Available online: www.notulaebiologicae.ro
Print ISSN 2067-3205; Electronic 2067-3264
Not Sci Biol, 2019, 11(4):333-336. DOI: 10.15835/nsb11410597
Original Article

Anticancer Activity of Water and Methanol Extracts of *Hypericum scabrum* L. on Different Cancer Cell Lines

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Abstract

Hypericum scabrum L. is an endemic medicinal plant with antimicrobial, anticancer and anti-inflammatory, antiviral effect. In this study, we aimed to investigate the cytotoxic effect of water and methanol extracts on different cancer cell lines such as osteosarcoma cancer, cervical cancer and prostate cancer. The data showed that the methanol extract had a highly effective cytotoxic effect on all three cell lines. Although water extract caused less cell loss than methanol extract, it was found that the cells were inhibited in all three cell lines. DU-145, which is the prostate cell line, was more effective in both extracts. As a result, the water and methanol extract of *Hypericum scabrum* L. have an anticancer effect on Saos-2, HeLa and DU-145 cells. There is a need for further and comprehensive studies by isolating the main active ingredient of this plant which is a candidate for drug of cancer in the future. Our study is expected to lead the studies in this direction.

Keywords: cancer; cytotoxic; DU-145; HeLa; *Hypericum scabrum*; Saos-2

Introduction

There are 89 species, 43 of which are endemic in our country and 484 species in the world belonging to Hyperiaceae which is an important family in terms of medicinal plants (Crockett *et al*., 2011; Cirak *et al*., 2014)

As a result of the studies, it has been reported that *Hypericum* plant is used in the treatment of many diseases such as gastric ulcer, menstrual disorders, hemorrhoids and stomach discomfort and Alzheimer's disease (Yesilada *et al*., 1995; Baytop, 1999; Cakir *et al*., 2003; Stojanovic *et al*., 2013). It is also known to have antioxidant, antimicrobial, antidepressant and anticancer effects. However, most of these studies are seen in *Hypericum perforatum* and *H. lydium* species (Ozturk *et al*., 2009; Tahir *et al*., 2017; Eruygur *et al*., 2019).

Hypericum scabrum L. is an endemic species which is perennial herbaceous plant, grows mostly in rocky areas (Tanker, 1971; Serbetci, 2002). Although this plant is known to have effect on many diseases (such as hepatitis, cystitis, chronic gastritis, epilepsy), medical studies in the field of cancer are limited (Kizil *et al*., 2004; Baris *et al*., 2011; Ebrahimzadeh *et al*., 2013; Hamzeloo-Moghadam *et al*., 2015; Keser *et al*., 2018).

Cell lines are *in vitro* mechanisms which used in the discovery of treatment of many diseases such as cancer and in the development of drug applications (Masters, 2000). These cell lines, which have pioneered in vivo studies, to some diseases whose formation processes are not clear contribute to the acquisition of medical knowledge (Neve *et al*., 2006). By way of example, the anti-polio vaccine was developed with the HeLa cervical cancer cell line. As a result of studies with K562, which is chronic myeloid leukemia cell line, the development of treatment protocols for these cancer patients has been achieved (Turner, 2012; Aktuna, 2018).

As a result, cancer cell lines have made a significant contribution to science in the preparation of appropriate conditions for the mechanism of disease formation and treatment process.

Received: 23 Oct 2019. Received in revised form: 15 Dec 2019. Accepted: 17 Dec 2019. Published online: 24 Dec 2019.

Many of the drugs used in also cancer treatment are of plant origin and it is important to determine the anticancer effects of plants *in vitro* to develop the active drug.

In this study, we aimed to determine the anticancer effect of *Hypericum scabrum* L. methanol and water extracts on the prostate cancer cell line DU-145, cervical cell line HeLa and osteosarcoma cell line Saos-2.

Materials and Methods

Collection and identification of plant material

The plant material was collected at the flowering form Yazibasi, Nuri Demirag Airport (Sivas, Turkey) at a height of 1590 m and identified by Prof. Dr. Akpulat HA, Department of Bıology, Sivas Cumhuriyet University.

Preparation of plant extracts

The aerial parts of plant were dried up constant weight in shade and grounded with blender (Blue house). 10 g of plant was soaked in 50 mL of deionized water for 24 h with intermittent shaking. At the end of extraction, it was filtrated by No. 1 Whatman filter paper. The filtrate was concentrated to dryness under reduced pressure with rotary evaporator at 40 °C and this was repeated for three times. The same procedure was followed for methanol extraction. The resulting solid extracts were stored in a freezer at -20 °C until use.

Cell culture

In this study, we used DMEM / F12 medium in Saos-2 osteosarcoma cell line, DMEM medium in HeLa cervical cancer cell line and EMEM medium in DU-145 prostate cancer cell line. We added 10% fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (10 mg/L) to all these mediums. Cells were grown in at 37 °C, 5% CO2 and 95% air in a humidified incubator. For each cell line, 70-80% confluent cell culture flask was trypsinized and cells were seeded in 96 well plates.

Cytotoxic effect of Hypericum scabrum L. methanol and water extracts on Saos-2, HeLa and DU-145 cells

Cytotoxicity of *Hypericum scabrum* L. methanol and water extracts against MDA-MB-231 cell lines was performed with the MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay according to the Skehan's method (Shekan *et al*., 1990). 1 µL of test substance at concentrations ranging between 10-800 µg/mL for water extract and 1-400 µg/mL for methanol extract were added into each well containing the cells. After mixing with a mechanical plate mixer for 15 min, the absorbance of plates were recorded at 570 nm on a microplate reader (Bio-Tek, USA). All drug doses were parallel tested in triplicate and were performed at least 3 times; control samples were run with 1% sterilized water.

Results and Discussion

Cytotoxic effect of Hypericum scabrum L. methanol extract on Saos-2, HeLa and DU-145 cells

Fig. 1 shows changes in cell inhibition for 24, 48 and 72 hours against increasing concentrations of Saos-2, DU-145 and HeLa cell lines. As a result of the data obtained, it was observed that the methanol extract of *H. scabrum* L. caused noticeable amount cell loss after 24, 48, 72 hours incubation when treated to three different cell lines. The methanol extract in all three cell lines was most active at 72 hours incubation (IC_{50} values for 72 hours; Saos-2: 22, 57±3,29; HeLa: 26,45±1,32; DU-145: 5,533±2,76) (Table 1).

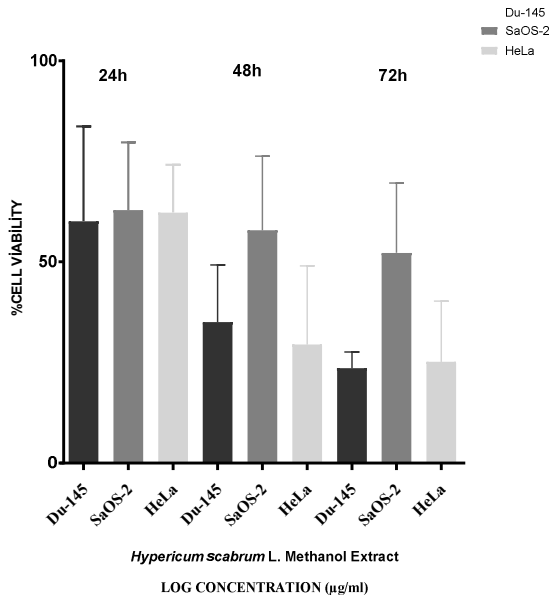

Fig. 1. Cytotoxity activities of methanol extract of *Hypericum scabrum* L. on Saos-2, HeLa and DU-145 cell lines

Table 1. Comparison of IC_{50} values between *Hypericum scabrum* L. methanol extract on different cells after 24 h, 48 h and 72 h of incubation

Cells	IC_{50} (µg/ml)		
	24 h	48 h	72 h
DU-145	66,72±3,12	24,08±2,48	5,533±2,76
HeLa	93,8±2,34	42,56±0,97	26,45±1,32
Saos-2	166,1±3,74	66,03±2,02	22,57±3,29

Cytotoxic effect of Hypericum scabrum L. *water extract on Saos-2, HeLa and DU-145 cells*

When the 24, 48 and 72 hours incubation results of the water extract of *Hypericum scabrum* on osteosarcoma, prostate and cervical cancer cell lines were examined, it was observed that the cells were inhibited at the maximum 72 hours incubation. In Table 2, IC_{50} values for 72 hours incubation were measured as 107.8 ± 4.78 for Saos-2, 149.8 ± 1.36 for HeLa, and 92 ± 3.89 for DU-145. The cell line in which the water extract is most active is DU-145, as in the methanol extract. However, it can be said that in all three cell lines, the water extract is less inhibiting than the methanol extract (Fig. 1 and Fig. 2).

Table 2. Comparison of IC_{50} values between *Hypericum scabrum* L. water extract on different cells after 24 h, 48 h and 72 h of incubation

Cells	IC_{50} (µg/ml)		
	24 h	48 h	72 h
DU-145	425,2±2,36	181,2±4,33	92±3,89
HeLa	420,2±3,19	379,3±2,11	149,8±1,36
Saos-2	380,6±1,07	113,6±3,21	107,8±4,78

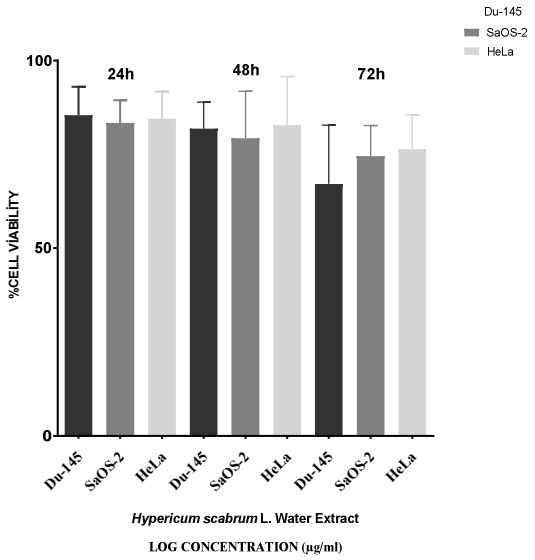

Fig. 2. Cytotoxity activities of water extract of *Hypericum scabrum* L. on Saos-2, HeLa and DU-145 cell lines

Conclusions

H. scabrum L. is an endemic medicinal plant with antioxidant, antimicrobial, antidepressant and anticancer properties. Studies on cancer with *Hypericum scabrum* are limited. However, there are in vitro studies conducted in different species belonging to the genus *Hypericum*. In a study of the ethanol extract of *Hypericum triquetrifolium* with HCT-116, a colon cancer cell line, it was claimed that this extract could be used as a potential therapeutic agent (Mahajna *et al*., 2019). It has been reported that the effect of ethanol extract of *Hypericum perforatum* on U87MG glioma cell line has anti-proliferative effect. However, the antitumor effect of three different extracts of this species on the K562 human erythroleukemic cell line was investigated and reported that these extracts reduce the growth of K562 cancer cells (Borawska *et al*., 2016; Valletta *et al*., 2018). In another study, *H. reflexum, H. canariense* and *H. grandifolium* species of Soxhlet extracts, A375 human malignant melanoma cell line, MDA-MB 231 human breast carcinoma cell line and HCT116 anticancer effect on human colon carcinoma cell line was investigated. *H. grandifolium* species has been reported to be most effective on all three cell lines (Zorzetto *et al*., 2015). However, there is no study on osteosarcoma, prostate and cervical cancer cell lines made with *Hypericum scabrum* L. All these studies can be considered as evidence of the anti-proliferative effect of the genus *Hypericum*.

According to the obtained data, it was determined that the water and methanol extract of *Hypericum scabrum* L. had inhibitory properties on different cancer cell lines. In order to obtain a future cancer drug candidate from *Hypericum scabrum* L., it is necessary to identify the active compounds from methanol and water extracts should be identified and to support the results with in vitro and in vivo studies.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest

The authors declare that there are no conflicts of interest related to this article.

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