Research Journal of Pharmacognosy (RJP) 4(4), 2017: 65-70
Received: 6 July 2017
Accepted: 27 Aug 2017

Original article

Cytotoxicity effect of *Zataria multiflora* Boiss. on two human colon carcinoma cell lines

F. Sharififar[1], M. Miri-Moghaddam[2], S.N. Nematollahi-Mahani[3], H. Forootanfar [4,5], M. Asgharian Rezee[2,5*]

[1]*Herbal and Traditional Medicines Research Center, Department of Pharmacognosy, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran.*
[2]*Department of Toxicology and Pharmacology, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran.*
[3]*Neuroscience Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran.*
[4]*Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran.*
[5]*Pharmaceutics Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran.*

Abstract

Background and objectives: Natural products are one of the major sources for investigations of novel medicines. *Zataria multiflora* Boiss (ZM) has shown pharmacological activities especially in gastrointestinal tract; however, there are limited studies about its cytotoxicity effects. In this study, the effect of *Zataria multiflora* was examined on two colon cancer cell lines (SW-48 and HT-29). **Methods:** Hydro-alcoholic extract of ZM and its fractions including chloroform, petroleum ether and methanol extract were prepared by warm maceration method. Different concentrations were prepared and examined on SW-48 and HT-29 cell lines using 2-(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyltetrazolium bromide (MTT) assay. **Results:** The results of the present study have shown the cytotoxic effect of some fractions of ZM. The most considerable cytotoxic effect was shown against HT-29 cell line. Also, total ZM extract and the petroleum ether fraction demonstrated cytotoxic effects with IC_{50} values of 44.22 and 33.42 µg/ml on SW-48 and HT-29 cell lines, respectively. **Conclusion:** *Zataria multiflora* was cytotoxic to against colon cancer cell lines HT-29 and SW-48.

Keywords: cytotoxicity, HT-29, SW-48, *Zataria multiflora* Boiss.

Introduction

Natural products have been proposed as one of the major sources of pharmacologic agents. Herbal extracts have been traditionally used in prevention and treatment of many chronic diseases such as inflammatory, cardiovascular, intestinal and bowl diseases, diabetes, allergies, *etc.* [1-3]. Since the spread of different cancer types with high rate of morbidity and mortality,

*Corresponding author: rezaeem@kmu.ac.ir, Tel: +9834-31325271, Fax: +9834-31325003

there has been growing interest in investigations of cytotoxic and anti-cancer activity of herbal extracts [1, 4]. In this regard, some potent anti-tumor compounds such as *Vinca* alkaloids, docetaxol and paclitaxel have been identified as natural based anti-cancer compounds [5-9].

Zataria multiflora Boiss. (ZM) is a thyme-like plant that belongs to the Laminaceae family growing in Iran, Pakistan and Afghanistan. ZM has an important role in traditional medicine of Iran and has been used as anti-septic and spasmodic diuretic, analgesic and anesthetic agent [10-12]. Modern pharmacological studies have reported various properties of ZM including anticonvulsant, antinociceptive, hepatoprotective, spasmolytic, anti-inflammatory, anti-viral and antimicrobial effects [13-20]. Our recent studies have indicated the antioxidant and anticholinesterase activity of the plant and its fractions [18, 21]. Moreover, the results of several studies have represented the anti-oxidant effect of ZM [2,21]. It seems that high percentage of monoterpenes such as thymol and carvacrol have roles in ZM anti-oxidant and anti-microbial effects of the species [10].

Colorectal carcinoma (CRC) is the fourth most common cancer worldwide. It's the second case of cancer related mortality [22]. Colorectal carcinoma is the most common cancer in developed countries. There are reports indicating the increase of CRC cases in Iran [23]. Genetic factors, the lifestyle and dietary habits are known to be associated factors in incidence of CRC [23,24]. Despite the recent improvement in diagnosis and treatment of CRC, there is poor prognosis in some cases and more investigations are needed to reduce the incidence and to improve the treatment. One proposed approach is the use of chemo-preventive agents that induce apoptosis in cancer cells with minimum side effects in normal cells [25,26].

Regarding the above-described findings and also the anti-oxidant and anti-microbial activity of ZM, in the presents study, the cytotoxicity of ZM hydro-alcoholic extract and fractions were examined on two human colon carcinoma cell lines.

Experimental

Plant material

Aerial parts of *Zataria multiflora* were collected from Kerman province, Iran in July 2016. A voucher specimen was deposited at the Herbarium of Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran (KF1375). Five hundred g of dried parts of the plant were milled, passed through sieve and extracted with methanol 80% by warm maceration method for 72 h [27]. The filtered extract was replaced with fresh solvent every 24 h. The obtained extracts were mixed and concentrated under vacuum and dried at below than 40 °C in oven. The other parts of the plant (about 1kg) were used for fractionation with petroleum ether, chloroform and methanol consecutively by warm maceration method. Each fraction was concentrated under vacuum and dried in oven (below 40 °C). The dried extract and fractions were weighed and stored at -20 °C until experiment.

Cell lines

Human colon carcinoma cell lines HT-29 and SW-48 were purchased from IBRC (Genetic and Biological Resource Center in Iran). The cells were grown in DMEM high glucose medium (Biosera, UK) supplemented with 10% fetal bovine serum (Biosera, UK). The cells were grown at 37 ° C humidified incubator with 5% Co2.

MTT cell proliferation assay

Cell proliferation was measured by the reduction of 2-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT) to formazan. Cells were exposed to 5, 10, 25, 50, 100, 150, 200 and 250 µg/mL of ZM hydro alcoholic extract and fractions (petroleum ether, chloroform, and methanol) and also 5-fluorouracil (10, 25, 50, 100 and 250 µg/mL) as a positive control, for 24 h. Each concentration was tested in triplicate. After treatment, the medium was removed and 20 µL MTT (5 mg/mL in PBS) was added to each well. After additional 4 h

incubation at 37 °C, the medium was removed and then 100 µL DMSO was added to each well. Formazan absorbance was assessed at 570 nm using microplate reader (BioTek, Germany). The results were expressed as a percentage of growth (% cell viability) with 100% presenting control cells treated by DMSO. Finally, the IC_{50} (he concentration of extract necessary to induce 50% inhibition in cell growth) was calculated through Probit analysis.

Statistical analysis

All analyses were performed using SPSS software (13.0 versions). The IC_{50} value was calculated through Probit analysis using SPSS. The differences between IC*50* of fractions were analyzed by One-way ANOVA followed by Tukey test. The results were expressed as the mean±SD. Differences were considered statistically significant if $p< 0.05$.

Results and Discussion

The inhibitory effect of different concentrations of total ZM extract and fractions was assessed on HT-29 and SW-48 cell lines. The IC_{50} values were calculated for each sample. The comparison of IC_{50} values showed that HT-29 cells treated with each extract represented greater growth inhibition than SW-48 indicating that HT-29 cells were more sensitive to the extracts than SW-48 (table 1). Also in the SW-48 cells, the IC_{50} value of the total ZM extract was lower than others samples while in the HT-29 cell line the lower IC_{50} resulted by petroleum ether and methanol fractions (table 1). The comparison of the results with 5-flourouracil (5-FU) as the positive control showed no significant difference on HT-29 cells but the IC_{50} of petroleum ether and chloroform extracts on SW-48 exhibited significant difference with 5-FU ($p<0.05$). These results indicated that the cytotoxic effect of the extract and fractions on HT-29 was comparable to inhibitory effect of 5-FU. Also, in SW-48 cells, the IC_{50} value of the total and methanol extracts were not significantly different from the IC_{50} of 5-FU.

Zataria multiflora has been used extensively in Iranian traditional medicine [10]. Also the new researches have shown the anti-inflammatory, anti-oxidant and anti-microbial effects of this plant [2,17,21]. In present study, the cytotoxicity of different concentrations of hydro-alcoholic extract of ZM and its fractions (methanol, chloroform and petroleum ether extracts) were evaluated on two colon carcinoma cell lines. Our results exhibited the inhibitory effect of ZM extracts on HT-29 and SW-48. The HT-29 and SW-48 cell lines are from different type of human colon carcinoma which explains different response to inhibitory effect of ZM extracts. The HT-29 cells represent chromosomal instability (CIN) whereas SW-48 cells are related to microsomal instability (MIN) [28]. The clinical studies have been shown that MIN^+ and CIN^+ tumors have different biological and clinical behavior [29]. *Zataria multiflora* has shown pharmacological effects and chemicals similarities to *Thymus vulgaris*, the well-known medicinal plant [10]. There are numerous studies on cytotoxicity of *Thymus vulgaris* but little investigation has been carried out on ZM. Rahimifard *et al*. have reported cytotoxic effects of essential oil of ZM on HeLa, Vero and Hep II cell lines using MTT assay [30].

Table 1. Growth inhibitory activity of hydro-alcoholic extract of *Zataria Multiflora* Bioss. and its three fractions in SW-48 and HT-29 cells

	IC_{50} (µg/mL)				
Cell line	**Total extract**	**Petroleum ether faction**	**Chloroform fraction**	**Methanol fraction**	**5-FU**
SW-48	44.22±6.7	62.48±9*	60.17±20*	54.1±15.8	25.74±0.5
HT-29	48.33±26.5	33.42±10.4	48.95±17.3	34.92±7.2	22.77±0.3

*Statistical compare represented $p<0.05$ between petroleum ether and chloroform extracts with 5-fu on SW48 cells (One-Way ANOVA). Each value indicated mean±SD.

Also we have previously showed antioxidant activity of ZM essential oil and also its anti-leishmanial effect [21].In other study the cytotoxicity of ZM hydro-alcoholic extract was evaluated on normal cells, Hep-G2 and SKOV-3 cell lines in comparison with cisplatin. The results showed that ZM extract in concentration of 5-150 µg/mL exhibited high IC_{50} compared to cisplatin [31]. However, our results showed that there was no significant difference between inhibitory effect of ZM extract and 5-FU especially on HT-29 cells. Regarding the difference between our results and the study of Shokrzadeh *et al.*[31] it seems that the cytotoxic effect of ZM extract was dependent to the cell type. Previous studies have reported the presence of phenolics and terpenoids as the major components of the plant [17]. The fractionation of the plant can separate these phytochemicals through solvents with different polarities and biologic activities [32-34]. Petroleum ether and somehow chloroform extracts can mostly extract plant terpenoids while phenolics enrich in polar solvents such as in methanol fraction. It could be concluded that cytotoxic constituents of the plant could belong to both polar and non-polar compounds and their additive effect might be represented in the total extract; however, more studies are needed to investigate *Zataria Multiflora* cytotoxic effects on colon carcinoma and its mechanisms.

Acknowledgments

The results described in this paper are part of a Pharm.D thesis. This work was supported by the Vice Chancellor of Research, Kerman University of Medical Science, Kerman, Iran (grant number: 940240).

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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