JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Modulating Effects of Thyme and Its Major Ingredients on Oxidative DNA Damage in Human Lymphocytes

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The present study was carried out to investigate the modulating effects of thyme and its major components against the oxidative DNA damage induced by H_2O_2. The human lymphocytes with thymol, carvacrol, and γ-terpinene incubated with or without 0.1 mM H_2O_2 for 30 min at 37 °C and the DNA damage were evaluated by singe cell gel electropheresis (comet assay). Concentrations above 0.1 mM thymol and γ-terpinene and 0.05 mM carvacrol significantly induced DNA damage in human lymphocytes, but at the smaller concentrations no additional DNA strand breakage has been observed. At the all concentrations studied, γ-terpinene did not show any protective effect against H_2O_2 induced oxidative DNA damage, but the phenolic compounds thymol and carvacrol at concentrations below 0.2 and 0.1 mM, respectively, significantly reduced the oxidative DNA damage ($p < 0.001$). The *n*-hexane and ethyl acetate fractions prepared from the methanolic extracts of *Thymus spicata* also were found to inhibit DNA damage.

KEYWORDS: Thyme; thymol; carvacrol; γ-terpinene; hydrogen peroxide; single-cell gel electrophoresis; comet assay

INTRODUCTION

There has been an increasing interest in the use of natural compounds and dietary components, and questions concerning the safety of synthetic agents have encouraged more detailed studies of plant resources. Phenolic phytochemicals are a large group of substances and are found in significant quantities in vegetables, fruits, spices, and seeds. Since they have been regarded as possible antioxidants, their roles in food industry and in chemoprevention of diseases resulting from oxidative stress have been of great concern. Thyme, rich in phenolic phytochemicals, has been commonly used in foods mainly for its flavor, aroma, and preservation and has also been used in folk medicine since the Greeks, Egyptians, and Romans. The leafy parts of thyme belonging to the Lamiacea family have been added to meat, fish, and food products for years. Turkey is regarded as an important gene-center for the Lamiacea family. Of 39 spices grown in Turkey, 19 are endemic, and *Thymus*, *Origanum*, *Thymbra*, *Coridothymus*, and *Satureja* species are known and used as thyme (*1*, *2*). They are regarded as oil-rich (>2%) on the basis of their essential oil content and are marketed under the same name "kekik", which is the name given those species with a thymol/carvacrol-type odor in Turkey (*3*).

The major constituents of the oils of the thyme and oregano species have been reported as thymol, carvacrol, and γ-terpinene (*3*). Thyme essential oil and its ingredients have been shown to exhibit a range of biological activities. Since essential oils of thyme and oregano possess strong antibacterial and antimicrobial activity, they can be used to delay or inhibit the growth of pathogenic microorganisms. These activities are mostly attributable to the presence of phenolic compounds, such as thymol and carvacrol, and to the hydrocarbons, such as γ-terpinene and *p*-cymene (*4*−*8*). Thymol and carvacrol can be used alone or combined during the treatment of oral infectious diseases since they showed an inhibitory activity on oral bacteria (*9*, *10*). Thyme and oregano have been found to inhibit aflatoxin production (*11*, *12*). Antispasmodic and antiplatelet aggregation activities were also reported with thyme constituents (*13*, *14*). Components of thyme, mainly thymol and carvacrol, were suggested to have antioxidant activity (*16*−*19*). In the search for natural replacements for synthetic antioxidant, thymol and carvacrol were found to be promising. Thyme oil and thymol dietary supplementations have been observed to have a positive effect on the antioxidant enzyme activities in animals (*20*, *21*).

As flavoring principles, thyme volatiles such as thymol and carvacrol are present in low concentrations in human food. However, if the use of these compounds is extended to other applications that may require higher doses as well, the increased exposure of humans to these compounds is a matter of concern. The data that are available in the literature mainly concern acute and short-term in vivo effects in different animal species and suggest that such compounds may not pose a risk for human health (*22*−*24*).

Very few studies have been performed on the mutagenicity and/or antimutagenicity of the ingredients of thyme. Although

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10.1021/jf0402375 CCC: $30.25
Published on Web 02/01/2005

the genotoxic potential of thymol and carvacrol at nontoxic doses in DNA repair test or SOS-chromotest have been suggested to be weak (*23*), contradictory results have also been reported with the Ames test (*23*−*25*).

In the present study, the mutagenic potential of major compounds of thyme oil (i.e., thymol, carvacrol, γ-terpinene) and the methanolics extract of thyme at different concentrations in human lymphocytes were investigated by single-cell gel electrophoresis (the comet assay). The effects of these substances on the H_2O_2 induced oxidative DNA damage were evaluated.

MATERIALS AND METHODS

Chemicals. The compounds thymol, carvacrol, and γ-terpinene used in the experiments were from Fluka AG Switzerland; the purity of the compounds were 99.5%, 98%, and 97%, respectively. The other chemicals used in the comet assay were purchased from the following suppliers. Normal melting agarose (NMA) and low melting agarose (LMA) were from Boehringer Manheim (Germany); sodium chloride (NaCl), hydrogen peroxide (H_2O_2), and sodium hydroxide (NaOH) were from Merck Chemicals (Darmstadt, Germany); dimethyl-sulfoxide (DMSO), ethidium bromide (EtBr), Triton X-100, and phosphate-buffered saline (PBS) tablets were from Sigma (St. Louis, MO); ethylenediamine tetraacetic acid disodium salt dihydrate (EDTA), *N*-lauroyl sarcosinate, and Tris from ICN Biochemicals (Aurora, OH).

Plant Material. *Thymus spicata*, which is known and used as "Karabaş Kekik", was collected from South Anatolia. The plant material was identified in the Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University. The sample was washed thoroughly with tap water, and 50 g of the plant material was extracted with 50% methanol in water at 40 °C and concentrated under vacuum. The concentrated methanolic extract was first extracted with *n*-hexane and then with ethyl acetate and evaporated separately. The fractions were kept in lyophilized and stored at −80 °C prior to testing. Before the experiments, the lyophilisates were dissolved in DMSO, but DMSO content of the solutions never exceeded 1%.

Thin Layer Chromatography (TLC) of the Fractions. The adsorbent used in TLC was Kieselgel GF_{254} coated on aluminum foil sheets 200 μm, 20 × 20 cm (Merck 5554). The solvent systems were given as follows: ethyl acetate:methanol:water (100:13.5:10), ethyl acetate:formic acid:glacial acetic acid:water (100:11:11:27), chloroform:methanol:water (61:32:7), and chloroform:methanol:water (80:20.2).

Detection. The spots were first studied directly on the chromatogram in daylight and under UV light. The following spray reagents were used for visualization: Naturstoff reagent (1% of diphenylboric acid aminoethyl ester in methanol); NH_3 vapor; 30% H_2SO_4 in water and heated to 100 °C; 1% vanillin in concentrated H_2SO_4 heated to 100 °C.

Blood Samples and Cell Preparation. For each experiment, 5 mL of heparinized (50 units/mol sodium heparin) whole blood was collected by venipuncture from a healthy 48-year-old nonsmoker female donor not exposed to radiation or drugs. Lymphocytes were isolated by Ficoll-Hypaque density gradient (*26*) and washed with PBS. Cell concentrations were adjusted to approximately 2×10^5/mL in the buffer. The cells were suspended in a total volume of 1 mL, and each reaction contained 50 μL of suspension (≈10^4 cells), varying microliter amounts of the test agent, and PBS in a total volume of 1 mL. The cells were incubated for 0.5 h at 37 °C in an incubator together with untreated control samples. All test substances were dissolved in DMSO, but DMSO content of the solutions never exceeded 1%. Control incubations contained the same concentrations of DMSO. After incubation, the lymphocytes were harvested by centrifugation at 800*g* for 3 min at 4 °C. Oxidative damage was introduced by replacing the medium with PBS containing at 0.1 mM concentration of H_2O_2, and the treatment was for 5 min on ice. Then the cells were harvested by centrifugation at 800*g* for 3 min at 4 °C. Again the cells were harvested by centrifugation at 800*g* for 3 min at 4 °C cells after washing with BPS. The lymphocytes were suspended in 75 μL of low melting point agarose (LMA) for embedding on slides. The replicate experiments were carried out with blood samples from the same donor collected at different time intervals. Cells were checked for viability by trypan blue exclusion.

Slide Preparation. The basic alkaline technique of Singh et al. (*27*), as further described by Anderson et al. (*28*) and Collins et al. (*29*), was followed. The microscopic slides had been each covered with 1% NMA at about 45 °C in Ca^{2+}- and Mg^{2+}-free PBS before the experiment. This layer was used to promote the attachment of the second layer. For the second layer, around 10 000 treated or control cells mixed with 75 μL of 0.5% LMA were rapidly pipetted onto this slide, spread using a cover slip, and maintained on an ice-cold flat tray for 5 min to solidify. After removal of the cover slip, the slides were immersed in cold lysing solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, 1% sodium sarcosinate, pH 10) with 1% Triton-X 100 and 10% DMSO added just before use for a minimum of 1 h at 4 °C, but the cells treated with H_2O_2 and embedded on slides were immersed in other cold lysing solution.

Electrophoresis. The slides were removed from the lysing solution, drained, and placed side by side avoiding space and with the agarose ends facing each other nearest the anode in a horizontal gel electrophoresis tank. The tank was filled with fresh electrophoresis solution (1 mM Na_2EDTA and 300 mM NaOH, pH 13) to a level approximately 0.25 cm above the slides. Before electrophoresis, the slides were left in the solution for 20 min to allow the unwinding of the DNA and expression of alkali labile damage. Electrophoresis was conducted at a low temperature (4 °C) for 20 min using 24 V and adjusting the current to 300 mA by raising or lowering the buffer level and using a compact power supply (Power Pack P 25 Biometra Analytic GmbH). All of these steps were conducted under dimmed light (tank was covered with a black cloth) to prevent the occurrence of additional DNA damage. After electrophoresis, the slides were taken out of the tank. Tris buffer (0.4 M Tris, pH 7.5) was added dropwise and gently to neutralize the excess alkali, and the slides were allowed to sit for 5 min. The neutralizing procedure was repeated 3 times.

Staining. To each slide, 65 μL of EtBr (20 μg/mL) was added. The slides were covered with a cover slip, placed in a humidified airtight container to prevent drying of the gel, and analyzed within 3−4 h.

Slide Scoring. For visualization of DNA damage, slides were examined at an 1000× magnification using a 100× objective (oil immersion) on a fluorescence microscope (Zeiss, Germany). Images of 200 randomly selected lymphocytes (i.e., 100 cells from each of two replicate slides) were analyzed from each sample, and the DNA damage was scored visually as described by Collins et al. (*29*), Breaks in the DNA molecule disturbs its complex super coiling, allowing liberated DNA to migrate toward the anode. Staining shows the DNA as "comets" with a briefly fluorescent head and a tail streaming away from it. Randomly selected lymphocytes by the slide reader were graded visually for each slide. A total damage score for the slide was derived by multiplying the number of cells assigned to each grade of damage by the numeric value of the grade and summing over all grades giving a maximum possible score of 400, corresponding to 100 cells of grade 4 (i.e., grade 0 (no damage), grade 1 (low damage), grade 2 (medium damage), grade 3 (high damage), grade 4 (very high damage)). One slide reader, thus minimizing variability due to subjective scoring, performed analysis.

Statistical Analysis. The SPSS for Windows 10.0 computer program was used for statistical analysis. Statistical comparisons of the results from negative controls and H_2O_2 groups were carried out by one-way analysis of variance (ANOVA) test. Post hoc analysis of group differences was performed by the LSD test. Results are expressed as mean ± SD.

RESULTS AND DISCUSSION

Cell viability, as tested using trypan blue dye exclusion of each treated group, was more than 90%. The effects of thymol, cavracrol, γ-terpinene, *n*-hexane, and ethyl acetate fractions of thyme on DNA damage in human lymphocytes are shown in **Table 1**. According to the data from three separate experiments, no additional DNA strand breakage at thymol concentrations

Table 1. Effects of Thymol, Carvacrol, γ-Terpinene, *n*-Hexane, and Ethyl Acetate Fractions of Thyme on DNA Damage in Human Lymphocytes in the Comet Assay

	damage in human lymphocytes[a]					
treatment	undamaged (0)	low damaged (1)	medium damaged (2)	high damaged (3)	very high damaged (4)	DNA damage (arbitrary units) (0–400)[a]
negative control (1% DMSO)	79	14	4	2	1	32
thymol 0.005 mM	84	11	3	1	1	25
thymol 0.01 mM	80	12	5	2	1	31
thymol 0.025 mM	78	13	4	4	1	37
thymol 0.05 mM	77	11	7	4	1	41
thymol 0.1 mM	78	8	9	3	1	40
thymol 0.2 mM	65	15	7	7	6	73[b]
thymol 0.5 mM	56	13	13	13	5	97[b]
thymol 1 mM	23	23	28	19	8	165[c]
thymol 2 mM	10	12	32	17	29	243[c]
negative control (1% DMSO)	89	8	3	1	0	16
carvacrol 0.0005 mM	87	10	2	1	0	17
carvacrol 0.001 mM	83	13	3	1	0	21
carvacrol 0.005 mM	81	13	4	1	0	27
carvacrol 0.025 mM	82	13	3	1	1	26
carvacrol 0.05 mM	90	6	3	2	0	17
carvacrol 0.1 mM	74	16	4	4	2	44[c]
carvacrol 0.2 mM	39	36	17	6	2	97[c]
carvacrol 0.5 mM	22	24	24	20	11	173[c]
carvacrol 1 mM	8	11	19	27	35	272[c]
carvacrol 2 mM	4	3	14	35	44	311[c]
negative control (1% DMSO)	91	7	2	0	0	13
γ-terpinene 0.00005 mM	87	9	3	1	0	19
γ-terpinene 0.0005 mM	89	9	2	0	0	13
γ-terpinene 0.005 mM	89	9	2	1	0	15
γ-terpinene 0.025 mM	87	12	0	0	0	13
γ-terpinene 0.05 mM	88	8	3	1	0	16
γ-terpinene 0.1 mM	85	10	4	1	0	20
γ-terpinene 0.2 mM	74	15	9	2	0	39[c]
γ-terpinene 0.5 mM	66	16	13	4	1	60[c]
γ-terpinene 1 mM	56	22	14	6	3	79[c]
negative control (1% DMSO)	89	9	2	0	0	13
HF 0.0075 mg/L	91	8	2	1	0	12
HF 0.075 mg/L	91	9	0	0	0	9
HF 0.75 mg/L	93	7	1	0	0	8
HF 7.5 mg/L	86	12	2	0	0	16
HF 15 mg/L	70	19	11	1	0	43[d]
HF 75 mg/L	20	24	29	17	10	173[c]
HF 150 mg/L	31	17	35	14	4	143[c]
HF 300 mg/L	23	13	18	31	17	206[c]
negative control (1% DMSO)	83	6	9	2	1	32
EAF 10 mg/L	83	14	3	0	0	20
EAF 50 mg/L	82	14	5	0	0	24
EAF 100 mg/L	75	11	8	7	0	47[b]
EAF 200 mg/L	40	20	33	6	1	108[c]
EAF 500 mg/L	25	11	29	30	5	179[c]
EAF 1000 mg/L	20	15	27	24	15	200[c]

[a] Values are the mean of three separate studies. [b] $p < 0.05$. [c] $p < 0.001$ compared with the negative control (1% DMSO). [d] $p < 0.01$.

of 0.005, 0.01, 0.025, 0.05, and 0.1 mM was observed; at higher concentrations of 0.2, 0.5, 1, and 2 mM significant increases in DNA damage were seen when compared with the negative control (1% DMSO). The numbers of damaged cells were significantly reduced when lymphocytes were incubated with H_2O_2 and thymol within the concentration range of 0.0005−0.1 mM as seen in **Figure 1**. Even at the concentration of 0.2 mM that caused DNA damage alone, thymol seemed to have a protective effect against H_2O_2 induced DNA breakage.

At the concentrations of 0.0005, 0.001, 0.005, 0.025, and 0.05 mM no additional DNA damage in human lymphocytes was observed with carvacrol since the number of damaged cells was not significantly different from the negative control. But it seemed that at the higher concentrations of 0.1, 0.2, 0.5, 1, and 2 mM carvacrol itself induced DNA strand breakage (**Table 1**). As seen in **Figure 2**, carvacrol protected lymphocytes from the mutagenic effects of H_2O_2 since a significantly reduced DNA damage was observed when carvacrol at concentrations of 0.00005, 0.0005, 0.005, 0.025, 0.05, and 0.1 mM was incubated with H_2O_2 ($p < 0.001$).

γ-Terpinene was also investigated in the dose range of 0.00005, 0.0005, 0.005, 0.025, 0.05, 0.1, 0.2, 0.5, and 1 mM. γ-Terpinene alone showed DNA damage in human lymphocytes at the concentrations starting from 0.2 mM ($p < 0.001$) (**Table 1**). However no protective effect against H_2O_2 induced DNA damage was observed with γ-terpinene at concentrations of 0.00005−0.1 mM. (**Figure 3**).

The major constituents of the plant extracts are well-known, and studies on evaluating its compounds are published elsewhere. In this paper, general thin layer chromatographic methods

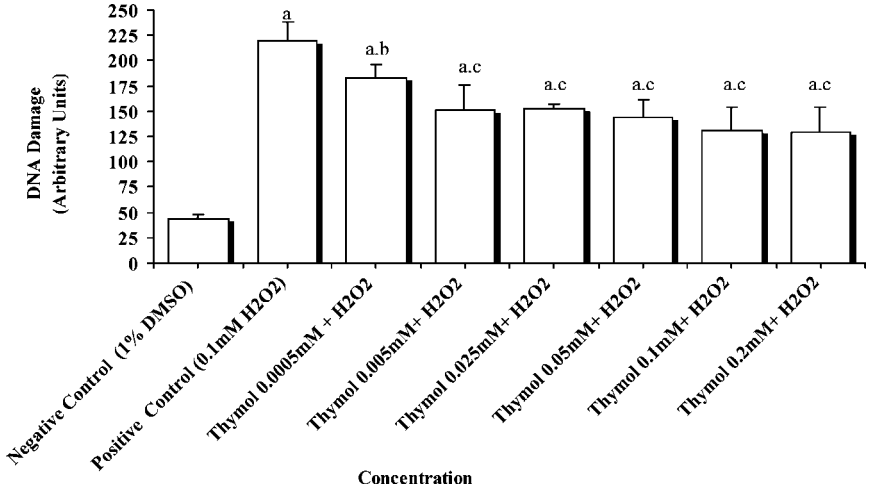

Figure 1. Protective effect of thymol on H_2O_2-induced oxidative DNA damage in human lymphocytes. Values are the mean of four separate studies. (a) $p < 0.001$ compared with the negative control (1% DMSO); (b) $p < 0.01$; (c) $p < 0.001$ compared with the positive control (0.1 mM H_2O_2). The statistic analysis is performed with ANOVA followed by LSD test.

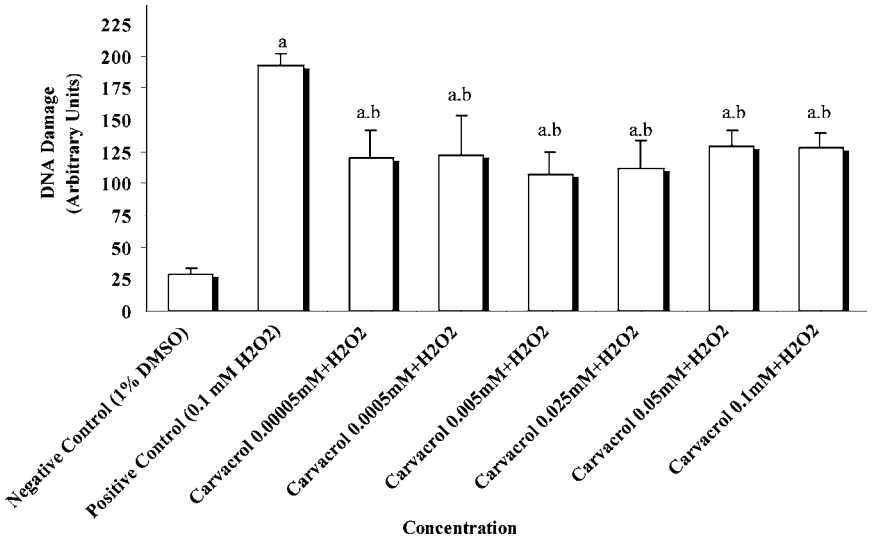

Figure 2. Protective effect of carvacrol on H_2O_2-induced oxidative DNA damage in human lymphocytes. Values are the mean of four separate studies. (a) $p < 0.001$ compared with the negative control (1% DMSO); (b) $p < 0.001$ compared with the positive control (0.1 mM H_2O_2). The statistic analysis is performed with ANOVA followed by LSD test.

were realized with different solvent systems and compared by authentic compounds. The results are assigned by the references done on this item (*2*, *3*, *9*). The major ingredients were established due to those chromatographic results obtained from the methanolic extract and the fractions as well as the data from the references. The constituents of the *n*-hexane fraction (HF) prepared from the concentrated methanolic extract of *Thymus spicata* was first examined by TLC method; carvacrol, thymol, and isomers of terpinene and some other terpenic substances were identified. The dilutions of the HF was prepared to give the concentrations of 0.0075, 0.075, 0.75, 7.5, 15, 75, 150, and 300 mg/L, which are the estimated equivalent concentrations of 0.00005, 0.0005, 0.005, 0.05, 0.1, 0.5, 1, and 2 mM according to thymol, carvacrol, and γ-terpinene content of the fraction. As seen in **Table 1** when compared with negative control, no significant DNA damaging effect was seen within the concentration at 0.0075−7.5 mg/L, but at the higher concentrations significant DNA strand breakage was observed in lymphocytes (15 mg/L, $p < 0.01$; 75, 150, 300 mg/L, $p < 0.001$). HF containing phenolic and terpenic compounds at the concentrations of 0.0075, 0.75, 15, 30, and 75 mg/L significantly protected lymphocytes against H_2O_2 induced DNA damage, and at concentrations of 0.75 and 15 mg/L, even an inhibition of DNA damage was observed (**Figure 4**).

The dried methanolic extract of *T. spicata* after fractioning with *n*-hexane was extracted with ethyl acetate. The ethyl acetate fraction (EAF) as examined by TLC was identified to contain flavonoids. The dilutions of the ethyl acetate fraction were prepared to give the concentrations of 10, 50, 100, 200, 500, and 1000 mg/L. At lower concentrations of 10 and 50 mg/L, the fraction when compared to negative control induced no DNA damage on human lymphocytes whereas, at the higher concentrations of the fraction containing flavonoids, DNA strand breakage was observed (**Table 1**). It seemed also that within the concentration range of 10−500 mg/L the ethyl acetate fractions protected lymphocytes from H_2O_2 induced DNA damage. At concentrations of 100 and 200 mg/L, which caused DNA damage alone, the fraction seemed to inhibit the DNA strand breakage induced by H_2O_2 ($p < 0.01$) (**Figure 5**).

The leaves and flowers of plants containing numerous aroma chemicals have been widely used in folk medicine and in modern aromatherapies. Modulation of carcinogenic and mutagenic effects by promoters or inhibitors from plant origin has been of crucial importance for the final outcome of some

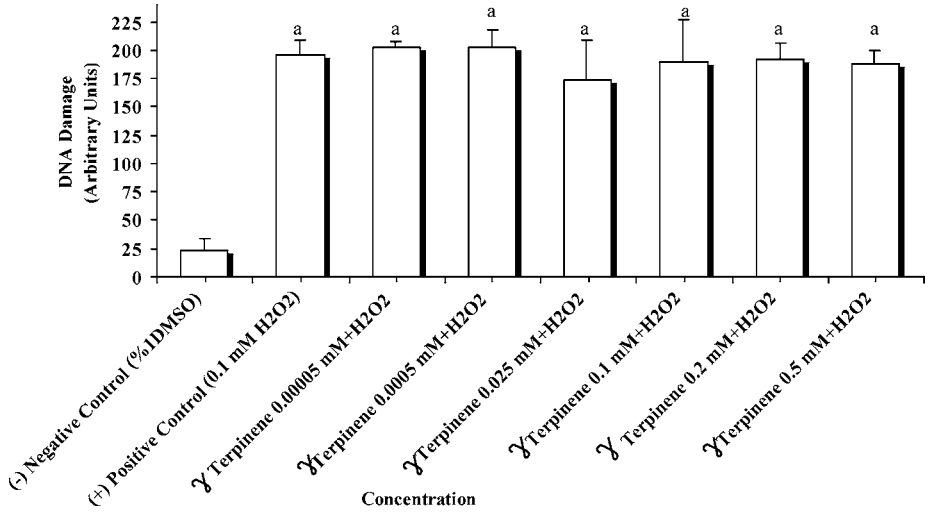

Figure 3. Protective effect of γ-terpinene on H_2O_2-induced oxidative DNA damage in human lymphocytes in the comet assay. Values are the mean of four separate studies. (a) $p < 0.001$ compared with the negative control (1% DMSO). The statistic analysis is performed with ANOVA followed by LSD test.

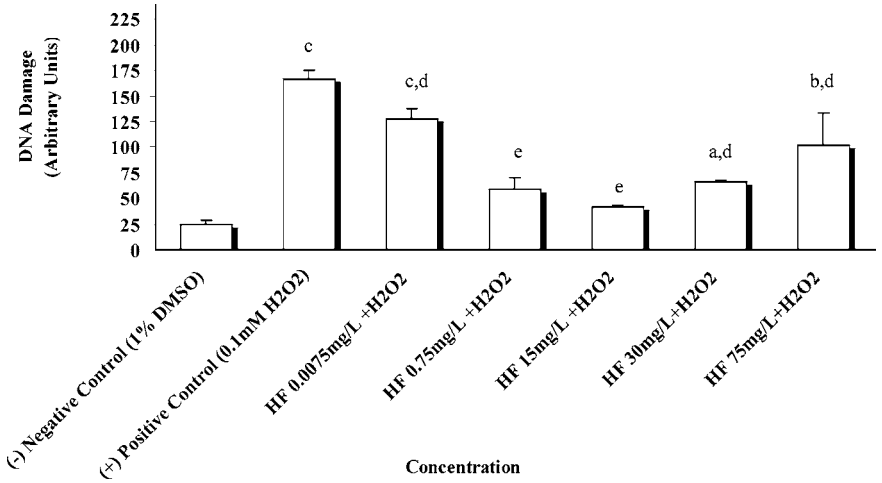

Figure 4. Protective effect of *n*-hexane fraction (HF) of thyme on H_2O_2-induced oxidative DNA damage in human lymphocytes. Values are the mean of three separate studies. (a) $p < 0.05$; (b) $p < 0.01$; (c) $p < 0.001$ compared with the negative control (1% DMSO); (d) $p < 0.01$; (e) $p < 0.001$ compared with the positive control (0.1 mM H_2O_2). The statistic analysis is performed with ANOVA followed by LSD test.

biological effects, particularly for cancer since available epidemiological evidence is exceptionally strong and consistent between consumption of high amounts of vegetables and fruits and a reduced risk of cancer (*30*−*33*). Phenolic phytochemicals are thought to promote optimal health partly via their antioxidant and free radical scavenging effects in protecting cellular components against free radicals. But due to their diverse chemical structures, they likely possess different degrees of antioxidant activity (*34*).

Few toxicological data are available for thyme and its ingredients. The only acute toxicity data available in vivo for thymol and carvacrol are those related to oral LD_{50}. In rats oral LD_{50} values are suggested to be 980 mg/kg body weight for thymol and 810 mg/kg body weight for carvacrol (*22*). In a later study it was found that thymol and carvacrol inhibited viability and proliferation of Hep 2 cells in a dose-dependent manner. IC_{50} ranged from 0.7 mM (thymol) in viability test to 0.2 mM (carvacrol) in the proliferation test. The morphological analysis suggested an involvement of apoptosis in the case of carvacrol (*24*).

Thymol sulfate was detected in plasma of humans, with peak concentrations of 93 ng/mL about 2 h after ingestion of a tablet containing the equivalent of 1 mg thymol; the mean terminal elimination half-life was 10.2 h (*10*). These data are particularly relevant for a risk assessment and provide a link between in vivo and in vitro studies. The peak levels in plasma after a therapeutic dose translate to less than 0.001 mM (i.e., much lower concentrations than those that induced DNA damage in human lymphocytes in this study).

Although it was suggested that no genotoxic property of the essential oil from *T. vulgaris* L. was found in *Bacillus subtilis* rec assay (*23*) and in *Salmonella typhimurium* strains TA 97, TA 98, and TA 100 with and without metabolic activation (*25*) but with thymol and carvacrol, the Ames test are somewhat ambiguous since it was found that they increased the number of revertants by 1.5−1.7 times regardless of the metabolic activation but not to a level generally considered significant (*24*). In the SOS-chromotest and also in the DNA repair test, the genotoxic potential of thymol and carvacrol was found to be very weak (*24*). On the other hand, thymol did not cause DNA single-strand breaks in human pulp cell cultures at cytotoxic (0.5 mM) concentrations (*35*). The broad range of concentrations of the compounds studied in this study have

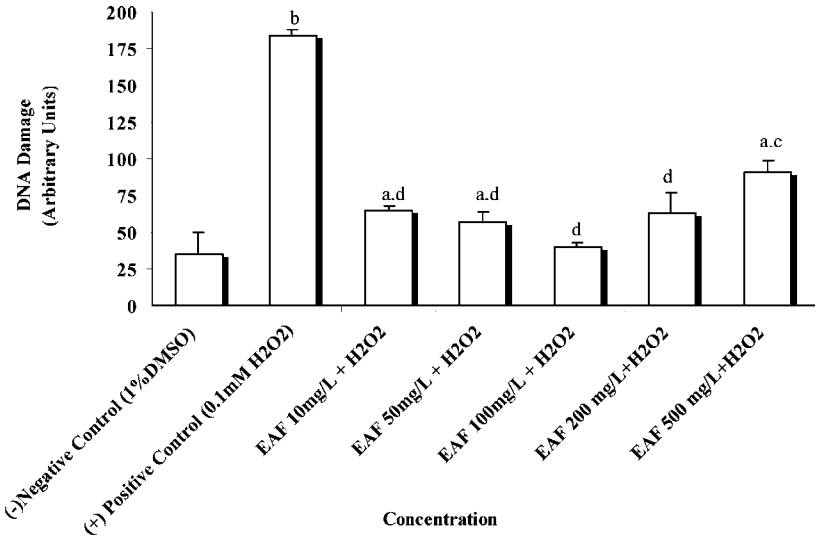

Figure 5. Effects of ethyl acetate fraction of thyme on H_2O_2-induced oxidative DNA damage in human lymphocytes. Values are the mean of three separate studies. (a) $p < 0.01$; (b) $p < 0.001$ compared with the negative control (1% DMSO); (c) $p < 0.01$; (d) $p < 0.001$ compared with the positive control (0.1 mM H_2O_2). The statistic analysis is performed with ANOVA followed by LSD test.

covered both the previous IC_{50} values and the concentrations evaluated in the genotoxicity studies previously.

In our study, the major constituents of thyme (i.e., thymol and γ-terpinene) did not seem to induce DNA strand breakage at lower concentrations than 0.1 mM in human lymphocytes, but at the concentrations starting from 0.2 mM both thymol and γ-terpinene showed DNA damage. Carvacrol, seemed to be more toxic than thymol since DNA damage was observed at the concentrations higher than 0.05 mM but at the concentrations below 0.05 mM no additional DNA damage in human lymphocytes was observed. These results are similar with the few toxicological data of carvacrol since the toxic effects at the cellular level could be rather different between even very closely chemically related compounds such as isomers (*22*, *24*). Both the *n*-hexane fractions containing phenolic and terpenic substances and also the ethyl acetate fractions containing flavonoids did not induce DNA breakage at concentrations below 15 and 50 mg/L, respectively. The results of our study are the first data that give the genotoxicity profile of the commonly worldwide used spice "thyme". At lower concentrations, the major compounds and constituents of thyme did not induce DNA damage in human lymphocytes as evaluated by the comet assay. On the other hand, it was found that at nontoxic concentrations thymol, carvacrol, and extracts protected lymphocytes from H_2O_2 induced oxidative DNA damage. Our results support the data of the antioxidant properties of thyme oil (*16*−*19*, *36*−*38*). Also our data are consistent with the findings that antioxidant effects were attributable to the presence of phenolic compounds since no protective effect was observed with the γ-terpinene. Studies about preventing the oxidation of blood plasma lipids, especially low-density protein (LDL), with phenolic compounds have been reported many times. Thymol and carvacrol are components of thyme that possess useful antioxidant properties since they decreased peroxidation of phospholipid liposomes ant the presence of iron(III) and ascorbate and they were found to be good scavengers of peroxyl radicals ($CCl_3O_2^{\bullet}$) radiated by pulse radiolysis. Antioxidant activity of volatile extracts from thyme was found to be similar in antioxidant activity to BHT and α-tocopherol as measured by the aldehyde/carboxylic acid and conjugated diene assay (*38*). Thymol was reported to inhibit malondialdehyde formation from blood plasma oxidation by 43% at the level of 400 μg/mL (*18*). Youdim and Deans (*20*, *21*) found also that thyme oil and thyme fed rats maintained significantly higher antioxidant enzyme activities such as superoxide dismutase, glutathione peroxidase and total antioxidant status.

In conclusion since the role of diet in the aetiology of cancer is extremely complex and our results concern only in vitro experiments with human lymphocytes, additional animal and human studies should be performed in order to clarify the antimutagenic potential of thyme and its components. It seems that our results highlight the potential benefit of thyme and its major components, such as thymol and carvacrol, as a dietary supplement and as a natural antioxidant.

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Received for review May 13, 2004. Accepted November 30, 2004. This study was supported by Hacettepe University Research Foundation (HUAF 01-02-301-003).

JF0402375