

RESEARCH ARTICLE

Portulaca oleracea Seed Oil Exerts Cytotoxic Effects on Human Liver Cancer (HepG2) and Human Lung Cancer (A-549) Cell Lines

Ebtesam Saad Al-Sheddi¹, Nida Nayyar Farshori¹, Mai Mohammad Al-Oqail¹, Javed Musarrat^{2,3}, Abdulaziz Ali Al-Khedhairi^{2,3}, Maqsood Ahmed Siddiqui^{2,3*}

Abstract

Portulaca oleracea (Family: Portulacaceae), is well known for its anti-inflammatory, antioxidative, anti-bacterial, and anti-tumor activities. However, cytotoxic effects of seed oil of *Portulaca oleracea* against human liver cancer (HepG2) and human lung cancer (A-549) cell lines have not been studied previously. Therefore, the present study was designed to investigate the cytotoxic effects of *Portulaca oleracea* seed oil on HepG2 and A-549 cell lines. Both cell lines were exposed to various concentrations of *Portulaca oleracea* seed oil for 24h. After the exposure, percentage cell viability was studied by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT), neutral red uptake (NRU) assays, and cellular morphology by phase contrast inverted microscopy. The results showed a concentration-dependent significant reduction in the percentage cell viability and an alteration in the cellular morphology of HepG2 and A-549 cells. The percentage cell viability was recorded as 73%, 63%, and 54% by MTT assay and 76%, 61%, and 50% by NRU assay at 250, 500, and 1000 µg/ml, respectively in HepG2 cells. Percentage cell viability was recorded as 82%, 72%, and 64% by MTT assay and 83%, 68%, and 56% by NRU assay at 250, 500, and 1000 µg/ml, respectively in A-549 cells. The 100 µg/ml and lower concentrations were found to be non cytotoxic to A-549 cells, whereas decrease of 14% and 12% were recorded by MTT and NRU assay, respectively in HepG2 cells. Both HepG2 and A-549 cell lines exposed to 250, 500, and 1000 µg/ml of *Portulaca oleracea* seed oil lost their normal morphology, cell adhesion capacity, become rounded, and appeared smaller in size. The data from this study showed that exposure to seed oil of *Portulaca oleracea* resulted in significant cytotoxicity and inhibition of growth of the human liver cancer (HepG2) and human lung cancer (A-549) cell lines.

Keywords: *Portulaca oleracea* - cytotoxicity - cellular morphology - cell viability - cancer cells

Asian Pac J Cancer Prev, 16 (8), 3383-3387

Introduction

Cancer is a leading cause of death worldwide, accounting for 8.2 million deaths in 2012 (IARC, 2012). Lung cancer (1.59 million deaths) and liver cancer (745 000 deaths) are the most common causes of cancer death (Fazeli et al., 2012; IARC, 2012). An accurate diagnosis of cancer diseases is much essential for adequate and effective treatment because of the specific type of cancer. Every cancer type requires a specific course of therapy which includes one or more modalities such as surgery, and/or radiotherapy, and/or chemotherapy. Chemotherapy is a category of cancer treatment that uses chemical substances, especially one or more anti-cancer drugs (chemotherapeutic agents). It is now considered as the most effective method of cancer treatment. Among the alternative traditional approaches, various plant products classified as alkaloids, saponins, triterpenes, glycosides, and polyphenols have shown very promising anticancer

properties in both *in vitro* and *in vivo* (Huang and Zou, 2011; Kma, 2013).

Portulaca oleracea (Family: Portulacaceae), is an annual green herbaceous medicinal plant widespread in temperate and tropical regions of the world (Yang et al., 2009). *Portulaca oleracea* is a fascinating plant recognised in most cultures for its extensive nutritional benefits. It has been used traditionally as a vegetable for human consumption (Bidhendi et al., 2014). The pharmacological potential of the *Portulaca oleracea*, such as anti-inflammatory (Chan et al., 2000), antioxidative (Dkhil et al., 2011), anti-bacterial (Zhang et al., 2002), skeletal muscle relaxant (Parry et al., 1993), wound-healing (Rashed et al., 2003), and *in vitro* anti-tumor (Yoon et al., 1999) activities have been reported. Recently we have also reported that seed extract of *Portulaca oleracea* induced cytotoxicity against human liver cancer cells (Farshori et al., 2014). Thus, the present investigation was carried out to study the anticancer activity of *Portulaca*

¹Department of Pharmacognosy, College of Pharmacy, ²Department of Zoology, College of Science, ³Al-Jeraisy Chair for DNA Research, King Saud University, Riyadh, Saudi Arabia *For correspondence: maqsoodahmads@gmail.com

oleracea seed oil on two cancer cell lines i.e. human liver cancer cell line (HepG2) and human lung cancer cell line (A-549) *in vitro*.

Materials and Methods

Reagents and consumables

DMEM culture medium, antibiotics-antimycotic solution, fetal bovine serum (FBS), and trypsin were purchased from Invitrogen, Life Sciences, USA. Consumables and culture wares used in this study were procured from Nunc, Denmark. All other specified reagents and solvents were purchased from Sigma Chemical Company Pvt. Ltd. St. Louis, MO, USA.

Cell cultures

Human liver cancer (HepG2) and human lung cancer (A-549) cell lines were cultured in DMEM, supplemented with 10% FBS, 0.2% sodium bicarbonate, and antibiotic/antimycotic solution (100x, 1 ml/100 ml of medium). Both the cell lines were grown in 5% CO₂ at 37°C in high humid atmosphere. Before the experiments, cell viability was assessed following the protocol of Siddiqui et al. (2008). Cells showing more than 95% cell viability and passage number between 20 and 22 were used in this study.

Plant material and extractions

The *Portulaca oleracea* seeds were obtained from the local market of Riyadh, Saudi Arabia. The seeds were screened manually to remove bad ones. They were then dried to constant weight in an oven at 70°C, ground using mechanical grinder, put in air-tight containers and stored in a desiccator. The oil from *Portulaca oleracea* seeds was extracted by continuous extraction in Soxhlet apparatus for 12 h using petroleum ether (60-80°C boiling range) as a solvent according to the method described by AOCS (Horwitz, 1980). At the end of the extraction the solvent was evaporated. The oil thus obtained was dried over anhydrous sodium sulphate and stored at -4°C for further analysis.

Experimental design

HepG2 and A-549 cells were exposed to various concentrations of *Portulaca oleracea* seed oil (10-1000 µg/ml) for 24h. Following the exposure of *Portulaca oleracea* seed oil, cells were subjected to assess the cytotoxic responses using MTT assay, NRU assay, and cellular morphological alterations using phase contrast inverted microscope.

Drug solutions

The extracts of *Portulaca oleracea* seed oil was not completely soluble in aqueous medium solution, therefore the stock solutions of all the extracts were prepared in Dimethylsulphoxide (DMSO) and were diluted in culture medium to reach the desired concentrations. The concentration of DMSO in culture medium was not more than 0.1% and this medium was used as control.

Cytotoxicity assessments

MTT assay: Percent cell viability was assessed using

the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay as described (Siddiqui et al., 2008). In brief, cells (1 x 10⁴) were allowed to adhere in 96 well culture plates in CO₂ incubator at 37°C for 24h. After the exposure, MTT (5 mg/ml) was added (10 µl/well in 100 µl of cell suspension), and plates were further incubated for 4h. Then, supernatants were discarded and 200 µl of DMSO were added to each well and mixed gently. The developed color was read at 550 nm using Multiwell Microplate Reader (Thermo Scientific, USA). Untreated sets were also run under identical conditions and served as control.

Neutral red uptake (NRU) assay: Neutral red uptake (NRU) assay was carried out following the protocol described (Siddiqui et al., 2010). Briefly, after the respective exposure with various concentrations of *Portulaca oleracea* seed oil, the medium was aspirated and cells were washed twice with PBS, and incubated for 3h in a medium supplemented with neutral red (50 µg/ml). Medium was washed off rapidly with a solution containing 0.5% formaldehyde and 1% calcium chloride. Cells were subjected to further incubation of 20 min at 37°C in a mixture of acetic acid (1%) and ethanol (50%) to extract the dye. The plates were read at 540 nm using multiwell microplate reader (Thermo Scientific, USA). The values were compared with the control sets run under identical conditions.

Morphological analysis

Morphological observation of cells treated with *Portulaca oleracea* seed oil were done to observe the alterations in the HepG2 and A-549 cells induced by *Portulaca oleracea* seed oil. Both the cell lines were exposed to increasing concentrations (10-1000 µg/ml) of *Portulaca oleracea* seed oil for 24h. The cell images were taken using an inverted phase contrast microscope (OLYMPUS CKX 41) at 20 x magnification.

Statistical analysis

Results were expressed as mean±SE of at least three independent experiments (each in triplicate). One way ANOVA was employed to detect differences between the groups of treated and control. The values showing p<0.05 were considered as statistically significant.

Results

Cytotoxicity assessment of *Portulaca oleracea* by MTT and NRU assays

The *in vitro* cytotoxic effects of *Portulaca oleracea* seed oil was evaluated by MTT and NRU assays. The cytotoxicity assessments in HepG2 cells exposed to *Portulaca oleracea* seed oil are summarized in Figure 1 and 2. HepG2 cells were exposed to various concentrations (10-1000 µg/ml) of *Portulaca oleracea* seed oil for 24h. Results showed that *Portulaca oleracea* seed oil induced statistically significant (p<0.001) decrease in the percentage cell viability of HepG2 cells in a concentration-dependent manner. The HepG2 cells exposed to *Portulaca oleracea* seed oil at 100 µg/ml and higher concentrations were found to be cytotoxic. The cell viability was recorded

as 86%, 73%, 63%, and 54% at 100, 250, 500, and 1000 $\mu\text{g/ml}$ of *Portulaca oleracea* seed oil, respectively by MTT assay (Figure 1) and 88%, 76%, 61%, and 50% at 100, 250, 500, and 1000 $\mu\text{g/ml}$ of *Portulaca oleracea* seed oil, respectively by NRU assay (Figure 2).

A-549 cells exposed to *Portulaca oleracea* seed oil at various concentrations (10-1000 $\mu\text{g/ml}$) for 24h also exhibited the statistically significant ($p < 0.001$) decrease in the percentage cell viability in a concentration-dependent manner (Figure 4 and 5). *Portulaca oleracea* seed oil exposed A-549 cells at 250 $\mu\text{g/ml}$ and higher

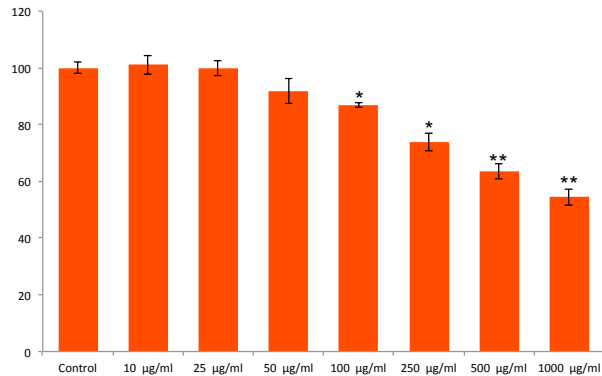


Figure 1. Percentage Cell Viability by MTT Assay in HepG2 Cells Exposed to Increasing Concentrations of Seed Oil of *Portulaca oleracea* for 24h. All values are mean+SD of three independent experiments. * $p < 0.05$; ** $p < 0.01$ vs control

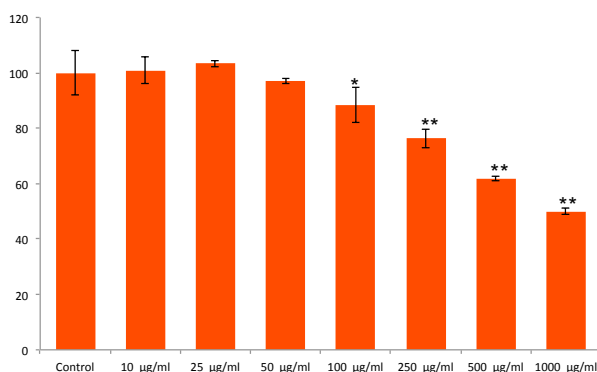


Figure 2. Percentage Cell Viability by NRU Assay in HepG2 Cells Exposed To Increasing Concentrations Of Seed Oil Of *Portulaca oleracea* for 24h. All values are mean+SD of three independent experiments. * $p < 0.05$; ** $p < 0.01$ vs control

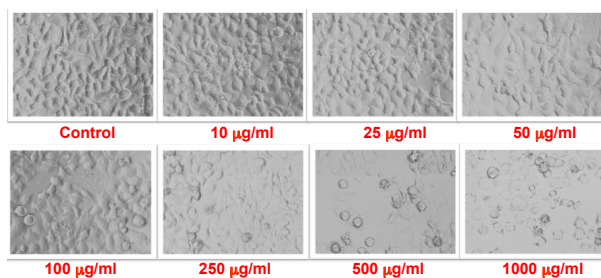


Figure 3. Morphological Changes in HepG2 Cells Exposed to Various Concentrations Of Seed Oil Of *Portulaca oleracea* for 24h. Images were taken using an inverted phase contrast microscope (OLYMPUS CKX 41) at 20 x magnification

concentrations decreased the cell viability. The decrease in the percentage cell viability at 250, 500, and 1000 $\mu\text{g/ml}$ of *Portulaca oleracea* seed oil was recorded as 82%, 72%, and 64% by MTT assay (Figure 4) and 83%, 68%, and 56% by NRU assay (Figure 5), respectively. The *Portulaca oleracea* seed oil at 100 $\mu\text{g/ml}$ and lower concentrations did not show any significant decrease in the percentage cell viability of A-549 cells as observed by MTT and NRU assays. The *Portulaca oleracea* seed oil extract was found more cytotoxic to HepG2 cells as compared to A-549 cell line.

Morphological changes

The morphological changes observed in HepG2 and A-549 cell lines are shown in Figures 3 and 6. The alterations in the morphology of HepG2 and A-549 cells exposed to *Portulaca oleracea* seed oil were found in a concentration dependent manner. HepG2 cells exposed to 100 $\mu\text{g/ml}$ and higher concentrations of *Portulaca oleracea* seed oil for 24 h lose the normal morphology and cell adhesion capacity as compared to control (Figure 3). However, *Portulaca oleracea* seed oil at 250 $\mu\text{g/ml}$ and higher concentrations lose the normal morphology and cell adhesion capacity of A-549 cell line as compared to control. Most of cells at higher concentrations appeared rounded in the shape (Figure 6). The HepG2 and A-549

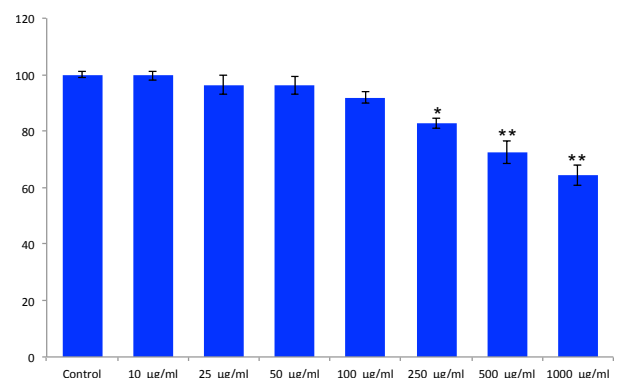


Figure 4. Percentage Cell Viability by MTT Assay in A-549 Cells Exposed to Increasing Concentrations of Seed Oil of *Portulaca oleracea* for 24h. All values are mean+SD of three independent experiments. * $p < 0.05$; ** $p < 0.01$ vs Control

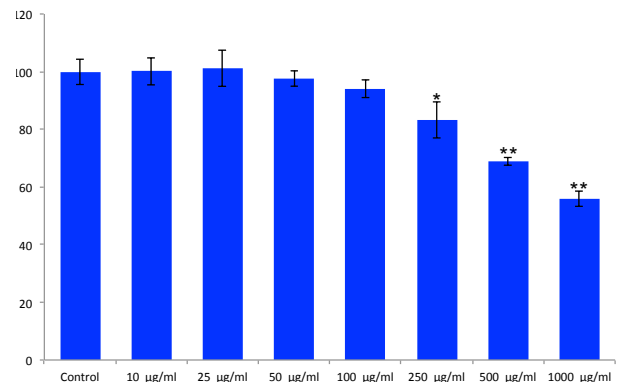


Figure 5. Percentage Cell Viability by NRU Assay in A-549 Cells Exposed to Increasing Concentrations of Seed Oil of *Portulaca oleracea* for 24h. All values are mean+SD of three independent experiments. * $p < 0.05$; ** $p < 0.01$ vs Control

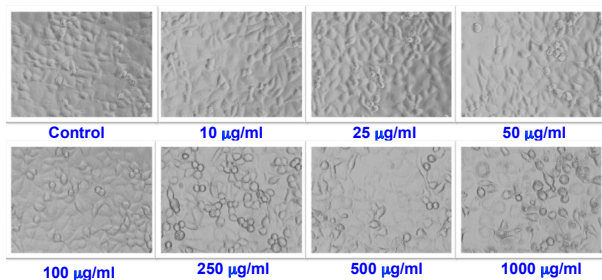


Figure 6. Morphological Changes in A-549 Cells Exposed to Various Concentrations of Seed Oil of *Portulaca oleracea* for 24h. Images were taken using an inverted phase contrast microscope (OLYMPUS CKX 41) at 20 x magnification

cell lines at 50 µg/ml and lower concentration did not cause any alterations in the cellular morphology.

Discussion

Plant derived natural products have received substantial consideration in recent times because of their diverse pharmacological and preventive properties, such as cytotoxic and cancer preventive effects (Pal et al., 2003; Al-Oqail et al., 2013; Farshori et al., 2013; Al-Sheddi et al., 2014). The natural products have been used as anticancer agents (Frei, 1982; Sasipawan et al., 2011), for example vincristine and vinblastine from *Catharanthus roseus* (Johnson et al., 1963), taxol and docetaxel from *Taxus brevifolia* (Wani et al., 1971), and camptothecins from *Camptotheca acuminata* (Wall et al., 1966). It is very well documented that vegetables and fruits may help in the reduction in the risk of cancers in human (Chen et al., 2006; Moon et al., 2011).

Portulaca oleracea, weedy plant in the purslane family (Portulacaceae), likely native to North Africa, the Middle East, and the Indian subcontinent and have been now naturalized in most parts of the world in tropical and subtropical regions (Sarah et al., 2013). It is considered quite nutritious because it is unusually high in omega-3 fatty acids (found mostly in fish and flax seeds) and contains significant amounts of vitamins A and C, as well as calcium, iron, magnesium and potassium and antioxidants (Alam et al., 2014; Uddin et al., 2014). Extensive modern pharmacological studies have attested its wide range of biological effects (Ezekwe et al., 1999). Many of these researches have verified the importance of *Portulaca oleracea* (Laitiff et al., 2010). Furthermore, with its high ethnopharmacological values and the present of cytotoxic polysaccharides and flavonoid especially luteolin, it is a promising plant in the investigation of cancer prevention (Liu et al., 2000). Therefore, the present study was designed to investigate *in vitro* anticancer activity of the *Portulaca oleracea* seed oil on human liver cancer (HepG2) and human lung cancer (A-549) cell lines. The cytotoxic effects of *Portulaca oleracea* seed oil were observed by MTT and NRU assays. We found that *Portulaca oleracea* seed oil significantly ($p < 0.01$) decreased the cell viability of both the cancer cell lines HepG2 and A-549 in a concentration-dependent manner. The results showed that 100-1000 µg/ml concentration

of *Portulaca oleracea* seed oil was found to be cytotoxic to HepG2 cells whereas 250-1000 µg/ml of *Portulaca oleracea* seed oil to A-549 cells. Our results are in well agreements with the previous studies showing *in vitro* anticancer activity of *Portulaca oleracea* at this concentration range against the various human cancer cell lines, i.e. HeLa and U14 (Zhao et al., 2013). The anti-proliferative effect of seed extracts of *Portulaca oleracea* have also been recently reported by us in HepG2 cells (Farshori et al., 2014). It has been also reported that water polysaccharides isolated from *Portulaca oleracea* possesses cytotoxic activity against cervical cancer (Chen et al., 2010). This cytotoxic effects observed in this study on HepG2 and A-549 cancer cell lines might be due to the presence of high antioxidant in *Portulaca oleracea*, which is mainly attributed to the rich source of omega-3 polyunsaturated fatty acids and flavonoid compounds; particularly kaempferol, apigenin, myricetin, quercetin, luteolin, carotene and alkaloids (Chan et al., 2000; Yang et al., 2009; Handique et al., 2012; Uddin et al., 2012). Despite of many beneficial effects of *Portulaca oleracea*, our findings demonstrate that *Portulaca oleracea* seed oil has anti-proliferative activity on human liver cancer and human lung cancer cells.

In conclusion, we have shown that *Portulaca oleracea* seed oil has anti-cancer activity on A-549 and HepG2 cell line. The results also demonstrated that *Portulaca oleracea* seed oil significantly decreased the percentage cell viability and altered the cell morphology of the cancerous cells in a concentration dependent manner. This study also provides preliminary screening of the cytotoxic potential of *Portulaca oleracea* seed oil on the human liver and lung cancer cell lines.

Acknowledgements

This work was supported by NSTIP strategic technologies program number (12-MED2491-02) in the Kingdom of Saudi Arabia.

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