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Original article

Impact of ethanolic extract of *Equisetum arvense* (EA1) on pancreatic carcinoma AsPC-1 cells

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ABSTRACT

The current research was focused on evaluation of the cytotoxic and suppressive action of ethanolic extract of *Equisetum arvense* (EA1) against human pancreatic carcinoma cell line ASPC-1 after treatment with 25 μg/mL, 50 μg/mL, 100 μg/mL and 200 μg/mL EA1, using MTT assay and Antioxidant activity. Detailed investigations led to reveal the ability of cell patronage through the dreadful upshot of free radicals. The current approach followed MTT assays to examine the long-lasting ability and growth of cells as EA1 restrained the cell viability and growth of ASPC-1. At the end, EA1 showed its potential cytotoxicity and reduced the cellular proliferation of ASPC-1 cells through a pattern, which appeared to be concentration dependent. Our results can form the basis to explore the molecular mechanisms underlying Ethanolic Extract of *Equisetum arvense* induced cell death in pancreatic cancer cell lines and may serve as an alternative anticancer agent for the treatment of pancreatic carcinoma (PC) with no or least side effects to the patient.

1. Introduction

Pancreatic cancer (PC) has become one of the major factors of cancer-associated mortality in USA ranked being fourth presently, with the most overwhelming divination of all solid tumors. It is approximately determined that in 2015, about 83% of individuals diagnosed with PC in the USA (Siegel et al., 2015). In spite of the progresses in understanding the genetics of pancreatic cancer and implementation of integrate chemotherapy and tested biological agents, the management of this lethal malignancy comes out to be an exceptional oncological confrontation (Hidalgo, 2010). Currently, the only affluent remedy to ameliorate for a local pancreatic tumor is abscission and ancillary eradicator after surgery, which is prone to delayed relapse; nevertheless, the effect is inadequate (Vincent et al., 2011). Patients with progressive and metastatic pancreatic cancer, the first-line treatment are chemotherapy and drug gemcitabine has become the popular and well-attended medication for the restriction and repression of pancreatic ductal adenocarcinoma (PDAC) (Burris et al., 1997; Berlin and Benson, 2010). However, in clinical research, the retaliation ratio of pancreatic ductal adenocarcinoma to the chemotherapy drug gemcitabine is <25%, and the patients emerge drug resistant throughout therapy (Ducreux et al., 2007; Ying et al., 2012). The consequential occurrence of expeditious resistance to gemcitabine may be associated with the influence of various factors including stem-like subpopulations of tumor cells with inborn resistance to chemotherapy. Also, the cancer cells may adopt molecular alterations, like transformations of transport and metabolism of gemcitabine or the DNA repair pathway or the upregulation of phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) (Ng et al., 2001; Davidson et al., 2004; Nakano et al., 2007; Itoi et al., 2007; Hagmann et al., 2010). Additionally, clinical treatments with gemcitabine in association with molecular targeted therapies using erlotinib, tipifarnib and gefitinib (Moore et al., 2007; Fountzilas et al., 2008) show unsatisfactory potency. Overall, there is a need to identify novel chemotherapeutical agents or composite scheme for the deteriorating malign PC.

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In Africa, dependency on such conventional medicines from locally available traditional plants is negatively regulated and dominated by meager access to allopathic medicines and the demanding obligations to combat against pandemics such as HIV/AIDS (Mahomoodally, 2013). Despite little setbacks such as inefficient management of traditional remedies, lack of information's and high demands for conventional medicines, many developed countries seem to be venturing into the use of traditional vegetative flora for the treatment of variety of infections throughout the world (Sawadogo et al., 2012; Mahomoodally, 2013).

Primarily, *Equisetum arvense* L. (horsetail) is familiar prescription for kidney problems, digestive and urinary problems and diuretics in Saudi Arabia since decades. Geographically Equisetum, genus of herbaceous plant, are distributed worldwide except Australia and have been are extensively utilized in traditional medicine. *Equisetum arvense* L. is relatively safe for use in cell lines and animal model as it has LD_{50} value of more than 5000 mg/kg body weight (Miwa and Sakuma 2009; Tago et al., 2010). Topical use of extracts or ointments based on field horsetails (*Equisetum arvense* L.) have shown to cure wounds in animals (Hayat et al., 2011) and humans (Asgharikhatooni et al., 2015). The phenomenon of wound healing improvements was associated with antioxidants present in the plants extracts, which specifically remove or hinder the generation of reactive oxygen species (Suntar et al., 2012). In the extracts of horsetail, it was identified that there is a connection between the phenolics content and antioxidant activity (Nunes et al., 2017). Also, many previous studies have reported tremendous potential of *Equisetum arvense* in countering oxidative stress related disorders and diseases (Patova et al., 2019; Kour et al., 2017; Pallag et al., 2018; Steinborn et al., 2018; Arbabzadegan et al., 2019).

Based on the mentioned facts and extensive past studies, we have selected *Equisetum arvense* L. as a model plant to plaid its pharmacological applications versus lethal pancreatic carcinoma AsPC-1 cells.

2. Materials and methods

2.1. Ethanol extract preparation of Equisetum arvense

E. arvense was acquired as a representative specimen. The collected and cleaned aerial parts of plant (leaves) were put under shade for drying up to seven days. The leaves were further converted into coarse powder by grinding with a blender. Approximately, 50 g of leaves powder were squeezed with 500 mL of 70% ethanol for a time span of five days under shade at room temperature. The extracted concentrate was purified and vaporized till removal of 100% humidity under lowered pressure at 40 °C. A suitable amount of the specimen was utilized according to the demand and suspended in 5% dimethyl sulfoxide (DMSO). The rest of the amount was kept at 4 °C for further assessment.

2.2. Cell lines and culture conditions

American type culture collection (ATCC, Manassas, VA, USA) was the designated source of human AsPC-1 cell line incubated in Dulbecco's modified Eagle's medium (DMEM; Gibco, Thermo Fisher Scientific, Inc., Waltham, MA, USA). The cell culture was supplemented with 10% fetal bovine serum (FBS) (Biochrom, Germany), and 1% (v/v) penicillin-streptomycin (Biochrom, Germany) incubated in a humidified environment at 37 °C with proper regulation of keeping CO_2 lower than 5%.

2.3. 3-(4, 5-Dimethylthiazolyl2)-2, 5-diphenyltetrazolium bromide (MTT) assay

The growth ability of the ASPC-1 cells was verified through MTT assay. At a density of 5×10^3 cells per well, cells were plated on 96-well plates with 3 replicates. Initially, cells were cultured in DMEM medium supplemented with 10% FBS for 12 h in order to obtain a confluent monolayer. After this, the medium was exchanged with DMEM containing 5% FBS and EAI for 24, 48 and 96 h. Subsequently, 10 μl MTT (5 mg ml^{-1} in PBS) was put in each well for a specified period of time. The culture medium was separated away and MTT formazan was disintegrated in 150 μl DMSO per well for about 4 h later. All the samples were agitated for about 15 min, and OD_{490nm} was noted using an absorbance reader.

2.4. Antioxidant activity: 2,2′-Azino-bis (3- ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging examination

ABTS radical scavenging activity was performed by the method of Delgado-Andrade et al. (2005) with some modification. In brief, ABTS was produced by combining 7 mM ABTS stock solution in 2.45 mM potassium persulfate. Prior to utilization, the mixture was allowed to be static at 25 °C for 12–16 h. The ABTS + solution (stable for 2 days) concentration was decreased with the help of 5 mM phosphate-buffered saline solution (pH 7.4) to a level of 730 nm of 0.70 ± 0.02. The absorbance was investigated at 30 min after the introduction of 0.01 mL of sample EA1 to 4 mL of diluted ABTS + solution. Three repetitive analyses were carried out. The ABTS radical-scavenging activity of the samples was expressed as

$$S\% = (\text{“A“ control} - -\text{“A“ sample})/\text{“A“ control}) \times 100,$$

where “A” control is the absorbance of the blank control (ABTS solution without test sample) and “A” sample is the absorbance of the sample under trial.

2.5. Superoxide radical scavenging assessment

Following a recently described procedure, this capacity was determined through alleviation of NBT (Fontana et al. 2001). The non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS/NADH) approach triggered the reactive superoxide anion radicals, which were converted nitro blue tetrazolium (NBT) to a purple formazan. The 1000 μl reaction suspension was composed of phosphate buffering agent (20 mM, pH 7.4), NADH (73 μM), NBT (50 μM), PMS (15 μM) as well as several components (0.25–2 mg/ml) of testing EA1 solution. To scale up the amount of formazan produced, the absorbance was calculated at 562 nm in comparison with a suitable blank after incubation of 5 min at optimum temperature. Entire tests were repeated carefully up to six repetitions while quercetin was taken as a positive standard.

2.6. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

The DPPH radical-scavenging magnitude of EA1was established related to the technique illustrated by Kao and Chen (2006) after few amendments. Briefly, 1000 μl of specimen EA1 solution with various concentrations (0.25–2 mg/ml) was included to 2.0 mL ethanolic DPPH radical emulsion (0.05 mM). The blend was agitated continuously at 25 °C. The control group comprised of just non-ionized water and concentrated DPPH solution blanks constituted exclusively of ethanol and the cells. Following centrifugation at 8000*g* for 10 min, the absorbance of the final mixture was

calculated in triplicates at 517 nm. The scavenging proficiency was defined as:

$$\text{Scavenging proficiency}\,(\%) = [1 - (\text{Asample} - \text{Ablank})/\text{Acontrol}] \times 100.$$

2.7. Statistical analysis

Student's *t*-test or one-way analysis of variance (Dunnett's test and Least Significant Difference test) was followed to scrutinize the obtained data. $P < 0.05$ was considered to support a statistically prominent difference. All the statistics were explained as means ± standard approximation of three replicates.

3. Results

3.1. Cell vitality

Undisciplined spreading power of cells results in cancer to be a deleterious disorder. The cancerous cells extend out beyond their capacity to any body part and then propagate to invade other adjacent organs systematically. The consequences on the proliferation and survival of ASPC-1 cell line against EA1 interaction were observed by MMT assay. A few doses of (100 μg/ml and 150 μg/ml) of EA1 were exercised against the ASPC-1 cell line for 24 h. EA1 depleted the cell growth of ASPC-1 cells. Further the cell vitality expedited remarkably up to 80% at 200 μg/ml of extract (**$P < 0.01$**), while more than 50% precipitation was inspected with 100 μg/ml of EA1, and above 95% cells in standard category appeared to be viable (**$P < 0.05$**). However, the effect with 25 μg/ml and 50 μg/ml was not noticeable (Fig. 1).

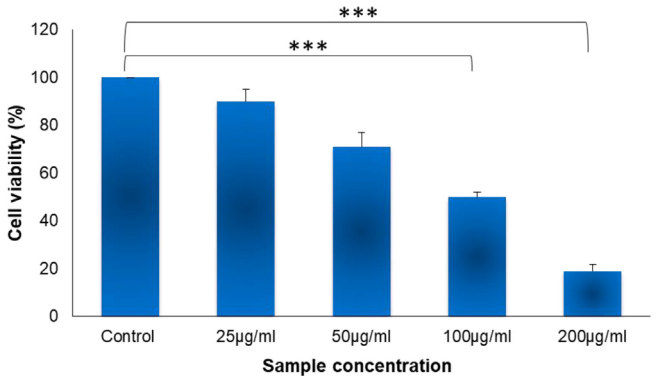

Fig. 1. Result of EAI on cell survival. Particulars are observed as average ± SD (n = 3). Values with *** are significantly different (P < 0.05).

3.2. Cytotoxic ability of EA1

Equisetum arvense L. an extensively cultivated therapeutic perennial herb in Saudi Arabia was found antagonist against ASPC-1 cells. The screening was carried out to see the capability of EA-extract to commemorate systematic cell-death pattern in ASPC-1. Cells were cultivated on suitable media and interacted with EA1 distillate. As obvious from Fig. 2, it was quantification-based potential of EA1 extract to initiate the programmed cellular death. The cells were analyzed with phase contrast microscope; standard ASPC-1 cellular growth was highly condensed, large in size, having sharp form and even margins. However, the cells were found floating once EA1 was applied to it at concentrations of 100, and 200 μg/mL, suggesting a preliminary evidence of apoptosis. Additionally, the extremities of several cells were found to be murky, and the cytoplasm was not as translucent enough as spotted in regular viable cells with no EA1 application. Altogether, the morphological features of cells were thoroughly disrupted revealing hard-shelled emergence (Fig. 2).

3.3. Antioxidant assay: ABTS radical scavenging assay

In ABTS assay, EA1 originated from the plant under study exhibited an elevated antioxidant value. However, the inhibition was based on quantity. Both ascorbic acid (200 μg/mL) and EA1 (200 μg/mL) manifested 83.66% and 63.3% inhibition, respectively (Fig. 3). However, the inhibition was less with 25 μg/mL and 50 μg/mL concentrations. The assay indicted successfully the EA1 potency and potential application as a source of antioxidants based on the ability of antioxidants compound to scavenge the long-life radical cation ABTS+. The radical anion ABTS•- is generated by ABTS2- oxidation by potassium persulfate.

3.4. Superoxide radical scavenging assessment

Various pathophysiological processes are subjected to association of superoxide anion. The hindrance of superoxide radical scavenging potential of gallic acid (200 μg/mL) and EA1 (200 μg/mL) are 85.35% and 64.18%, respectively. The consequences demonstrate that the aftermath of gallic acid and EA1 are concentration dependent as disclosed in Fig. 4. The minimum percentage inhibition was shown with 25 μg/mL of EA1.

3.5. DPPH free radical scavenging evaluation

DPPH free radical scavenging assay is considered a simple, rapid, and sensitive method with better reproducibility. Free radicals are harmful to cell and tissue and can cause their damage; therefore, radical scavengers may induce a vital part in shielding cells and tissues. The DPPH free radical scavenging analysis of

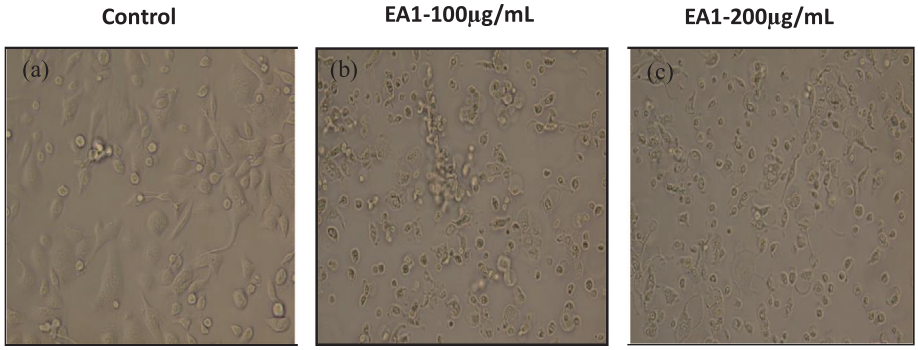

Fig. 2. Cytotoxic ability of EA1 on human ASPC-1 cells. (a). Control. (b). EA1-100 μg/mL. (c). EA1-200 μg/mL.

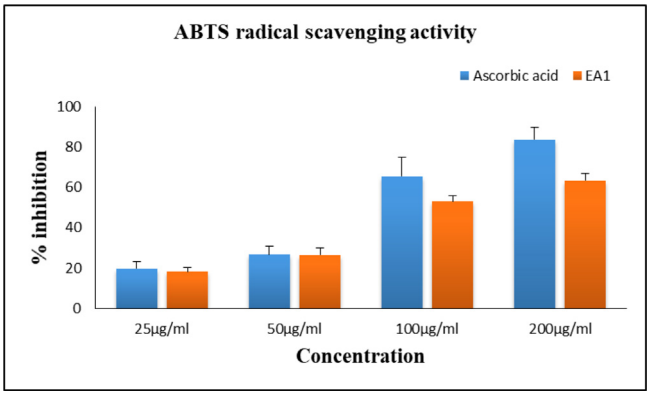

Fig. 3. ABTS radical scavenging activity of EA1 and standard antioxidant ascorbic acid.

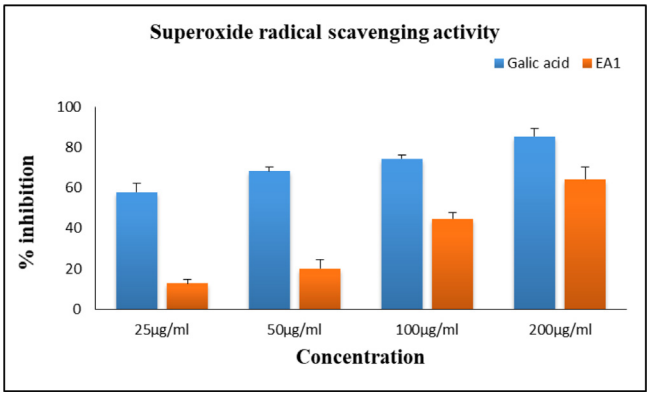

Fig. 4. Superoxide radical scavenging potential of EA1 and standard compound gallic acid.

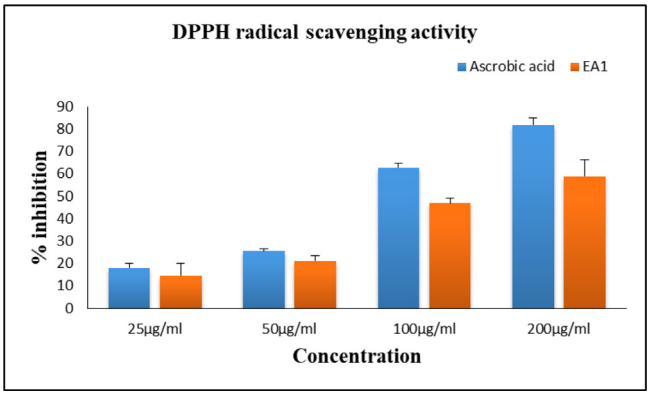

Fig. 5. DPPH radical scavenging ability of EA1 and standard compound ascorbic acid.

the EA1 and ascorbic acid is depicted in Fig. 5. The final outcome indicates that EA1 (200 µg/mL) along with ascorbic acid illustrated the excessive radical-scavenging ability 81.81% and 58.88%, respectively.

4. Discussion

Although there is much advancement in the treatment of different cancers, there is still a long way to go to improve the pancreatic cancer treatment and patient survival; thus make the identification and development of novel therapeutic compounds with improved efficiency for treatment of human pancreatic cancer cardinal. Pancreatic carcinoma is estimated to be amongst the leading cause of death amongst cancer patients in the coming decade (Rahib et al., 2014). Pancreatic cancer is a very aggressive and heterogeneous disease resistant to the available conventional treatment methods. Moreover, chemotherapeutic agents used to counter pancreatic cancer are highly toxic (Goldsmith et al., 2015; Siegel et al., 2012; Chang et al., 2008). Thus, it becomes crucial to control and manage the development of pancreatic cancer as well as to look for novel therapeutic strategies against it (Biankin et al., 2012).

Naturally, occurring products are known to have role in the prevention and treatment of various ailments in humans and animals. With ever increasing awareness of molecular science and advancement in isolation and structure elucidation techniques, various anti-cancer plant and plant products have been identified as well (Kour et al., 2017). From the beginning of human existence, plants and their derivatives with various biological effects are utilized as remedies in one way or the other. In the modern technological age, plant-based natural products have disclosed promising anti-pancreatic cancer effect both in vitro and in vivo (Yu et al., 2013).

Equisetum arvense L. have shown effective anticancer properties (Huda et al., 2017). The Ethanol extract (EA1) extracted from *Equisetum arvense* L. has cytotoxic effect on the A549 lung carcinoma cell line (Huda et al., 2017). So far, there are no reports on the effect of EA1 in pancreatic cancer cells. Therefore, this study was conducted to analyze the cytotoxic effect of ethanol extract (EA1) extracted from *Equisetum arvense* L. on the human pancreatic cancer cells, AsPC-1. Here, we observed that EA1 activate cell death in AsPC-1 through induction of apoptosis. Our results were in agreement with the study of Pallag et al., 2018.

In the ongoing study, the effect of EA1 on AsPC-1 cells was detected to be a quantificational-effective agent. Moreover, the antioxidant capacity of EA1 suggested it as unique and neoteric therapeutics for the obstruction and cure of pancreatic cancer. Nevertheless, the results further require quantification using animal trial and technique by which EA1 works warrant more study. In many approaches, apoptosis is related with the depletion of efficiency of deeper layers of mitochondria that might be termed as a regulating feature in the systematic and programmed cell death (Bossy-Wetzel and Green, 1999).

Pancreatic cancer is currently one the major death warrant in cancer-associated mortality in industrialized countries, with an incidence rate almost equivalent to its mortality rate which displays its detrimental strength. Despite research efforts, the progress regarding recovery of this disease is too little; therefore, it increases the necessity of modern and up to date action plan to discover and identify any corrective remedial drug for cancer.

5. Conclusions

In the present study, EA1 extracted from *Equisetum arvense* L. depicted a powerful suppressive effect against AsPC-1 cell growth. Furthermore, the extract inhibited noteworthy antioxidant restrictive potentiality. These findings underpin that EA1 is probably novel initiator for the restriction of pancreatic cancer and simultaneously a legitimate and reasonable antioxidant for its application in nutritional and pharma industries. However, further research analysis to distinguish and explore efficiency mechanism of EA1 is an obligatory requirement.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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