



# International Journal of Pharmacology

ISSN 1811-7775

**science**  
alert

**ansinet**  
Asian Network for Scientific Information

## Evaluation of Cytotoxic Effect of *Teucrium polium* on a New Glioblastoma Multiforme Cell Line (REYF-1) Using MTT and Soft Agar Clonogenic Assays

<sup>1</sup>Hossein Eskandary, <sup>1</sup>Saeed Rajabalian, <sup>1</sup>Touraj Yazdi,  
<sup>2</sup>Mohammad Eskandari, <sup>2</sup>Khadijeh Fatehi and <sup>1</sup>Narges Ashraf Ganjooei  
<sup>1</sup>Kerman Neuroscience Research Center, Kerman University of Medical Sciences, Kerman, Iran  
<sup>2</sup>Afzal Research Institute (NGO), Kerman, Iran

**Abstract:** In the present study we evaluated the *in vitro* cytotoxic activity of aqueous and methanolic extract of *T. polium*. In order to evaluate, first we established a new glioblastoma multiforme cell line, which was designated as REYF-1 cell. Cytotoxic activity of the extracts was evaluated on this cell line using both MTT assay and clonogenic cell assay. Present results show that methanolic extract of *T. polium* exhibits dose dependant cytotoxic effect. The IC<sub>50</sub> values of methanolic extract were 95 and 69  $\mu\text{g mL}^{-1}$  using MTT assay and Clonogenic assay, respectively. The IC<sub>50</sub> for aqueous extract was 1400  $\mu\text{g mL}^{-1}$ . These findings show a cytotoxic activity of *T. polium* methanolic extract on glioblastoma multiforme cells *in vitro*. Further studies need to be done to find out the active compound(s) of this plant as a cytotoxic agent.

**Key words:** *Teucrium polium*, cytotoxicity, *Glioblastoma multiforme*, MTT assay, clonogenic assay

### INTRODUCTION

It is well established that plants have been always a useful source of antitumor and cancer prevention compounds (Reddy *et al.*, 2003).

More than one thousand plants have been found to possess significant anticancer properties. Some of the plant-derived anticancer compounds have been beneficial to treatment of cancers. For example, some phytochemicals such as; taxol, taxotere, vincristine, navelbine, etoposide, teniposide, topotecan and irinotecan are used in clinic for cancer treatment (Pezzuto, 1997; Mukherjee *et al.*, 2001). There have been worldwide efforts to discover new anticancer agents from plants (Hayashi *et al.*, 2002; Tai *et al.*, 2004). *Teucrium polium* L. (Labiatae) has been long used in Iran as an anti-inflammatory, antibacterial, antihypertensive and antinociceptive agent (Rasekh *et al.*, 2001; Esmaili and Yazdanparast, 2004). This wild-growing flowering plant is also found abundantly in south western Asia, Europe and North Africa. Based on some compounds isolated from this plant such as Diterpenoids, Flavonoids, Iridoids, Sterols and Terpenoids (Tariq *et al.*, 1989; Bedir *et al.*, 2003), in the present study, we evaluated the *in vitro* cytotoxic activity and anti-tumor properties of aqueous and methanolic extracts of *T. polium* on a new cell line derived from human Glioblastoma multiforme, which is one of the most aggressive primary brain tumors, with a

grim prognosis. Developments in the past decades have not significantly increased the overall survival of patients with this disease. Further research efforts in all aspects of glioblastoma multiforme treatment remains essential to improve the overall prognosis of the disease (Hou *et al.*, 2006).

### MATERIALS AND METHODS

**REYF-1 cell line:** REYF-1 cell line was established in the cell culture lab of the Neuroscience Research Center of Medical Science University of Kerman from the surgical specimen of a glioblastoma multiforme of a 60 year old woman. The REYF-1 cells were grown in culture for over 150 passages and have a doubling time of 19 h (Gunawan *et al.*, 1998).

**Cell culture:** The cells were grown in DMEM medium supplemented with 10% heat-inactivated Fetal Bovine Serum (FBS), 100  $\mu\text{g mL}^{-1}$  streptomycin and 100 U  $\text{mL}^{-1}$  penicillin (all from Gibco-BRL, UK). The cultures maintained in 25 cm<sup>2</sup> flasks in 37°C humidified atmosphere of 5% CO<sub>2</sub> and 95% air.

**Preparations of extracts:** *T. polium* aerial parts were collected during the flowering period (spring) from Koohpayeh region of Kerman province in south east of Iran. After definite identification of species in

pharmacognosia Lab of Faculty of Pharmacy; Kerman University of Medical Sciences, the plant materials were dried at room temperature and powdered. The dried plant samples (50 g) were separately extracted by maceration with 400 mL of methanolic or distilled water twice at room temperature for 48 h. The water of the extract was filtered and then dried by freezing.

The dry methanol extract was obtained after removing the solvent by evaporation under reduced pressure at 40°C. The yields of the methanolic and water extracts were 10.2 and 8%, respectively. The dry methanolic and aqueous extracts were then dissolved in dimethyl sulfoxide (100 mg mL<sup>-1</sup>) and distilled water (50 mg mL<sup>-1</sup>), respectively.

**Antiproliferative assay:** The cells were seeded onto 96-well plates at 2500 cells per well and incubated for 24 h. After incubation, the medium was replaced with fresh one containing different concentrations of extracts or the vehicle. The plates were then incubated for another 72 h. The final concentrations of the extracts were 10 to 1000 µg mL<sup>-1</sup>. After incubation, 30 µL of a 2.5 mg mL<sup>-1</sup> solution of MTT was added to each well and the plates were incubated for 1 h. Then the culture medium was removed and the formazan crystals were dissolved in 100 µL of dimethyl sulfoxide (Sigma-Aldrich, USA). The plates were then read on a microplate reader at a 587 nm wavelength (Christopher and Hand, 1988).

**Soft agar clonogenic cell assay:** Soft agar clonogenic cell assay was performed using a modification of the Hamburger and Salmon method. Briefly, a 1 mL base layer containing 0.6% agar and different concentrations of methanolic extract in enriched DMEM medium was prepared in 35 mm-diameter sterile dishes and after gelling, base layers were overlaid with 1 mL of a plating layer consisting of 1000 cells suspended in complete DMEM medium containing 0.3% agar. The plates were incubated after gelling at 4°C in a 5% CO<sub>2</sub> humidified atmosphere at 37°C. A set of three plates were then prepared for each concentration. The extract concentration range was between 30 to 200 µg mL<sup>-1</sup>. The cell aggregates of 50 µm or greater in diameter were counted after 6 days (Ali-Osman and Beltz, 1988) and the survival rate was calculated using the following formula:

$$\text{Survival rate (\%)} = \frac{\text{Mean No. of test colonies}}{\text{Mean No. of control colonies}} \times 100$$

We compared the sensitivity of the extracts using IC<sub>50</sub> values, corresponding to 50% growth inhibition, obtained from the fitted concentration response curves.

## RESULTS AND DISCUSSION

The extracts exhibit dose dependant cytotoxic effect on REYF-1 cell line. The IC<sub>50</sub> values were 95 µg mL<sup>-1</sup> for the methanolic extract and 1400 µg mL<sup>-1</sup> for the aqueous extract as evaluated by MTT assay (Fig. 1). The methanolic extract at 30, 60, 100 and 200 µg mL<sup>-1</sup> decreased the colony formation of REYF-1 cells to 91, 65, 35 and 0.006% of control, respectively (IC<sub>50</sub> = 69 µg mL<sup>-1</sup>). The cloning efficiency of REYF-1 cell in complete culture medium was assessed to be 70.77±5.27% (mean±SD).

The methanolic extract was more potent than the aqueous extract which is related to better solubility of the active chemicals in methanol. In addition, present study suggests that clonogenic assay leads to better result than MTT assay. Considering that our sample was crude, it is important to note that the pure active compound(s) would possibly show stronger effects. It has been reported that some of terpenoids and flavonoids have anti-tumor properties (Wada *et al.*, 1998; Reddy *et al.*, 2003; Miyata *et al.*, 2005; Wada and Tanaka, 2005). The presence of these classes of constituents in this plant may play a role in the observed cytotoxic effects. We suggest that there may be synergistic or additive effects between toxic compounds of *T. polium* which exhibited potent cytotoxic effects.

Current Studies demonstrated that *T. polium* species possess antioxidant activity (Kadifkova *et al.*, 2005; Ljubuncic *et al.*, 2005). Therefore, based on these studies and our results, methanolic extract of *T. polium* may present cancer preventive properties as well as its cytotoxic activity.

Many side effects of common used chemotherapy agents (e.g., Cisplatin) are because of their oxidative

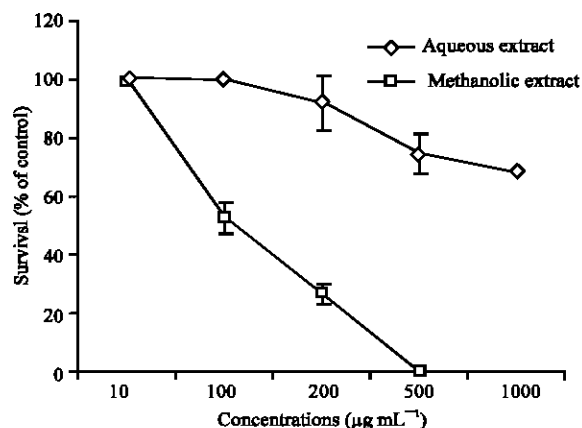


Fig. 1: Effect of aqueous and methanolic extracts on REYF-1 cells tested by MTT assay

stress (Taguchi *et al.*, 2005) and in some reports it is demonstrated that antioxidant food and plants can prevent this side effect (Premkumar *et al.*, 2003; Cetin *et al.*, 2006). According to our result and the fact that *T. polium* has antioxidant effect we suggest further studies to investigate the effect of *T. polium* as a supplement to those who may go through chemotherapy treatment and find out the active compound(s) of the extract as a cytotoxic agent.

#### ACKNOWLEDGMENTS

This research was supported by Kerman Neuroscience Research Center, University of Medical Sciences. We thank to Dr. Mohsen Mohammadi for assistance with English editing of the manuscript.

#### REFERENCES

- Ali-Osman, F. and P.A. Beltz, 1988. Optimization and characterization of the capillary human tumor clonogenic cell assay. *Cancer Res.*, 3: 715-724.
- Bedir, E. and R. Manyam *et al.*, 2003. Neo-clerodane diterpenoids and phenylethanoid glycosides from *Teucrium chamaedrys* L. *Phytochemistry*, 8: 977-983.
- Cetin, R. and E. Devrim *et al.*, 2006. Cisplatin impairs antioxidant system and causes oxidation in rat kidney tissues: Possible protective roles of natural antioxidant foods. *J. Applied Toxicol.*, 1: 42-46.
- Christopher, M. and J.R.V.P.B. Hand, 1988. Chemotherapy in experimental brain tumor. Part 1: *In vitro* colorimetric MTT assay. *J. Neuro-Oncol.*, 1: 1-6.
- Esmaili, M.A. and R. Yazdanparast, 2004. Hypoglycaemic effect of *Teucrium polium*: Studies with rat pancreatic islets. *J. Ethnopharmacol.*, 1: 27-30.
- Gunawan, B. and S. Braun *et al.*, 1998. Characterization of a newly established endometrial stromal sarcoma cell line. *Int. J. Cancer*, 3: 424-428.
- Hayashi, K. and Y. Nakanishi *et al.*, 2002. Antitumor agents. Part 212. Bucidasins A-C, three new cytotoxic clerodane diterpenes from *Bucida buceras*. *Bioorg. Med. Chem. Lett.*, 3: 345-348.
- Hou, L.C. and A. Veeravagu *et al.*, 2006. Recurrent Glioblastoma multiforme: A review of natural history and management options. *Neurosurg. Focus*, 4: E5.
- Kadifkova Panovska, T. and S. Kulevanova *et al.*, 2005. *In vitro* antioxidant activity of some *Teucrium* species (Lamiaceae). *Acta Pharm.*, 2: 207-214.
- Ljubuncic, P. and H. Azaizeh *et al.*, 2005. Antioxidant activity and cytotoxicity of eight plants used in traditional Arab medicine in Israel. *J. Ethnopharmacol.*, 1: 43-47.
- Miyata, Y. and T. Sato *et al.*, 2005. Triptolide, a diterpenoid triepoxide, induces antitumor proliferation via activation of c-Jun NH<sub>2</sub>-terminal kinase 1 by decreasing phosphatidylinositol 3-kinase activity in human tumor cells. *Biochem. Biophys. Res. Commun.*, 4: 1081-1086.
- Mukherjee, A.K. and S. Basu *et al.*, 2001. Advances in cancer therapy with plant based natural products. *Curr. Med. Chem.*, 12: 1467-14686.
- Pezzuto, J.M., 1997. Plant-derived anticancer agents. *Biochem. Pharmacol.*, 2: 121-133.
- Premkumar, K. and S.K. Abraham *et al.*, 2003. Protective effects of saffron (*Crocus sativus* Linn.) on genotoxins-induced oxidative stress in Swiss albino mice. *Phytother. Res.*, 6: 614-617.
- Rasekh, H.R. and M.J. Khoshnood-Mansourkhani *et al.*, 2001. Hypolipidemic effects of *Teucrium polium* in rats. *Fitoterapia*, 8: 937-939.
- Reddy, L. and B. Odhav *et al.*, 2003. Natural products for cancer prevention: A global perspective. *Pharmacol. Ther.*, 1: 1-13.
- Taguchi, T. and A. Nazneen *et al.*, 2005. Cisplatin-associated nephrotoxicity and pathological events. *Contrib. Nephrol.*, 148: 107-121.
- Tai, J. and S. Cheung *et al.*, 2004. *In vitro* culture studies of *Sutherlandia frutescens* on human tumor cell lines. *J. Ethnopharmacol.*, 1: 9-19.
- Tariq, M. and A.M. Ageel *et al.*, 1989. Anti-inflammatory activity of *Teucrium polium*. *Int. J. Tissue React.*, 4: 185-188.
- Wada, S. and R. Tanaka *et al.*, 1998. *In vitro* inhibitory effects of DNA topoisomerase II by fernane-type triterpenoids isolated from a *Euphorbia* genus. *Bioorg. Med. Chem. Lett.*, 20: 2829-2832.
- Wada, S. and R. Tanaka, 2005. Synthetic lanostane-type triterpenoids as inhibitors of DNA topoisomerase II. *Bioorg. Med. Chem. Lett.*, 12: 2966-2969.