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The effects of thyme volatiles on the induction of DNA damage by the heterocyclic amine IQ and mitomycin C

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Abstract

The leafy parts of thyme and its essential oil have been used in foods for its flavour, aroma and preservation for many years. In the present study the genotoxic potential of major compounds of thyme oil, i.e. thymol, carvacrol, and γ-terpinene and of the methanolic extracts of thyme, were investigated in human lymphocytes by single-cell gel electrophoresis. Also, the effects of these substances on the induction of DNA damage by 2-amino-3-methylimidazo[4,5-f]-quinoline (IQ) and mitomycin C (MMC) were evaluated. No increase in DNA strand breakage was observed at thymol and γ-terpinene concentrations below 0.1 mM, but at the higher concentration of 0.2 mM significant increases in DNA damage were seen. Thymol and γ-terpinene significantly reduced the DNA strand breakage induced by IQ and MMC at the lower concentrations studied. Carvacrol, which is an isomer of thymol, seemed to protect lymphocytes from the genotoxic effects of IQ and MMC at non-toxic concentrations below 0.05 mM, but at the higher concentration of 0.1 mM carvacrol itself induced DNA damage. Also the constituents of the *n*-hexane and ethyl acetate fractions prepared from the concentrated aqueous methanolic extracts of *Thymus spicata* protected lymphocytes against IQ- and MMC-induced DNA damage in a concentration-dependent manner.

Keywords: Thyme; Thymol; Carvacrol; γ-terpinene; Mitomycin C; 2-Amino-3-methylimidazo[4,5-f]-quinoline; Single-cell gel electrophoresis; Comet assay

1. Introduction

The renewed interest in natural substances, rather than in synthetic agents has focused attention on plants used as food or spices, which are a rich source of bio-nutrients or bio-active photochemicals. Questions concerning the safety of these compounds have encouraged more detailed studies of plant resources. Some aroma extracts and essential oils isolated from plants, formerly considered only as flavours and fragrances, are now considered as natural remedies. Thyme or oregano has been commonly used in foods mainly for the flavour, aroma and preservation and also in

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folk medicine since the ancient 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 and of 39 spices grown in Turkey, 19 are endemic. *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 in Turkey to those species with a thymol/carvacrol type odor [3].

The major constituents of the oils of thyme and oregano species have been reported to be thymol, carvacrol and γ-terpinene. 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 hydrocarbons like γ-terpinene and *p*-cymene [3–8]. Thymol and carvacrol can be used alone or in combination during the treatment of oral infectious diseases because of their inhibitory activity on oral bacteria [9,10]. Components of thyme, mainly thymol and carvacrol, were suggested to have antioxidant activity [11–14] and thyme and oregano were found to inhibit aflatoxin production [15,16]. Antispasmodic and antiplatelet aggregation activities were also reported with thyme constituents [17,18].

As flavouring 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 few data available in the literature mainly concern acute and short-term effects in vivo on different animal species, and suggest that such compounds may not pose a risk to human health [19–21].

Very few studies have been performed on the mutagenicity and/or antimutagenicity of the ingredients of thyme. Although the genotoxic potential of thymol and carvacrol at non-toxic doses has been suggested to be weak in the DNA-repair test and the SOS-chromotest [21], contradictory results have been reported with the Ames mutagenicity assay [21–23].

In the present study, the genotoxic potential of major compounds of thyme oil, i.e. thymol, carvacrol and γ-terpinene and of the methanolic extract of thyme were investigated at different concentrations in human lymphocytes by use of the single-cell gel electrophoresis (comet) assay. The effects of these substances on DNA-damage induction by mitomycin C (MMC) and 2-amino-3-methylimidazo[4,5-f]-quinoline (IQ) were also evaluated.

2. Material and methods

2.1. Chemicals

The pure compounds thymol, carvacrol and γ-terpinene used in the experiments were from Fluka AG Switzerland. The other chemicals used in the comet assay were purchased from the following suppliers: normal melting agarose (NMA) and low melting agarose (LMA) from Boehringer Mannheim (Germany); sodium chloride (NaCl) and sodium hydroxide (NaOH) from Merck Chemicals (Darmstadt, Germany); mitomycin C (MMC), dimethyl sulfoxide (DMSO), ethidium bromide (EtBr), Triton X-100, and phosphate-buffered saline (PBS) tablets from Sigma (St. Louis, USA); ethylenediamine-tetraacetic acid disodium salt dihydrate (EDTA), 2-amino-3-methylimidazo[4,5-f]-quinoline (IQ), *N*-lauroyl sarcosinate and Tris from ICN biochemicals (Aurora, OH, USA).

2.2. Plant material

Thymus spicata, known and used as 'karabaş kekik' was collected from South Anatolia, the plant material was identified in Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy. The sample was washed thoroughly with tap water and 50 g of 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 form and were stored at −80 °C prior to testing. Before the experiments the lyophilized material was dissolved in DMSO, the DMSO content of the solutions never exceeding 1%.