Cytotoxic Effects of Essential Oils and Extracts of some *Mentha* species on Vero, Hela and Hep2 Cell Lines

Rahimifard N (Ph.D.)[1,2,3], Hajimehdipoor H (Ph.D.)[4,5*], Hedayati MH (M.Sc.)[6], Bagheri O (M.Sc.)[7], Pishehvar H (Pharm.D.)[3], Ajani Y (M.Sc.)[8]

1- Department of Microbiology, Food and Drug Laboratory Research Center (FDLRC), MOH & ME, Tehran, Iran
2- Department of Microbiology, Food and Drug Control Laboratories (FDCLs), MOH & ME, Tehran, Iran
3- Department of Microbiology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran
4- Traditional Medicine and Materia Medica Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
5- Department of Traditional Pharmacy, School of Traditional Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
6- Department of Biotechnology, Pasture Institute of Iran, Tehran, Iran
7- Molecular Biotechnology Research Center, Baqiatalah University of Medical Sciences, Tehran, Iran
8- Department of Pharmacognosy and Pharmaceutics, Institute of Medicinal Plants, ACECR, Tehran, Iran
* Corresponding author: Traditional Medicine and Materia Medica Research Center and Department of Traditional Pharmacy, School of Traditional Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Tel & Fax: +98 – 21 - 88776027
E.mail: Hajimehd@tums.ac.ir, hmehdipoor@itmrc.org

Receive: 4 Jun 2010 **Acceptance:** 28 Aug. 2010

Abstract

Background: ***Mentha*** **species are widely used in traditional medicine mostly as anti-flatulence. Nowadays, their usage as flavor and preservative in food, cosmetic and pharmaceutical industries has been developed. Moreover, cytotoxic effects of some** ***Mentha*** **species have been reported.**

Objective: In this study, cytotoxic properties of ***Mentha piperita, M. spicata, M. aquatica, M. crispa, M. pulegium*** **and** ***M. longifolia*** **have been investigated.**

Methods: Different concentrations of essential oils and total extracts of six ***Mentha*** **species were tested by MTT assay against Vero, Hep2 and Hela cell lines.**

Results: The results showed that all samples were toxic against Vero, Hela and Hep2 cell lines (IC_{50} 28.1-166.2 µg/ml).

Conclusion: All examined ***Mentha*** **species extracts and essential oils have cytotoxic effects but some of them could be considered as potent toxic agents.**

Keywords: ***Mentha*****, Cytotoxic effects, MTT, Vero, Hela, Hep2 cell line**

Introduction

Cancers belong to the group of disorders with difficulty in treatment and sometimes are incurable. Several investigations have been performed in order to find new drugs in treatment of cancers but unfortunately, many of drugs are not efficient enough and there is a lack of information about prevention and treatment of this kind of disease yet. Medicinal plants are source of natural compounds and nowadays many researchers have been interested to them in order to find efficient plants to cure cancers [1]. *Mentha* is a world wide plant and contains several species which are used in traditional medicine and phytotherapy mainly for gastrointestinal disturbances. The essential oils of *Mentha* spp. are used in aromatherapy as well. This genus contains flavonoids, caffeic acid derivatives and essential oil (menthol or carvone as major compounds) [2]. Since many pharmacological and cytotoxic activities have been reported from *Mentha* spp. [3 - 8], in this investigation cytotoxic effects of six *Mentha* species named *M. piperita, M. spicata, M. longifolia, M. crispa, M. pulegium* and *M. aquatica* collected from north of Iran have been studied by using MTT assay against three cell lines of Vero, Hela and Hep2. This method is usually used for preliminary evaluation of anti-tumor compounds.

Materials and Methods

Plant material

Aerial parts of *Mentha piperita, M. spicata, M. longifolia, M. aquatica, M. pulegium* and *M. crispa* were collected from Gorgan (Golestan province) in May 2008 and identified by Y. Ajani, Institute of Medicinal Plants (ACECR).

Preparation of extracts

Dried and milled aerial parts of each plant were extracted with methanol 80% (1:10) by using maceration method for 4 days. After every 24 h, the mixture was filtered and new solvent was added to the plant powder. The combined extracts were concentrated under reduced pressure to dryness and different concentrations of each extract were prepared in DMSO 10%.

Preparation of essential oils

The air dried and powdered aerial parts of the plants were subjected to hydro-distillation for 4h using a Clevenger type apparatus. The obtained essential oils were dried with anhydrous sodium sulphate and stored at +4°C before using. In order to prepare different concentrations of essential oils, DMSO 10% was used as solvent.

Cell lines

Hela (human malignant cervix carcinoma), Hep2 (human laryngeal carcinoma) and Vero (green African monkey kidney) cell lines were obtained from Pasture Institute of Iran, Tehran, Iran. Each cell line was cultured in suitable medium for desired growth, plus 10% FBS and 1% penicillin-streptomycine in a humidified incubator at 37°C in an atmosphere of 95% O_2 and 5% CO_2. Then the growth curve of each cell line was plotted.

MTT assay

Cell viability was assessed by MTT assay (microculture tetrazolium/formazan assay) according to the method which proposed by Alley et al [9] with some modification, in the

presence and absence of different concentrations of the plants extracts and essential oils. The cells were seeded in 96-well plates. Four wells for each concentration were seeded and triplicate plates were used for each cell line. Then, the cells were incubated at 37°C. After 24 h the medium was replaced by fresh medium containing different concentrations of the plants extracts or essential oils. Then, the medium was changed by fresh medium containing MTT ([3-(4,5-dimethylthiazol-2-yl)-2,4-diphenyltetrazolium bromide]) with a final concentration of 0.5 mg/ml (after 24 h). The cells were incubated for another 4 h in a humidified atmosphere at 37°C and after that the medium containing MTT was removed and remaining MTT-formazan crystals were dissolved in DMSO. The absorbance was measured at 570 nm immediately using an ELISA reader. IC_{50} was defined as the concentration of the extracts or essential oils that produced a 50% decrease in cell viability relative to the negative control which was wells exposed to the solvent without any extract or essential oil [10, 11].

Results

The results of cytotoxic tests have been shown in table 1.

Discussion

The results demonstrated that all six *Mentha* species extracts and essential oils were toxic against Vero, Hela and Hep2 cell lines in different concentrations (IC_{50} 28.1-166.2 µg/ml). The essential oils of the plants showed more toxic effects in Hela cell line (IC_{50} ≤42.3 µg/ml), but in Hep2 cell line, both of extracts and essential oils showed cytotoxic effects in high dosages (IC_{50} about 100 µg/ml). It was found that the extracts of *Mentha* species were more toxic against Vero cell line (IC_{50} ≤54.6 µg/ml), whereas the essential oils have the less effect on Hep2 cell line (IC_{50} ≥ 94.3 µg/ml) compared to other cell lines. Among all examined samples, essential oil of *M. spicata* was found to be less toxic against Hep2 cell line. In an investigation which was performed on *M. pulegium*, methanolic extract of the plant showed no cytotoxicity against SK-OV-

Table 1- Cytotoxic activities of some *Mentha* species extracts and essential oils

Plant name	IC_{50} (µg/ml)					
	Vero		Hela		Hep2	
	*Ext**	*Ess***	*Ext*	*Ess*	*Ext*	*Ess*
M. piperita	52.4	35.5	104.4	31.2	111.7	125.0
M. spicata	54.6	59.9	99.7	42.3	106.8	166.2
M. pulegium	51.2	54.8	114.4	30.6	126.4	109.3
M. longifolia	51.8	36.8	119.1	35.9	89.9	94.3
M. aquatica	53.0	42.7	97.8	28.1	98.8	145.5
M. crispa	53.6	36.7	102.4	31.4	97.7	108.4

*Extract
**Essential oil

3, Hela and A549 cell lines but the essential oil of the plant was proved to be a potent cytotoxic agent against above-mentioned cell lines with LD_{50} of 14.1, 59.1 and 18.76 µg/ml, respectively [6]. Another study by Arumugam et al. on *Mentha spicata* demonstrated the potent cytotoxic effect of the chloroform fraction of the plant against PC-3 cell line [7]. In addition, it has been proved that *M. spicata* oil has anti-proliferative activity on KB and P388 cell lines [8]. It is concluded that *Mentha* species have potent cytotoxic properties on some cell lines but show weak activity or no effect on other cell lines. *M. piperita, M. spicata, M. longifolia, M. aquatica, M. crispa* and *M. pulegium* extracts and essential oils from Iran have cytotoxic properties but regarding to the toxic concentrations, some of them could be considered as potent cytotoxic agents.

Acknowledgments

The authors wish to thank Dr. M.H. Soleimani from Giyah Essence Company for collecting the plants.

References

1. Rahimifard N, Pakzad SR, Shoeibi Sh, Hedayati MH, Hajimehdipour H, Motaharinia V, Mehrafshan L, Javadi A, Pirali Hamedani M. Effects of essential oil and extract of *Thymus vulgaris, Zataria multiflora* and *Eugenia carryophilata* on Vero, Hela, Hep2 cell lines by MTT assay. *J. Med. Plants* 2009; 8 (30): 152 - 6.

2. Fleming T. PDR for herbal medicines. Medical Economics Company. Montvale. 2000, pp: 275.

3. Hafedh H, Fathi BA, Mejdi S, Emira N, Amina B. Effect of *Mentha longifolia* L. ssp. *longifolia* essential oil on the morphology of four pathogenic bacteria visualized by atomic force microscopy. *Afr. J. microbiol. Res.* 2010; 4 (11): 1122 - 7.

4. Sousa PJC, Linard CFBM, Azevedo-Batista D, Oliveria AC, Coelho-de-Souza AN, Leal-Cardoso JH. Antinociceptive effects of the essential oil of *Mentha×villosa* leaf and its major constituent piperitone oxide in mice. *Braz. J. Med. Biol. Res.* 2009; 42: 655 - 9.

5. Londonkar RL, Poddar PV. Studies on activity of various extracts of *Mentha arvensis* Linn. Against drug induced gastric ulcer in mammals. *World J. Gastrointes. Oncol.* 2009; 1 (1): 82 - 8.

6. Hosseini Shirazi F, Ahmadi N, Kamalinejad M. Evaluation of northern Iran *Mentha pulegium* L. cytotoxicity. *Daru* 2004; 12 (3): 106 - 10.

7. Arumugam P, Ramamurthy P, Ramesh A. Antioxidant and cytotoxic activities of lipophilic and hydrophilic fractions of *Mentha spicata* L. *Int. J. Food Prop.* 2010; 13 (1): 23 - 31.

8. Manosroi J, Dhumtanom P, Manosroi A. Anti-prolifrative activity of essential oil extracted from Thai medicinal plants on KB and P388 cell lines. *Cancer Lett.* 2006; 235: 114 - 20.

9. Alley MC, Scudiero DA, Monkes A, Hursey ML, Czerwinski MJ, Fine DL, Abbott BJ, Mayo JG, Shoemaker RH, Boyd MR. Feasibility of drug screening with panel of human tumor cell lines using a microculture tetrazolium assay. *Cancer Res.* 1988; 48: 589 - 601.

10. Mosaddegh M, Ostad SN, Naghibi F, Hamzeloo Moghadam M. Cytoxic effects of

five species of *Inula* against some tumor cell lines. *IJPR.* 2006; 2 (4): 203 - 8.

11. Mosaddegh M, Hamzeloo Moghadam M, Ghafari S, Naghibi F, Ostad SN, Read RW. Sesquiterpene lactones from *Inula oculus-christi*. *NPC*. 2010; 5 (4): 511 - 4.