



Cytotoxic effect of *Prangos Pabularia* extract on HeLa cell line a medicinal plant

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ABSTRACT

Cancer is regarded as a complex disease which is non-curable. Traditional medicines have been applied for the treatment of cancers in the world. Prangospabularia DC. (Apiaceae) is a perennial herb native to mountain slopes of the central and western Asia countries. Prangos species are widely used in folk medicine. Anticancer activity of Prangospabularia extract against HeLa line was studied using cytotoxicity effect. Many antitumor agents, have been reported to induce apoptotic cell death. This cell death plays a critical role in killing tumor cells in cancer therapy⁽¹⁾. In order to evaluate of cytotoxicity and antioxidant properties of the plant, dried powdered of roots of P.pabularia were Soxhlet extracted successively with n-hexan, dichloromethane (DCM) and methanol. Viability and cytotoxicity of HeLa cell line were measured by MTT assays. The antioxidant potential of the plant extracts was evaluated by DPPH assay. Additionally characteristic of cell death were using H&E morphological staining. The DCM extract showed a significant antioxidant effect with RC_{50} value of 0.08 mg/ml and our results showed that DCM extract displayed a significant cytotoxic activity against HeLa cell line in culture with a IC_{50} value of 0.526 mg/ml¹ in 24h with MTT assay. Additionally characteristic of aponecrotic cell death confirmed using H&E morphological staining. This study reveals biological effects and antitumor activity of Prangospabularia DC.(Apiaceae) extract against HeLa line.

Keywords: cytotoxicity, cell line, *Prangospabularia*, antioxidant

INTRODUCTION

Cancer is one of the main causes of death in United States and many parts of the world. According to a report dealing with the incidence and mortality of cancers in the USA, a total of 1,665,540 new cancer cases and 585,720 deaths from cancer were projected to occur in 2014⁽²⁾. Cancer is regarded as a complex disease which is non-curable in most cases⁽³⁾. Breast and cervical cancers are the most common cancers in women in Iran and worldwide⁽⁴⁾. Conventional medicine, surgery, chemotherapy and radiotherapy are primary approaches for cancer treatment, but they are not always effective.

For many years, traditional medicines (TM) have been applied for the treatment of cancers in the world. Herbal medicines are generally low in cost, plentiful, and show very little toxicity or side effects in clinical practice.

Genus *Prangos* (family Apiaceae), consists of more than 36 species and are mainly distributed in mediterranean region and western and central Asia. *Prangos* is herbaceous, perennial plant growing up to 1m high and its roots and

fruits have medicinal value⁽⁵⁾. Some *Prangos* species have been applied in the traditional medicine as carminative, antiflatulent, antihelminthic, antifungal, and antibacterial agents^(6, 7).

Prangos produces a large number of coumarins and secondary metabolic products⁽⁸⁾. Coumarins and their derivatives have considerable medicinal functions^(8, 9) and antiproliferative effects against malignant cell lines⁽¹⁰⁾ by inducing apoptosis. Apoptosis is a programmed cell death leading to cell death in response to environmental or developmental signals. Outstanding morphological characteristics of apoptosis are cytoplasmic blebbing, cell shrinkage, chromatin condensation and nucleosomal fragmentation⁽¹¹⁾. Many antitumor agents, have been reported to induce apoptosis cell death and therefore plays a critical role in killing tumor cells in cancer therapy⁽¹⁾. Currently, using medicinal plants is regarded as an effective strategy for treatment of various cancers. So, using some compounds that are derived from plants has been established in various cancer therapeutic regimens. The study was undertaken to determine cytotoxic effects of *prangospabularia* extracts on HeLa cell lines.

MATERIALS AND METHODS

General experimental procedures

An experimental study was designed to examine the effect of *prangospabularia* extract on cancer cells and were assigned into experimental and control group

Cell line and culture conditions

HeLa cancer cell line were obtained from Pasteur Institute of Iran . Hela (NCBI:C115) cell lines were grown in RPMI 1640 medium (Gibco, No. 51800-019). Each 500 ml of the medium was supplemented with 10% ml heat inactivated fetal calf serum (FCS)⁽¹²⁾, in 25 cm²culture flasks at (37°C) in a incubator with humidified atmosphere and 5% CO₂. In the following assays, allcells had a passage number of 3- 5.

Plant material

The roots of *P. pabularia* were collected from the MishovDagh Mountains, EastAzerbaijan, Iran, in September 2006. The plant was identified by the Department of Biology, Faculty of Sciences, University of Mohaghegh Ardabili. A voucher specimen(No: 1386-1) has been deposited at the Herbarium of the University. Dried, powdered roots of *P. pabularia*(100 g) were Soxhlet extracted successively with n-hexan, dichloromethane (DCM) and methanol.^(13, 14)

Cytotoxicity assay

HeLa cancer cell line were obtained from Pasteur Institute of Iran . Hela (C115) cell lines were grown in RPMI 1640 medium (Gibco, No. 51800-019). Each 500ml of the medium was supplemented with 10% ml heat in activated fetal calf serum (FCS) in deionized water. The stock solutions of extract were prepared by dissolving the compound indimethylsulfoxide (DMSO) 100 mL. The final concentration of the compound was (0.05,0.10, 0.50, 0.75 and 1mg ml⁻¹).Cells were plated in the appropriate media on 24-well plates in a 500 ml total volume at a density of 3*10⁵cells/well. The plates were incubated at 37 °C in 5% CO₂ for a time period of (4, 8,16 and 24h). The experiments were performed in triplicate to confirm the accuracy of the results. cytotoxicity were evaluated by a colorimetric assay using 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) (Sigma cat. 2128)⁽¹⁵⁾ In MTT assay, the OD570 nm was determined using a spectrophotometer and the 50% inhibitory concentration (IC₅₀) was defined as the concentration that reduced the absorbance of the untreated wells by 50%,and the viability percentage was evaluated as OD treatment/ODcontrol⁽¹⁶⁾.

Antioxidant assay

Serial dilutions were carried out with the stock solutions (1 mg ml⁻¹) of 8-geranyloxyxpsoralen to obtain concentrations 0.5, 0.25, 0.125, 0.0625, 0.0313 and 0.0156 mg ml⁻¹. Diluted solutions (5 ml each) were mixed with 5 mL of 2,2-diphenyl-1-picryl hydrazyl (DPPH, Sigma) and allowed to stand for 3 min for any reaction to occur. The UV absorbance was recorded at 517 nm. The same procedure was followed for the positive control Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid, Sigma-Aldrich, UK). The experiment was performed in duplicate and the average absorption was noted for each concentration. The RC50 value, which is the concentration of the test material that reduces 50% of the free radical concentration, was calculated as milligram per millilitre⁽¹⁷⁾

Morphological staining

To observe the morphological changes of the cells an inverted phase contrast microscope was used . Cells were

inoculated at 3×10^5 cell/well in 24 wellmicroplates and treated mentioned manner. Other culture wells were treated by Actinomycin D (50 μ M) and H₂O₂(100 μ M) as positive control of apoptosis and necrosis, respectively, as negative control some culture wells were prepared without any treatment⁽¹⁸⁾. After being cultured for 16h, the culture media removed and cells fixed and stained by the standard hematoxylin-eosin method. The prepared samples were photographed at $\times 100$.

Statistical analysis

One-way analysis of variance (ANOVA), Duncan and Wilcoxon test were used for data analysis. All the results are expressed as the mean \pm SEM, and p-values below 0.05 were considered statistically significant.

RESULTS

Antioxidant activity

In this study antioxidant assay showed that the dichloromethane extract of *P. pabularia* roots significantly exhibited antioxidant activity. The DPPH assay indicated that DCM extract of *P. pabularia* roots displayed high free radical scavenging activity with a RC₅₀ value of 0.17 mg ml⁻¹. The n-hexane and Methanol extracts exhibited modest antioxidant properties with a RC₅₀ value of 1.38 and 0.17 mg ml⁻¹ (Table1).

Table 1: Reduction of DPPH by different extracts

| Extracts | RC ₅₀ value mg ml ⁻¹ |
|----------|--|
| DCM | 0.08 |
| Metanol | 0.17 |
| n-hexane | 1.38 |

Cytotoxic evaluation

The cytotoxic effect of DCM extract was evaluated using MTT assay⁽¹⁹⁾. RC₅₀ extract showed that DCM extract the highest antioxidant effect with RC₅₀ 0.08 mg ml⁻¹. Cytotoxic studies for a time period of 4, 8, 16 and 24h assayed, DCM extract of *P. pabularia* displayed cytotoxic effect in a dose dependent manner that the highest concentration in 1 mg ml⁻¹, the plant extract inhibited the growth of HeLa cancer cell line and this effect by reducing the concentrations of the extract (0.05, 0.1, 0.5, 0.75, 1 mg ml⁻¹) are decreased, A concentration of 1 mg ml⁻¹ in treatment times of 4, 8, 16, 24 hours cells had 57%, 35%, 21%, 20% the ability to viability Which is most obvious in the period of 16 hours. IC₅₀ for 4h treatments is 1.64 mg ml⁻¹, for the treatment of 8h is 0.66 mg ml⁻¹, , for the treatment of 8his 0.63 mg ml⁻¹ and for the treatment of 24his 0.52 mg ml⁻¹. A 100% cytotoxicity was found when the extract concentrations were increased to above 1 mg/ml(Figure 1).

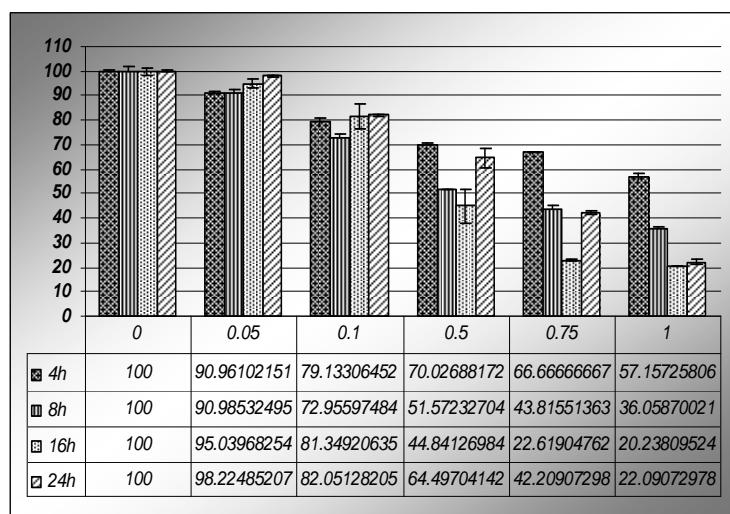


Fig.1: Cytotoxicity effect of *P.pabularia* on Hela cell line in dose-dependent and time

Morphological staining

Morphological study of cell shape changes was performed by direct microscopy, hematoxylin and eosin staining. Using an inverted phase-contrast microscope (400X), it was found that the untreated cells exhibited normal shapes, with clear outline. Although the growth of the DCM-extract-treated cells was obviously inhibited. The extract-treated cells were round, proliferation was inhibited and slowed (Figure 2).

To examine morphological changes, the treated cells were stained with haematoxylin and eosin. The shape of control cells was normal and the nuclei were round, homogeneous and dark blue, while the cells treated with 0.5 and 1mg ml^{-1} after 24 h exhibited typical characteristic of aponecrosis⁽²⁰⁾, such as nuclear condensation was stained dark blue, as shown in positive control of apoptotic cells (Figure 3)

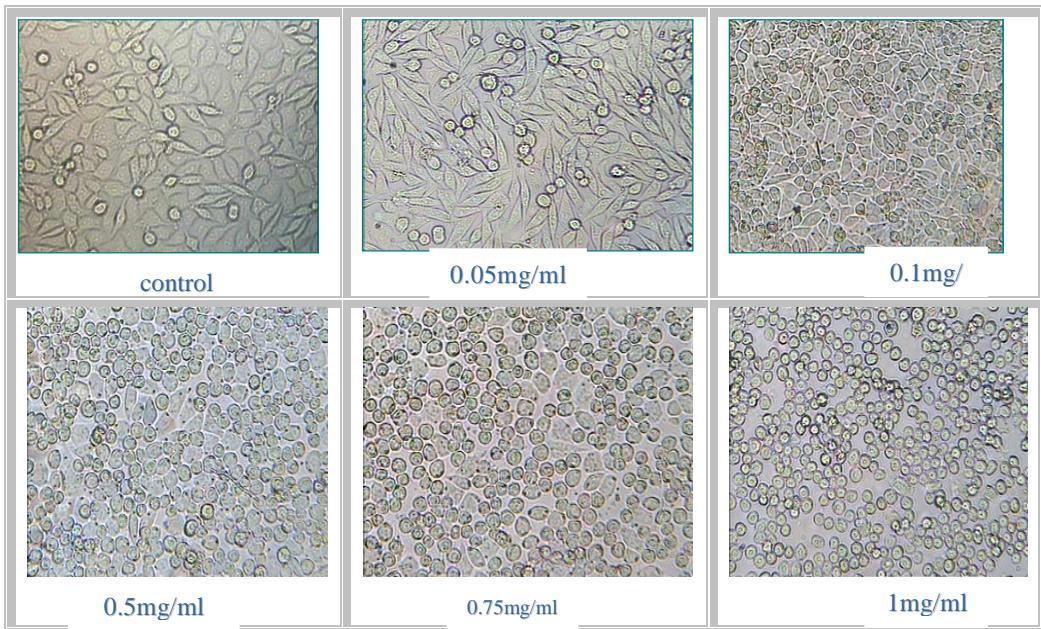


Fig.2: Inverted phase-contrast microscope (400X)Effect of *P. papularia* extract on HeLa cell line dose- dependent in 24h

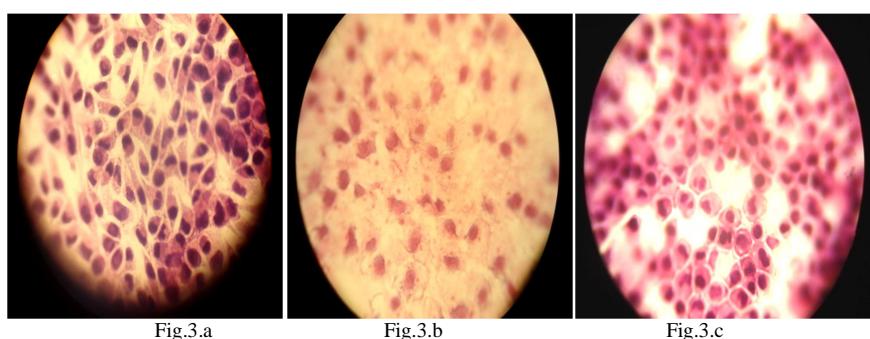


Fig.3: Fig.3.a: control , Fig.3.b: positive control of necrosis, Fig.3.c: positive control(H&E,100 X)



Fig.3.d

Fig.3.e

Fig.3.f

Fig.3.d: Treated with 0.5 mg ml^{-1} DCM extract, **Fig.3.e:** Treated with 0.75 mg ml^{-1} DCM extract, **Fig.3.f:** Treated with 1 mg ml^{-1} DCM extract (H&E,100 X)

DISCUSSION

Natural extracts offer a vast variety of phytochemicals with diverse biological functions. These phytochemicals play a significant role in plant physiology including antioxidants and cytotoxic effect involving different cellular systems⁽²¹⁾. Among their various biological properties, the antiproliferative effects and antitumor activities have been extensively studied⁽²²⁾. It seems likely that rational strategies to manipulate programmed cell death will produce new therapies that are less toxic than current treatment regimens⁽²³⁾. In the current study, the following alcoholic extracts were extracted. Among the extracts, DCM showed the highest antioxidant effect and cytotoxic effects with inhibitory potency against the growth of human HeLa cell lines. Our results show that the DCM-extract of *P. pabularia* has significant antiproliferative and cytotoxic effects on HeLa cell line. The concentration of 1mg/ml of the extract showed 20% by MTT test. The present findings corroborate the findings reported by Farooq et al., who evaluated chemical constituents and cytotoxicity effect from *Prangos pabularia* on human cancer cell lines[lung (A549 and NCI-H322), epidermoid carcinoma (A431), melanoma (A375), prostate (PC-3) and colon(HCT-116)⁽¹⁹⁾. Direct microscopic study of the treated cell cultures showed changes in cell shape,discontinuity in cell-cell adhesion and slowing down of the growth rate in a dose dependent manner. Detail studies using hematoxylin-eosin staining showed . Rupture of the cellular membrane is one of the crucial criteria used to distinguish necrosis from apoptosis⁽²⁴⁾. Thus, it means that the cell membrane was disrupted and the cells died by necrosis at higher concentrations of the extract.The study results showed staining cells were treated with 1 mg ml^{-1} concentrations are aponecrotic , chromatin compaction in these cases is low⁽²⁰⁾ , It seems cell death may be initiated by applications and the end-stage controlled activation of caspase and necrosis can be seen as an end in a concentration of 1mg ml^{-1} Thus, these findings confirmed that the DCM extract of *P. pabularia* induces aponecrosis In higher concentrations. Identification and isolation of natural chemical agents from this extract and evaluation of their probable effects on cancerous cell lines is in progress.

CONCLUSION

In conclusion, it was approved that the extract of DCM produced by the *P. pabularia* roots possesses a considerable cytotoxic potential on carcinoma HeLa cell line. Accordingly, the extract could be regarded as an antiproliferative and cytotoxic agent might be used for the control growth of cell line.

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