RESEARCH ARTICLE

Cytotoxicity Assessments of *Portulaca oleracea* and *Petroselinum sativum* Seed Extracts on Human Hepatocellular Carcinoma Cells (HepG2)

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Abstract

The Pharmacological potential, such as antioxidant, anti-inflammatory, and antibacterial activities of *Portulaca oleracea* (PO) and *Petroselinum sativum* (PS) extracts are well known. However, the preventive properties against hepatocellular carcinoma cells have not been explored so far. Therefore, the present investigation was designed to study the anticancer activity of seed extracts of PO and PS on the human hepatocellular carcinoma cells (HepG2). The HepG2 cells were exposed with 5-500 µg/ml of PO and PS for 24 h. After the exposure, cell viability by 3-(4,5-dimethylthiazol-2yl)-2,5-biphenyl tetrazolium bromide (MTT) assay, neutral red uptake (NRU) assay, and cellular morphology by phase contrast inverted microscope were studied. The results showed that PO and PS extracts significantly reduced the cell viability of HepG2 in a concentration dependent manner. The cell viability was recorded to be 67%, 31%, 21%, and 17% at 50, 100, 250, and 500 µg/ml of PO, respectively by MTT assay and 91%, 62%, 27%, and 18% at 50, 100, 250, and 500 µg/ml of PO, respectively by NRU assay. PS exposed HepG2 cells with 100 µg/ml and higher concentrations were also found to be cytotoxic. The decrease in the cell viability at 100, 250, and 500 µg/ml of PS was recorded as 70%, 33%, and 15% by MTT assay and 63%, 29%, and 17%, respectively by NRU assay. Results also showed that PO and PS exposed cells reduced the normal morphology and adhesion capacity of HepG2 cells. HepG2 cells exposed with 50 µg/ml and higher concentrations of PO and PS lost their typical morphology, become smaller in size, and appeared in rounded bodies. Our results demonstrated preliminary screening of anticancer activity of *Portulaca oleracea* and *Petroselinum sativum* extracts against HepG2 cells, which can be further used for the development of a potential therapeutic anticancer agent.

Keywords: HepG2 cells - cytotoxicity - cellular morphology - anticancer activity

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Introduction

Hepatocellular carcinoma, is the sixth most common cancer worldwide, continues to have high prevalence in many Asian countries (Fazeli et al., 2013). Due to the poor prognosis, hepatocellular carcinoma is the fourth fatal cancer in the world (Mohamad et al., 2013). It is also the third most frequent cause of cancer deaths among men worldwide (Parkin et al., 2005). Usually, males are more affected than females and, are most common between the 30-50 years of age (Kumar et al., 2003). Chemotherapy is the most common treatment for the cancer. Unfortunately, many of the chemotherapeutic drugs are non-specific and cause severe side effects. Therefore, searching for new alternative strategies for the treatment and prevention of heptocellular carcinoma is necessary. Natural products have been considered as a valuable source for the anticancer drug discovery (Svejda et al., 2010; Khan et al., 2011; Randhawa and Alghamdi, 2011; Sharma et al., 2011; Thoppil et al., 2013; Al-Oqail et al., 2013; Farshori et al., 2013, Al-Sheddi et al., 2014).

Portulaca oleracea (purslane), is an annual green herbaceous medicinal plant widespread in temperate and tropical regions of the world (Yang et al., 2009). 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. The *Petroselinum sativum* (PS) or parsley, a member of the family of Umbelliferae, has also been reported to have antioxidant (Kreydiyyeh et al., 2001; Ahmed et al., 2010), antidiabetic (Yanardag et al., 2003), anti-inflammatory, antiedema antihypertensive, antimicrobial (Wahba et al., 2010) activities. Recently we have also reported that seed and oil extracts of *Petroselinum sativum* induced cytotoxicity against human breast cancer cells (Farshori

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et al., 2013).

Due to the diverse pharmacological and preventive properties of these plant extracts, present investigation was carried out to screen the anticancer activity of seed extracts of *Petroselinum sativum* and *Portulaca oleracea* against hepatocarcinoma cells (HepG2). The HepG2 cell line has been well established *in vitro* model for anti hepatocellular carcinoma (Li et al., 2014).

Materials and Methods

Chemicals and consumables

Dulbecco's Modified Eagle's Medium (DMEM) culture medium, antibiotics-atimycotic solution, fetal bovine serum (FBS) and trypsin were purchased from Invitogen, Life Sciences, USA. Consumables and culture wares used in the study were procured from Nunc, Denmark. All other specified reagents and solvents were purchased from Sigma Chemical Company Pvt. Ltd. St. Louis, MO, USA.

Plant material and extractions

The seeds of *Portulaca oleracea* and *Petroselinum sativum* and used in this study were obtained from the local market of Riyadh, Saudi Arabia. The seeds were screened manually to remove bad ones. For the preparation of alcoholic extract, the seeds were macerated in alcohol and then filtered. The procedure was repeated several times. The solvent was then evaporated using a rotary evaporator and the residue so obtained was called as the alcoholic extract.

Cell culture

HepG2, human hepatocellular carcinoma cells were cultured in DMEM, supplemented with 10% FBS, 0.2% sodium bicarbonate and antibiotic/antimycotic solution (100x, 1ml/100 ml of medium). Cells were grown in 5% CO_2 at 37°C in high humid atmosphere. Before the experiments, viability of cells was assessed following the protocol of (Siddiqui et al., 2008). HepG2 cells showing more than 98% cell viability and passage number between 20 and 22 were used in the present study.

Experimental design

HepG2 cells were exposed to various concentrations (5-500 µg/ml) of seed extracts of *Portulaca oleracea* and *Petroselinum sativum* for a period of 24 h. Following the exposures, HepG2 cells were subjected to assess the cytotoxic responses using 3-(4, 5-dimethylthiazol-2yl)-2, 5-biphenyl tetrazolium bromide (MTT), neutral red uptake (NRU) assay, and cellular morphology by phase contrast inverted microscope.

Drug solutions

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

Cytotoxicity screening

MTT assay: 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). Briefly, HepG2 cells (1x104) were allowed to adhere for 24 h CO2 incubator at 37°C in 96 well culture plates. After the respective exposure, MTT (5 mg/ml of stock in PBS) was added (10 µl/well in 100 µl of cell suspension), and plates were incubated for 4 h. 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 (Siddiqui et al., 2010). Briefly, after the exposure, the medium was aspirated and cells were washed twice with PBS, and incubated for 3 h 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 changes in HepG2 cells exposed to increasing concentrations (5-500 µg/ml) of PO and PS extracts were taken using an inverted phase contrast microscope (OLYMPUS CKX 41) at 20 X magnification.

Statistical analysis

The results were expressed as mean and standard error of means (SEM). 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 assessments by MTT and NRU assay

The cytotoxicity assessments by MTT and NRU assay in HepG2 cells exposed to PO extract are summarized in Figure 1 and 2. HepG2 cells were exposed to various concentrations (5-500 µg/ml) of PO for 24 h. Results showed that PO induced statistically significant ($p<0.001$) decrease in the cell viability of HepG2 cells in a concentration dependent manner. The HepG2 cells exposed to PO at 50 µg/ml and higher concentrations were found to be cytotoxic. The cell viability was recorded as 67%, 31%, 21%, and 17% at 50, 100, 250, and 500 µg/ml of PO, respectively by MTT assay (Figure 1) and 91%, 62%, 27%, and 18% at 50, 100, 250, and 500 µg/ml of PO, respectively by NRU assay (Figure 2).

HepG2 cells exposed to PS extract at various concentrations (5-500 µg/ml) for 24 h also exhibit the

statistically significant (p<0.001) decrease in the cell viability in a concentration dependent manner (Figure 4 and 5). PS exposed HepG2 cells with 100 µg/ml and higher concentrations decreased the cell viability. The decrease in the cell viability at 100, 250, and 500 µg/ml of PS was recorded as 70%, 33%, and 15% by MTT assay (Figure 4) and 63%, 29%, and 17% by NRU assay (Figure 5), respectively. The PO and PS at 25 µg/ml and lower concentrations did not show any decrease in the cell viability of HepG2 cells as observed by MTT and NRU assays. The PO extract was found to be more cytotoxic to HepG2 cells as compared to PS extract.

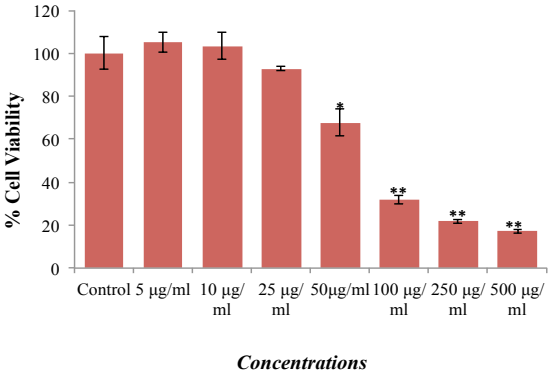

Figure 1. Cytotoxicity Assessment by MTT Assay in HepG2 Cells following the Exposure of Various Concentrations of Portulaca oleracea (PO) seed Extracts for 24 h. Values are mean±SE of three independent experiments. *p<0.05, **p<0.01 *vs* control

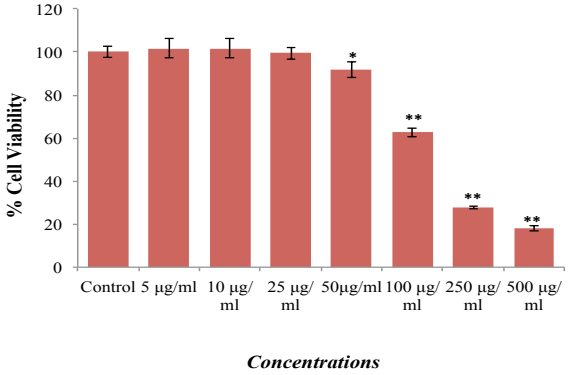

Figure 2. Cytotoxicity Assessment by NRU Assay in HepG2 cells Following the Eexposure of Various Concentrations of *Portulaca oleracea* (PO) seed Extracts for 24 h. Values are mean±SE of three independent experiments. *p<0.05, **p<0.01 *vs* control

Morphological changes

The morphological changes observed in HepG2 cells are shown in Figures 3 and 6. Alterations in the morphology of HepG2 cells exposed to PO and PS were found in a concentration dependent manner. Cells exposed to 50 µg/ml and higher concentrations of PO for 24 h lose the normal morphology and cell adhesion capacity of

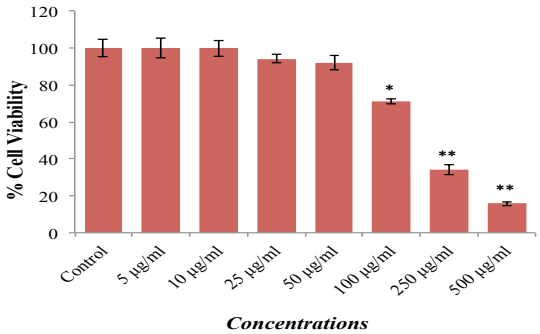

Figure 4. Cytotoxicity Assessment by MTT Assay in HepG2 Cells Following the Exposure of various Concentrations of Petroselinum sativa (PS) seed Extracts for 24 h. Values are mean±SE of three independent experiments. *p<0.05, **p<0.01 *vs* control

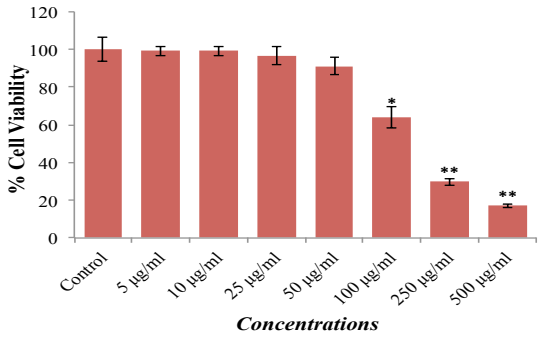

Figure 5. Cytotoxicity Aassessment by NRU Assay in HepG2 Ccells Following the Exposure of various Concentrations of Petroselinum sativa (PS) seed Extracts for 24 h. Values are mean±SE of three independent experiments. *p<0.05, **p<0.01 *vs* control

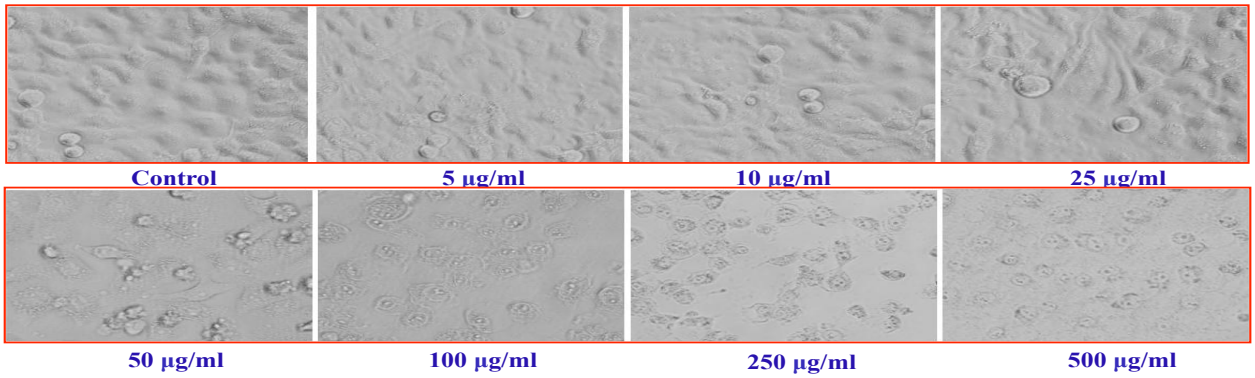

Figure 3. Morphological Changes in HepG2 Cells Following the exposure of Various Concentrations of *Portulaca oleracea* (PO) Seed Extracts for 24 h. Images were taken using an inverted phase contrast microscope (OLYMPUS CKX 41) at 20 X magnification

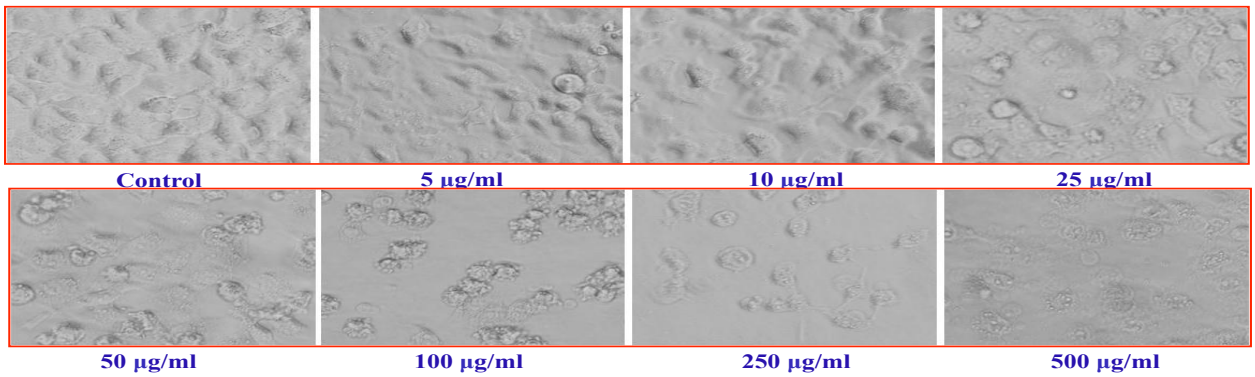

Figure 6. Morphological Changes in HepG2 Cells following the Exposure of Various Concentrations of Petroselinum Sativa (PS) Seed Extracts for 24 h. Images were taken using an inverted phase contrast microscope (OLYMPUS CKX 41) at 20 X magnification

HepG2 cells as compared to control (Figure 3). However, PS at 100 µg/ml and higher concentrations lose the normal morphology and cell adhesion capacity of HepG2 cells as compared to control. Most of cells at higher concentrations appeared rounded in the shape (Figure 6). The PO and PS at 25 µg/ml and lower concentration did not effect on the morphology of HepG2 cells.

Discussion

Several studies demonstrate that traditional medicine could be a promising source in the development of a potential therapeutic anticancer drug (Pan and Ho, 2008; Al-Oqail et al., 2013; Farshori et al., 2013, Al-Sheddi et al., 2014). Herbal medicines have long been viewed as a source of curative remedy based on religious and cultural traditions (Ghazanfer, 1994; Pal and Shukla, 2003; Saetung et al., 2005). *Portulaca oleracea* (purslane), is well known for its preventive and pharmacological potential, such as anti-inflammatory (Chan et al., 2000), antioxidative (Dkhil et al., 2011; Erkan et al., 2012), anti-bacterial (Zhang et al., 2002) activities. Thus, in order to provide comparative data on *in vitro* anticancer activity of the extracts of *Portulaca oleracea* (PO) and *Petroselinum sativum* (PS) on human hepatocellular carcinoma cell lines (HepG2), the present study was designed. The cytotoxicity of PO and PS extracts were observed by MTT and NRU assays. The results indicated that PO and PS seed extracts decreased the cell viability of HepG2 cells in a concentration-dependent manner. The study demonstrated that 50–500 µg/ml of PO and 100-500 of PS concentrations significantly ($p<0.01$) decreased the cell viability. Our results are in accordance with the previous studies showing *v* anticancer activity of PO at 50-500 µg/ml concentrations against the human cervical cancer (HeLa) and the mouse cervical carcinoma (U14) cells (Zhao et al., 2013). The protective potential of PO against Ehrlich ascites carcinoma bearing albino mice have also been reported (Ali et al., 2014). Our results from present study suggest that the cytotoxicity of the PO might be due to free radical scavenging property of extract in the presence of antioxidant phytochemicals (Han et al., 2003; Peksel et al., 2006; Ebrahimzadeh et al., 2009) and the presence of active compounds (Huang and Zou, 2011; Kma, 2013), which is showing anticancer activity on HepG2 cells. Studies also showed that constitutes of various plant extracts also inhibit the growth of other cancerous cells (Li et al., 1995; Kim et al., 2002; Kumi-Diaka and Butler, 2000). Recently, we have also shown that plant extracts decreased the cell viability of various cancer cells, including human epidermoid cancer cells (HEp2), human breast adenocarcinoma cells (MCF-7), human amniotic epithelial cells (WISH), and human lung cancer cells (Al-Oqail et al., 2013, Farshori et al., 2013, Al-Sheddi et al., 2014). The alterations in the morphology of HepG2 exposed to various concentrations of seed extracts of PO and PS observed by phase contrast inverted microscope showed the detachment of the cells with the increasing concentrations. These kinds of alterations in the cellular morphology induced by plant extracts at higher concentration have also been reported (Berrington and Lall, 2012; Al-Oqail et al., 2013, Farshori et al., 2013, Al-Sheddi et al., 2014).

Our results also showed that PS extract significantly decreased the cell viability of HepG2 in a concentration dependent manner. The antioxidant (Kreydiyyeh et al., 2001; Ahmed et al., 2010), antidiabetic (Yanardag et al., 2003), anti-inflammatory, antiedema antihypertensive, and antimicrobial (Wahba et al., 2010) activities of PS extracts have previously been reported. Our results are in well correspondence with the previous findings of anticancer activity of seed extract of PS against MCF-7 cells (Farshori et al., 2013). Many studies have also shown that this kind of effects towards cancerous cells may be due to the presence of bioactive components such as, polysaccharides, flavonoids, coumarins, monoterpene glycoside and alkaloids in these plant extracts (Xiang et al., 2005, Xin et al., 2008, Li et al., 2009, Tan et. al., 2013). Various plant extracts have also been found to induce cytotoxicity on human breast cancer T47D cells (Abdolmohammadi et al., 2008), human gastric adenocarcinoma (MK-1), human uterus carcinoma (HeLa), and murine melanoma (B16F10) cells (Fujika et al., 1999). Recently, we have also shown that PS seed extracts decreased the cell viability of human breast cancer cells (MCF-7) (Farshori et al., 2013). The PO extract showed higher cytotoxicity in HepG2 cells as compared to the PS extract. The difference in the response of plant extracts towards the HepG2 cells could be due to the presence of active components (Samarakoon et al., 2010)

and flavanoids (Das et al., 2010).

In conclusion, our results showed that *Portulaca oleracea* (PO) and *Petroselinum sativum* (PS) extracts significantly reduced the cell viability, and altered the cellular morphology of HepG2 cells in a concentration dependent manner. The data also revealed that HepG2 cells were more sensitive to PO than the PS extract. Further molecular studies are undergoing to elucidate the mechanism(s) of action of these extracts on human hepatocellular carcinoma cells.

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