a period of five days in the dark at room temperature in a conical flask. After five

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days, the extract was filtered and evaporated to dryness under reduced pressure at 40°C. A yield of 4.6g was obtained. A desired concentration of sample was used as per requirement and dissolved in 5% dimethyl sulfoxide (DMSO). The remaining sample was stored at 4°C for future use. Further, the sample in DMSO was filter-sterilized using 0.22 µm pore size filter and used as such.

Preparation of A549 human lung carcinoma cell line

A549 lung carcinoma cell line was cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum in the relative humidified atmosphere with 96% air and 5% CO₂ at 37°C. The study protocol was approved by Institutional Ethics Committee.

Cell viability assay

The cytotoxicity of the EA1 extract on cancer cell line was measured by MMT assay. In brief, 96-well micro plates were seeded with 0.1mL of cell suspension in a medium. The cells were incubated for 20 hours for attachment and treated with various concentrations (100, and 150µg/ml) of EA1 and serially diluted in reverse order to lower the dosage concentration. The cells were incubated for 48 hours and cell viability was checked by spectrophotometer using tetrazolium salt as a cytotoxicity indicator. The reading was taken at 590nm.

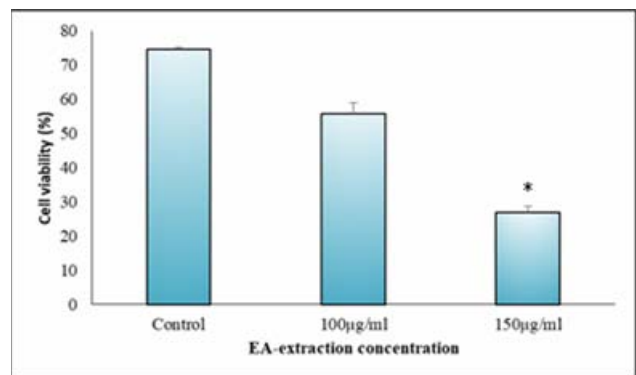


Fig. 1: Effect of EA-extraction on A549 cell viability. Values are means ± standard error. *P<0.05 versus control group.

Acridine orange staining

The A549 cells were seeded in tissue culture plates at a concentration of 10⁶ cells per well. The cells were divided in different groups treated without or with EA1 at a concentration of 100, 150µL per ml and incubated for 20 hours and finally washed with phosphate buffer (PBS). Further, the washed cells were stained with acridine orange at a concentration of 3µg/ml and immediately observed under fluorescence microscope.

STATISTICAL ANALYSIS

The data were evaluated by one way analysis of variance followed by Student's t-test. P values <0.05 were considered as statistically significant.

RESULTS

Cell viability

Cancer is one of the deadly diseases with the uncontrolled growth of cells. The cells grow beyond their limits in a particular organ and spreads to other organs. The effect of EA-1 on survival of A549 lung carcinoma cell line was evaluated by MMT assay. The A549 cell line was treated with various concentrations (100µg/ml and 150µg/ml) of EA1 for 24 hours. EA1 decreased the cell viability of A549 lung carcinoma cells. The cell viability precipitated by more than 75% at 150µg/ml of EA1, while more than 40% precipitation was observed with 100µg/ml of EA1, and more than 80% cells in control group remained viable (fig. 1).

Cytotoxicity effect of EA1

Equisetum arvense, a widely grown medicinal plant in Saudi Arabia was screened against A549 lung carcinoma cell line. The screening was carried out with an aim to find out whether the EA- extract can induce apoptosis in A549 lung carcinoma cell line. The cells were grown and treated with EA-extract. As shown in fig. 2, the effect of EA-extract inducing apoptosis in the lung carcinoma cell line was concentration dependent. The cells were observed under phase contrast microscope, normal cells of A549 were densely populated, polygonal or fusiform in shape with clear outlines and smooth edges. However, after treating with EA1 at a dosage of 100 and 150µg/mL, the cells were floating which is the sign of early apoptosis. In addition, the edges of many cells were not clear and the cytoplasm was not as transparent as seen in control cells without any treatment. Overall, the cell structure was completely desegregated with a hard-shelled appearance (fig. 2). In many systems, apoptosis is associated with the loss of mitochondrial inner membrane potential which may be regarded as a limiting factor in the apoptotic pathway (Bossy and Green, 1999).

Acridine orange staining

Acridine orange staining was used to verify the cytotoxicity or apoptosis in A549 cell line. A549 cells were treated with two different concentrations of EA1 (100, and 150µg/mL) and incubated for 24 hours. The cells taken as control exhibited green fluorescence. However, 50% cells developed orange fluorescence after treated with EA1 at 100µg/mL. Nevertheless, the effect was seen as concentration dependent, more than 70% cells developed orange fluorescence after treated with 150 µg/mL EA1. The development of orange or orange-red color indicated disruption of cell membrane (fig. 3).

DISCUSSION

Medicinal plants have been used for a long time for the prevention of cancer; however, there are still many plants which are not much explored. In many countries, herbal medicines are used as a traditional therapy to treat many

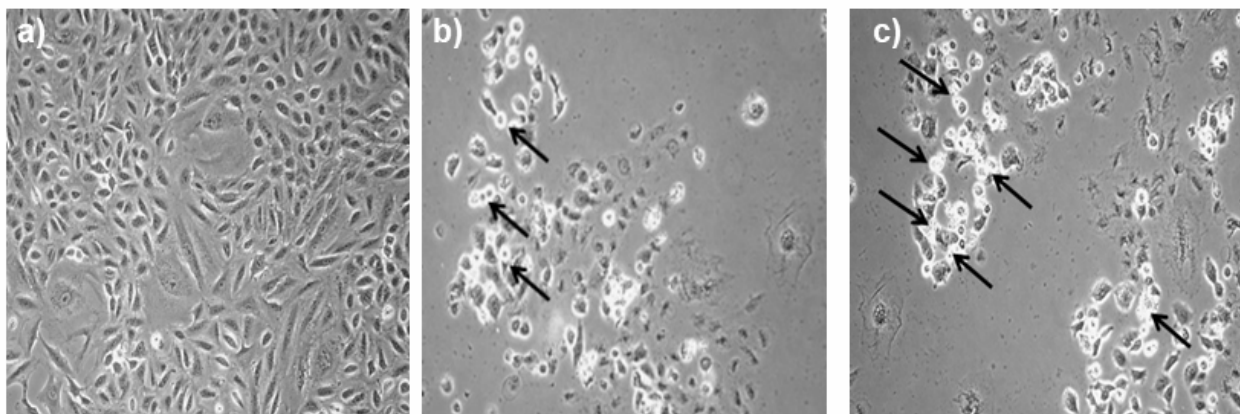


Fig. 2: Cytotoxicity effect of EA1 on A549 cells. a) Control. b). Treated with 100µg/mL of EA1, c). Treated with 150 µg/mL EA1.

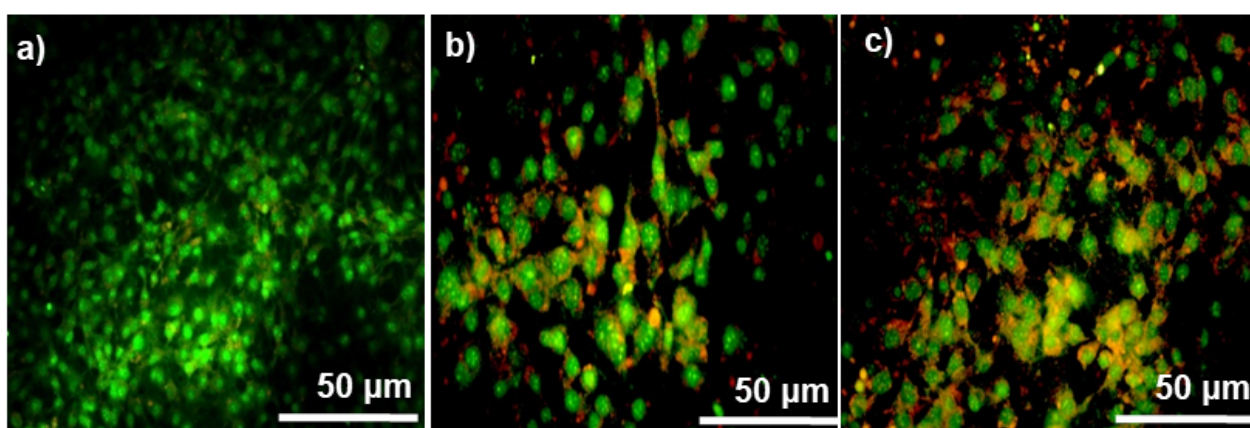


Fig. 3: A549 cells stained with acridine orange after incubation with various concentrations of EA1. a) Control. b). Treated with 100µg/mL of EA1, c). Treated with 150µg/mL EA1.

diseases and disorders. Cancer is a generic term for a large group of diseases that can affect any part of the body. The main feature of cancer is the rapid abnormal growth of cells beyond their usual limits, which can invade neighboring tissues or spread to other organs. Many cancers are fatal. In fact, cancer is a leading cause of death worldwide and accounts for around 7 million deaths every year (around 13% of all deaths).

Plants have been used for medicinal purposes as long as history has been recorded. Herbal medicine is a complementary therapy that uses plants to treat some disorders. In various countries, a large number of plants have been used as therapeutic agents in the traditional medicine system (Kamboj *et al.*, 2013; Sierpiana, 2001).

The plant contains abundant minerals such as calcium, potassium, phosphate, iron, manganese and silica (Gierlinger *et al.*, 2008; Law and Exley, 2011), as well as small amounts of pharmacologically active compounds (tannin, resin, cellulose, pectic substances, fatty acids, alkaloids, glycosides) and flavonoids (Asgarpanah and Roohi, 2012; Radulovic *et al.*, 2006). In addition, this plant has been used as a traditional medicine in

respiratory tract infections, bone tissue regeneration (Bessa *et al.*, 2012; Ferraz *et al.*, 2008), skin conditions (Asgharikhatooni *et al.*, 2015) and kidney diseases (Rao, 2002).

CONCLUSION

The study was carried out to evaluate the anticancer effect of EA1 extracted from *Equisetum arvense* on A549 lung carcinoma cell line. The results depict that *Equisetum arvense* has a potential apoptotic and cytotoxic effect on A549 lung carcinoma cell line, suggesting the presence of anti-cancer compounds in the extract. However, the isolation and identification of the active compound (s) would be beneficial for the development of biological therapy for lung cancer.

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