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FULL PAPER

Investigating anti-cancer mechanism of phenolic compounds of water mint in cancer cells

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Mentha species are among the prevalent plants found worldwide. Some evidence has shown its anti-cancer effects. However, the underlying molecular mechanism of such effects is vague. Therefore, the current study aimed at evaluating the possible anti-cancer effects of phenoliccompounds of water mint (*Mentha aquatic)* as the most common species in vitro. For this purpose, the phenolic compounds of *Mentha aquatica* were prepared. Hella and MCF-7 cells were incubated with RPMI culture medium containing 10% fetal bovine serum (FBS), 100 U/mL of penicillin, and 100 µg/mL of streptomycin. Then, the cells were treated with different concentrations. After the incubation period, an MTT assay was utilized to determine cytotoxicity. Also, the changes in cellular ROS and glutathione levels were determined. According to our result, the ethanolic extract of *Mentha aquatica* could significantly reduce cell viability after 48 h. In addition, the level of ROS was substantially elevated while intracellular glutathione levels exhibited a downward trend. Although further screening of active components of the extract responsible for these effects is required, the results of the current study propose that *Mentha aquatica* may be beneficial as a possible anti-cancer agent.

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KEYWORDS

Mentha aquatica; anticancer; water mint.

Introduction

According to the World Health Organization reports, cancer is the leading cause of death worldwide [1]. Aging and related signaling pathways and extended exposure to carcinogenic agents have been considered the leading vital causes for the direct association of life expectation and the incidence of cancer [2]. -Researchers are still seeking more selective anti-cancer agents with less toxic effects concerning chemotherapy. In this context, many researchers have focused on natural compounds [3-5]. Mentha aquatica L. originated from the European continent is globally found in moist regions [6,7]. Its aerial segments have been used to treat several diseases such as pulmonary, gastrointestinal, and mental problems [8-11]. Its chemical structure contains different phenolic compounds, flavonoids, and triterpenoids [12]. From the pharmacological point of view, prohibitory effects on monoamine oxidase (MAO) and affinity to the GABA-A receptor have been identified [13]. Furthermore, menthe aquatica significantly inhibits the lipase and amylase enzymes of the pancreas and shows antibacterial and anti-inflammatory activities [14-16]. Also, the cytotoxic effects of the extracts against some cancer cell lines have been reported [17]. Therefore, the current study aimed to assess the anti-proliferative effects of the ethanolic

extract of Mentha aquatica L. aerial parts via in vitro tests.

Materials and methods

Chemicals

All the chemicals used were high-quality analytical-grade reagents.

Plant materials

Mentha aquatica (water mint) was collected from the mountains around Maragheh, Iran. The aerial parts of the plant, including leaves and stems, were dried in a 60° incubator after washing. To prepare the alcoholic extract, the samples were ground, and 50 g of it was extracted in 500 mL of 80% ethanol for 68 h on an Erlenmeyer shaker at 38-60 °C at 200 rpm by massaging. Using soaking did not damage the substances in the extract prepared from plants. An attempt was made to obtain a uniform extract by stirring during this process. The resulting extract was filtered with 0.6 µm Whatman paper (Watman UK). The ethanolic extract was concentrated under vacuum at 80 °C at 8 rpm by a rotary apparatus poured into sterile Petri dishes and dried at 60 °C. The extracts were then collected. Phenolic compounds were extracted according to Riberean-Gayon. The chemical composition of Mentha aquatica was determined using GC-MS analysis, and the volatile compounds were identified based on literature and the retention index (RI).

Evaluation of biological activity in cellular assays

Cell culture and treatments

The MCF-7 and Hela cells were seeded on 96 and 6 well culture plates to assess the viability and ROS formation, respectively. The hydroethanolic extracts of M. Aquatica, (final concentrations of 100–200 µg/mL) were added to the culture medium, and the cells were incubated for 48 h. Then, the culture medium was replaced with fresh ones.

MTT assay

MTT assay based on the generation of formazan from tetrazolium salt from viable cells was used to determine cell proliferation after treatment [18]. Accordingly, the percentages of the living cells in comparison with the control were utilized to represent cell viability.

Intracellular ROS production

The non-fluorescent dye, 2′,7′dichlorofluorescin diacetate (DCFHDA), was applied to measure relative levels of cellular ROS production [19]. After treatment, cells were detached by trypsin-EDTA and were washed by PBS. Then the washed cells were incubated in FBS-free DCFHDA (50 µM)–containing culture medium for 30 min. Then the washing step was repeated, and the cells were centrifuged at 412 g for 10 min. Finally, the cell plates were dissolved with 1% Triton X100. And fluorescence alterations were determined via a Jasco R_FP-750 spectrofluorometer (Jasco Corporation, Tokyo, Japan).

Determination of reduced glutathione

The technique of Popet *et al*. [20] was applied to assess the reduction in glutathione level. DTNB reagent containing (12 mM NADPH, 50 U/mL GSH reductase 0.1 mM DTNB, in 0.1 mM sodium phosphate buffer with 1 mM EDTA, pH 7.5) was incubated with 50 µL of the cell lysate for half an hour. Then, an ELISA microplate reader was utilized to measure the absorbance at 415 nm.

Results and discussion

Empirical evidence proposes that dietary components, predominantly phytochemicals, can modify the multifaceted process of cancer induction via interfering with different signaling pathways. A wide diversity of phenolic elements own potent anti-mutagenic and anti-tumorigenic properties. Mentha

species are among common phytochemicals worldwide, and some experiments recommend that it might have anti-cancer effects. However, very little is known regarding the molecular mechanisms they may exert their anti-tumourigenic effect. The current investigation evaluated the potential anti-proliferative effects of Mentha aquatica aerial parts as adjuvant therapy against tumor cell growth. Ethanol was selected as a solvent in the maceration process since it is less polluting and exhibits a better safety profile [21,22]. The in vitro cytotoxic effects were assessed against hella and MCF-7 cell lines at varying concentrations, which displayed a significant cytotoxic effect against the studied cell line. The results showed the occurrence of considerable cell death evident after 48 h. Figure 1-a represents the changes in the cell viability percentage in Hella cells and MCF-7.

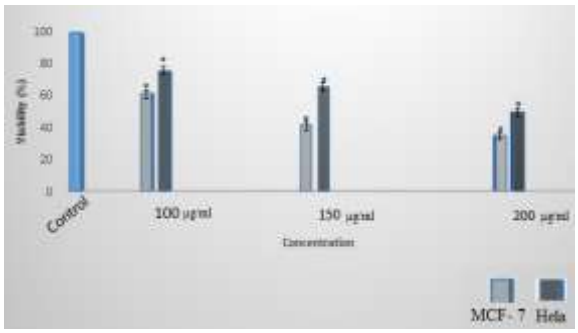

FIGURE 1 The figure represents the changes in the cell viability percentage in Hella cells and MCF-7 are after 48 h treatment with water mint. The values are represented as mean ± S.D. *P < 0.05 versus control

It has been shown in the previous reports that fractions of mentha extracts can prohibit cell proliferation in a concentration-dependent manner in different cell lines [23]. These results are in line with other studies that reported the loss of cell growth ability [24]. Also, it has been shown that mentha extract fractions significantly inhibited colony formation in non-small lung (NCI-h460) and mcf-7 cell lines, indicating the arrest of the cell cycle in the G0 step [25]. However, these effects were not observed in dimethyl sulfoxide (DMSO)-stored fractions. Moreover, it has been documented that the anti-proliferative effects of mentha in breast cancer are modulated by its agonism towards the estrogen receptors, which has been confirmed via E-screen assay (26). Similarly, DMSO-stored fractions did not show any effect 17b-estradiol-related cell growth, although DMSO could enhance the solubility and dispersion of the samples.

Our results indicated that incubation of Hela and MCF-7 cells with ethanolic extract of Mentha after 48 h led to a dose-dependent increment of the ROS formation (Figure 2).

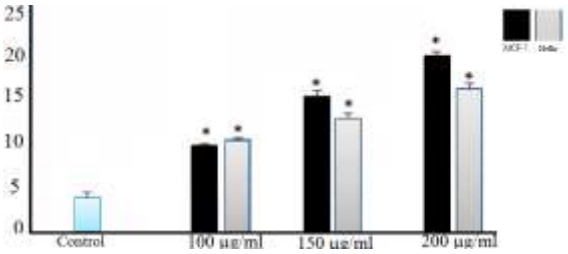

FIGURE 2 Hela and MCF-7 cells with ethanolic extract of Mentha after 48 hours led to a dose-dependent increment of the ROS formation. ROS is measured as pmole DCF formed/mg protein. The values are represented as mean ± S.D. *P < 0.05, versus control

Thus, the current results suggest the involvement of ROS in the possible anti-cancer effects of Mentha in these cell lines. In addition to ROS formation, it is also well known that cancer cell death is induced by the depletion of endogenous antioxidants, mainly glutathione [27]. We have also assessed the intracellular glutathione levels in both cell lines post-mentha treatment. Accordingly, the glutathione level substantially plummeted compared to the control group (Figure 3).

It was found that the glutathione was meaningfully lower compared with the control cells. Depleting glutathione has been reported to increase vulnerability to oxidative hazard-related cell death.

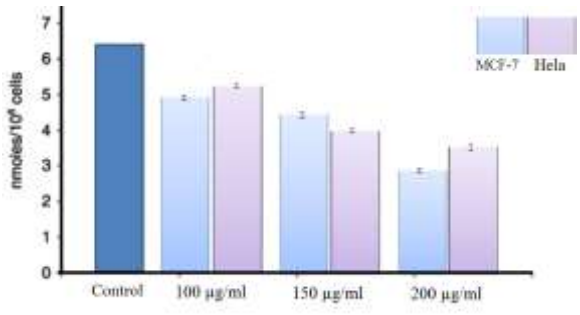

FIGURE 3 Effect of 48 h treatment with different doses of water mint on the glutathione levels in the Hela and MCF-7 cells. Changes are measured as the nmoles of glutathione in cells. The values are represented as mean ± S.D. *P < 0.05, versus control

Conclusion

In summary, mentha induces cytotoxicity of hella and MCF-7 cells through the elevation of ROS formation and glutathione depletion. This might be due to phytochemicals existing in the extract, which should be further fractionalized. Although additional studies are required to approve these features of water mint in vivo, the present investigation opens new windows of cancer treatment.

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