ORIGINAL ARTICLE

Radioprotective Effects of Thymus Vulgaris L. Essential Oil on Human Peripheral Blood Mononuclear Cells

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Received: 30 October 2022 / Accepted: 21 December 2022

Abstract

Purpose: Generated free radicals by ionizing radiations, as powerful cytotoxic agents, can damage DNA and proteins. Thymus vulgaris L (thyme) plant is a rich source of antioxidant phenolic compounds, which makes it a preferable candidate for medical applications. Given this, we set out the present study to investigate the effectiveness of thyme essential oil on Human Peripheral Blood Mononuclear Cells (PBMCs) as a radioprotector agent against ionizing radiations.

Materials and Methods: We extracted the thyme essential oil by the conventional Clevenger extraction method. Heparinized peripheral blood samples were also collected from five male volunteers, aged 22-25, without a history of smoking and irradiation. PBMCs were isolated and the maximum nontoxic concentrations (85µg/ml (of thyme essential oil were determined based on the result of the MTT method. In the next step, the PBMCs were cultured in the presence of thyme essential oil before and after X-irradiation with doses of 0.25 and 2.00 Gy.

Results: The most radioprotective effect was observed in the dose of 2.00 Gy for thyme-treated cells 24 hours before the irradiation (p-value ≤ 0.001) by a survival enhancement factor of 1.67, compared to the control group.

Conclusion: Our results showed that thyme essential oil can be used as an effective radioprotector agent for PBMCs against ionizing radiations. The most radioprotective effect was observed in the presence of thyme essential oil during irradiation.

Keywords: Radioprotector; Antioxidant; Thyme Essential Oil; Ionizing Radiations; Human Peripheral Blood Mononuclear Cell.

DOI: https://doi.org/10.18502/fbt.v11i2.15345

1. Introduction

Enzymatic and non-enzymatic antioxidant defense mechanisms are sufficient to balance the amount of free radicals, but various stresses can upset the balance of free radicals and cause tissue damage [1]. One of the popular stresses is ionizing radiation that its increasing applications in medicine lead to increased oxidative stress and metabolic change [2, 3]. In order to reduce the harmful effects of ionizing radiations, the evaluation of various types of nontoxic radioprotectors has been concerned by researchers [4- 9]. Nontoxic radioprotector agents have no or little toxic effects on the biological systems while reducing the damage of ionizing radiations. Radioprotectors reduce the radiation damage by different mechanisms. One of the mechanisms is based on the scavenging of free radicals which are produced due to the interactions of ionizing radiations with the biological systems [10, 11].

From thousands of years ago, natural herbal products have shown a great role in the treatment and health of human beings. Plants with antibacterial and antioxidant properties could be suitable options as radioprotector agents [12, 13]. Thymus vulgaris (thyme) is an aromatic plant from the family of Lamiaceae. It grows in the form of exuberant at dry hillsides of Mediterranean, Asian, South European, and North African regions [12]. This plant has more than 100 species with anti-bacterial, anti-fungal, anti-oxidant, anti-seizure, anti-cough, and anti-inflammation properties [14-18]. Furthermore, its anti-cancer effect has been approved by several studies [19].

The effective material of the thyme plant, thyme essential oil, is a yellow or dark reddish-brown liquid with a strong pleasant and cooling smell, which is extracted by distillation of the leaves and flowered branches of the plant. Thyme essential oil is obtained by water vapor distillation and deteriorates in the presence of light. Its specific weight is between 0.915 and 0.935. Thyme contains 0.8 to 2.6 % (normally 1 %) of essential oil, and its main part includes phenols (20-80 %), monoterpene hydrocarbons, and alcohols [20]. Thymol (2-isopropyl-5-methylphenol) and carvacrol are the main and subsidiary phenol component of thyme, respectively [21-25].

Various studies have revealed several biological properties of Thymol, including anti-inflammatory, antibacterial, and antioxidant activities. Hosseinimehr *et al*. investigated the effect of zataria multiflora (Avishan–e Shirazi) as a radioprotector on Human Blood Lymphocytes. Zataria-treated and untreated samples have been irradiated with 150 cGy of cobalt-60 γ source. The results of the micronuclei assay showed a reduction of 32% the micronuclei formation in the zataria-treated groups. They have reported that zataria extract with a high concentration of thymol can reduce the side effects of ionizing radiations as a radioprotector [26]. In the study of Ahmed *et al.*, the evaluation of antioxidant properties of thyme oil showed that the histological lesions induced by Doxorubicin (a common drug used in chemotherapy) in the kidney and heart can be reduced in the presence of thyme oil due to the enhancement of antioxidant defense mechanism and suppression of oxidative stress [18]. Tawfik *et al*. evaluated the antioxidative role of administrated thyme oil in γ-irradiated rats. Their results showed that thyme oil can minimize induced cell damage by free radicals based on the comparison of serum, liver, kidney, and heart tissues in treated and untreated groups [27]. Aljabeili *et al.* confirmed the impressive role of thyme oil as a powerful radical scavenger that can be applied commercially as an antioxidant agent [11]. In-vivo study by Archana *et al.* clearly documented the radioprotective potentials of Thymol by a dose reduction factor of 1.25 [28]. Nada *et al.* evaluated the radiation-induced injury to the kidney and liver cells in the presence of thyme oil with a single dose of gamma radiation. Their results confirmed the reduction of cellular injuries by the radioprotective effects of thyme oil [3].

Although the radioprotective effect of thyme essential oil has been investigated in several studies, and this reduces the innovation of similar works, the evaluation of the effect of thyme essential oil on various cell lines (in this study, human blood mononuclear cell line (PBMCs)) and in radiation doses selected based on scientific and practical reasons (0.25 and 2 Gy) can provide more information about its exact performance. The investigation of the radioprotective effects of thyme essential oil on PBMCs and in a dose range of the present study has not been clarified in biomedical sciences.

Human Peripheral Blood Mononuclear Cells (PBMCs) are immune cells with a single, round nucleus that originate in the bone marrow and are secreted into the peripheral circulation [29]. These cells are critical components of the immune system with high radiation sensitivity, which have been investigated in various studies related to the effect of ionizing radiations [30, 31].

The present study is designed to investigate the radioprotective effects of thyme essential oil on PBMCs against ionizing radiations.

2. Materials and Methods

2.1. Extraction of Thyme Essential Oil

All components of collected Thymus vulgaris including leaves, flowers, and stems were dried and powdered. The extraction of essential oil was performed by Clevenger apparatus using distilled water [14]. 40 gr dried thyme was weighed, powdered, and mixed in one liter of distilled water. During the distillation procedure, the liquefied vapor containing the molecules of essence was collected. The essence floated on the water surface was separated, and purified using a centrifuge (Eppendorf 5810R, Germany). The container of thyme essential oil was covered by parafilm and foil to maintain the concentration stability of essential oil.

2.2. Preparation of PBMCs Samples

Hepatized peripheral blood samples were taken from five normal volunteers aged 25–35 years, without a history of smoking and irradiation. Samples were centrifuged at 1800 rpm for 30 min. PBMCs were separated and cultured in RPMI-1640 medium supplemented with 10% Fetal Bovine Serum (FBS), 50 µg/ml streptomycin, and 50 iu/ml penicillin (Gibco, Germany). Plates were incubated at 37 C in 5% CO_2.

2.3. Evaluation of Thyme essential Oil Toxicity

For the evaluation of thyme essential oil toxicity, PBMCs were seeded in 96-well plates (10^5 cells/well). After their attachment to the bottom of the wells, they were treated with thyme concentrations of 10, 20, 50, 100, 200, and 400 µg/ml in triplicate groups. Ninety-six hours later, the toxicity was evaluated using MTT assay and the nontoxic concentration (IC10) of thyme essential oil was determined. A group of cells without thyme essential oil was taken as the control group.

2.4. Irradiation Set-Ups

Samples were irradiated by 6 MV photon beams of an ELEKTA Compact medical linear accelerator (Compact, ELEKTA-England). A tissue equivalent solid water slab phantom with a thickness of 5 cm was placed on the coach of treatment and the plates with PBMCs were placed on it. Then, two solid slab phantom sheets with a thickness of 5 cm, 10 cm in total, were inserted on the top of the plates. The irradiation was based on the Source-to-Axis Distance (SAD) set-up of 100 cm. The irradiation was conducted in the doses of 0.25 (low dose region) and 2.00 (high dose region in the interventional radiology) Gy at the gantry angle of 180 degrees and the field size of 20 × 20 cm^2 with a dose rate of 2.5 Gy/min at room temperature.

2.5. Evaluation of the Radioprotective Effect of Thyme Essential Oil

Enriched PBMCs were divided into three groups: first, the control group (without the thyme essential oil); second, thyme-treated cells before exposure to radiation (with incubation of 24 hours); and third, thyme-treated cells one hour after exposure to radiation.

The groups were irradiated by X-ray photon beams with doses of 0.25 and 2.00 Gy. 72 hours after irradiation, cell viabilities were evaluated by using the MTT method. Cells were incubated 4 hours in 37 C with a concentration of 5 mg/ml MTT (Sigma-Aldrich, Munich, Germany). Then, the medium was replaced with 100 µl of DMSO and the spectral absorption was measured with an ELISA microplate reader (TECAN infinite M200, Switzerland) at the 570-630 nm wavelengths.

2.6. Statistical Analysis

The statistical package of SPSS 16.0 was used for data analysis. Statistical differences of multiple groups were calculated via one-way Analysis of Variance (ANOVA). Probabilities of p-value<0.05 were considered statistically significant. Results have been reported as mean ± Standard Deviation (SD) of triplicates.

3. Results

3.1. Thyme Essential Oil Toxicity

The results of the evaluation of thyme essential oil toxicity with MTT assay have been shown in Figure 1. A Thyme concentration of less than 85 µg/ml (the peak survival on the toxicity curve), has led to the growth and reproduction of PBMCs. Beyond this concentration,

the cell viability has been decreased. Based on the cell viability results, the value of IC10 has been obtained at 135 µg/ml. In the present study, Thyme concentration at the peak of the toxicity curve (equal to 85 µg/ml) has been used for the evaluation of the effectiveness of thyme essential oil as a radioprotector agent.

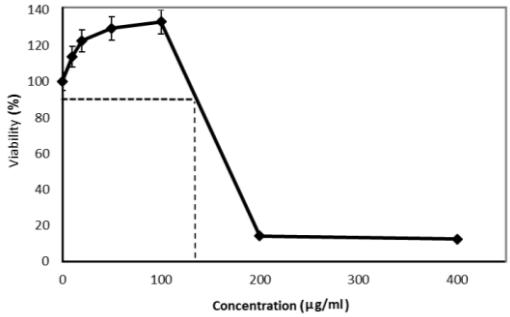

Figure 1. The viability of PBMCs in the presence of thyme essential oil after 96 hours incubation, obtained from MTT assay

3.2. Radioprotective Effects of Thyme Essential Oil

Cell viability results and the statistical differences of control and thyme-treated groups, irradiated with various doses of 6 MV X-ray photon beams, have been presented in Table 1 and Figure 2. The percentage viabilities of thyme-treated cells 24 hours before irradiation with doses of 0.25 and 2.00 Gy have been obtained 74.2 ± 9.9 and 72.7 ± 5.0, respectively.

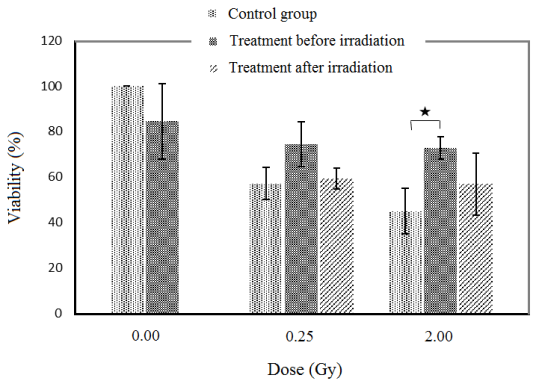

Figure 2. Cell viability and the statistical difference of control and thyme-treated groups irradiated with doses of 0.25 Gy and 2 Gy. (*p-value $\leq$ 0.05)

Furthermore, the percentage viabilities of thyme-treated cells that receive thyme essential oil one hour after irradiation with doses of 0.25 and 2.00 Gy have been obtained 59.3 ± 4.6 and 56.8 ± 13.6, respectively.

The Survival Enhancement Factor (SEF) and the statistical difference between various treated and control groups have been shown in Table 2 and Figure 3. The SEF values in groups which received thyme essential oil 24 hours before the radiation doses of 0.25 and 2.00 Gy, have been obtained 1.27 and 1.67, respectively. The SEF for those groups, which received thyme essential oil 1 hour after radiation doses of 0.25 and 2.00 Gy, have been obtained 1.02 and 1.29, respectively.

Table 1. Cell viability and the statistical difference of control and thyme-treated groups irradiated with doses of 0.25 Gy and 2 Gy

Group	0 Gy	0.25 Gy	2 Gy	p-value*	
				0.25 Gy	2 Gy
Control (without Thyme essential oil)	100	58.4 ± 7.1	43.5 ± 10.0	-	-
treatment before irradiation	84.7 ± 16.5	74.2 ± 9.9	72.7 ± 5.0	0.07	0.03
treatment after irradiation	-	59.3 ± 4.6	56.8 ± 13.6	0.1	0.3

*The statistical difference of various groups compared to the control group

Table 2. Survival enhancement factor and the statistical difference of thyme-treated groups before and after irradiation with doses of 0.25 Gy and 2 Gy

Group	0.25 Gy	2 Gy	p-value*	
			0.25 Gy	2 Gy
Control (without Thyme essential oil)	1	1	-	-
treatment before irradiation	1.27	1.67	0.05	0.001
treatment after irradiation	1.02	1.29	0.6	0.08

*The statistical difference of various groups compared to the control group

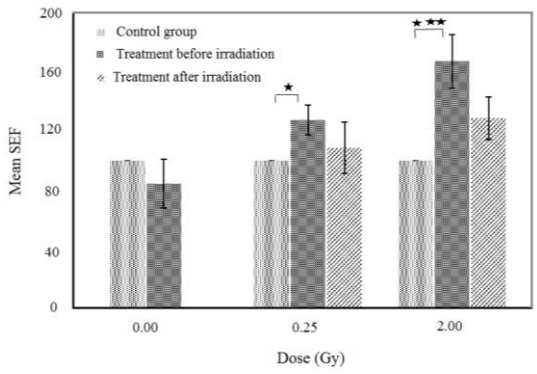

Figure 3. Survival enhancement factor and the statistical difference of control and thyme-treated groups irradiated with doses of 0.25 Gy and 2 Gy. (**p*-value ≤ 0.05; ***p*-value ≤ 0.01; *** *p*-value ≤ 0.001)

4.Discussion

With the advancement of technology, the use of ionizing radiation in diagnostic and therapeutic applications of medicine is increasing. Ionizing radiations, despite their advantages and extensive applications, lead to biological and cellular damage. Several studies have assessed the various kinds of radioprotector agents to introduce or develop chemical and herbal drugs for the reduction of ionizing radiation injuries. The evidence of synthetic chemicals toxicity leads to an interest in herbal drugs. Thyme plant with the main components of thymol and carvacrol phenol compositions, has demonstrated strong antibacterial and antioxidant properties [19]. In the present study, the toxicity and the radioprotective effect of thyme essential oil on PBMCs in radiation doses of 0.25 and 2.00 Gy have been evaluated.

Due to the adaptive response, bystander effect, and hormesis effect, fewer data repeatability is expected in a low-dose region (0-100 mSv). Therefore, a dose of 0.25 Gy, the outside of low-dose region, was selected in the sublethal dose range. On the other hand, the effective dose of adults from the most common techniques of interventional procedures, computed tomography, and nuclear medicine based on the report of Radiological Society of North America, have been estimated 5-70 mSv, 2-20 mSv, and 0.3-20 mSv, respectively [32]. These ranges imply that the dose ranges in the diagnostic procedures of developed countries can reach 7 cGy. It could be expected that these doses are higher in developing countries. Even, based on international reports, an effective dose of patients in the fluoroscopy and angiography procedures can be higher in some cases [32, 33]. In this study, a dose of 2 Gy is considered a representative of high dose region in the interventional radiology procedures so that the results can be generalized to the staff and patients with any diagnostic procedure.

The calculation of SEF (Figure 3) shows that there are significant differences between the groups which received thyme essential oil before the irradiation compared to the control group (p-value ≤ 0.05). The difference is higher in irradiated groups of 2.00 Gy compared to a low dose of 0.25 Gy. This means that the thyme essential oil has radioprotective effect against ionizing radiations, especially at the dose of 2.00 Gy. Comparing the results of the groups which received thyme essential oil before and after the irradiation, shows that percent viability and SEF is higher in the groups that receive thyme essential oil before the irradiation. Therefore, the highest level of its radioprotective effect is provided in the presence of thyme essential oil in the cell culture medium during the irradiation.

As it has been shown in Table 2, the SEF at the dose of 2 Gy is higher than the dose of 0.25 Gy. This difference is more obvious in the groups that received thyme essential oil before irradiation with significant differences of p-value ≤ 0.001 and p-value ≤ 0.05 compared to the control group, for doses of 2 and 0.25 Gy, respectively. The obtained results for the radioprotective effect of thyme essential oil at 0.25 Gy dose can be extended to the common radiological diagnostic procedures for the patients and staff. The dose of 2.00 Gy has been used as the representative of high dose levels in interventional radiological procedures and the borderline between the lethal and sublethal doses.

The results of this study, in full agreement with previous studies [11, 18, 26-28] confirm the effect of thyme essential oil as an effective antioxidant and appropriate radioprotector agent. Archana *et al.* reported the radioprotective potentials of Thymol by a Dose Reduction Factor (DEF) of 1.25 at a dose of 2.17 Gy in the in-vivo study [28]. In our study, a SER of 1.69 has been obtained for a dose of 2 Gy. This enhancement of SER compared to the DEF of Archana's study is related to the various conditions of the in-vitro and in-vivo studies. Additionally, the evaluation of the radioprotective effect of several plants like crochromine on mice [4], essential oil of achillea millefolium on blood lymphocyte5, extract of fenugreek6, the essence of rosemary [7- 9] has been evaluated and showed significant results in agreement with the result of our study.

The effect of essential oil depends on the type of species, the parts of the plant used for the oil extraction and the method of extraction [13]. A study about the effect of four species of thyme plant on PBMCs indicated that the root of the plant had the highest effect on the growth enhancement of PBMCs population [14]. The effect of thyme essential oil as a radioprotectors from different parts of the plant such as flower, leaf, stem, and root can be examined separately. Some studies have reported that the use of the essential oils in combination with nanoparticles can increase the effectiveness and efficiency of treatment, which is recommended for future work for more evaluation of thyme essential oil [34].

Several studies have pointed to the aggravating properties of the apoptotic process and growth inhibiting of cancer cells in the presence of thyme essential oil in comparison with normal cells [34, 35]. This means that this essence, besides the radioprotective role, can intensify the process of apoptosis in cancer cells. According to these studies, the effects of thyme essential oil in the process of radiation therapy need further investigation.

As limitations of the study, we can point out the small number of samples of only men and also the performance of only one test (MTT test). Unfortunately, due to some problems in the process of the study, it was not possible to conduct more tests. Certainly, more samples from men and women, as well as conducting more cellular tests, can provide more details about the radioprotective effect of thyme essential oil on PBMC cells.

5. Conclusion

The results of this study showed that thyme essential oil can be used as an effective radioprotector agent in the dose ranges of common and interventional radiology procedures for patients and staff when it is administrated before exposure to radiation.

Acknowledgments

The authors appreciate Shahid Sadoughi University of Medical Sciences for the financial support of this work.

The project was found to be in accordance with the ethical principles and the national norms and standards for conducting medical research in Iran [Approval ID: IR.SSU.MEDICINE.REC.1396.107].

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