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ORIGINAL ARTICLE

Antigenotoxic potential of curcumin and carvacrol against malathion-induced DNA damage in cultured human peripheral blood and its relation to *GSTM1* and *GSTT1* polymorphism

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Abstract The aerial application of malathion, a widely used organophosphate insecticide, has raised public concerns about its potential adverse health effects. We, therefore, studied the antigenotoxic potential of curcumin and carvacrol against malathion-induced DNA damage using sister chromatid exchange (SCE) as a biomarker of genotoxicity. To observe the antigenotoxic potential of curcumin and carvacrol, heparinized fresh blood from healthy individuals was treated with 30 μg/mL of malathion in the presence of curcumin and carvacrol. Curcumin at concentrations of 25 μg/mL and 50 μg/mL had significantly reduced ($p < 0.05$) the frequency of SCE as compared to malathion-exposed sample. Similarly, carvacrol showed significant ($p < 0.05$) antigenotoxic effect at concentrations of 2.5 μg/mL and 5.0 μg/mL against malathion. We also studied the effect of *GSTT1* and *GSTM1* on the genotoxicity of malathion and antigenotoxic potential of curcumin and carvacrol. We observed that there is a statistically significant ($p < 0.05$) reduction in the frequency of SCE in case of curcumin and carvacrol as compared to malathion, but we did not observe any significant relationship ($p > 0.05$) between *GSTT1* and *GSTM1* polymorphism and the genotoxicity of malathion and antigenotoxic potential of curcumin and carvacrol.

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Introduction

Pesticides are widely used across the world, and in recent years their utilization has increased. Exposure to pesticides is omnipresent, not because of application of pesticides in agriculture and contamination of foods, but due to the widespread use of these products in and around households. Malathion is an organophosphate insecticide that is extensively used in gardens and orchards, to control various pests including the Mediterranean fruit fly. Positive consequences for chromosomal damage were shown by technical-grade malathion in five *in vivo* animal studies, while two studies using purified malathion showed negative results.[1] Studies have generally focused on pesticide applicators, that are exposed to other pesticides in addition to malathion and have substantiated for increased sister chromatid exchanges (SCEs) and/or chromosome aberrations (CAs).[2] Our study focused on DNA damage caused by malathion and its prevention by using curcumin and carvacrol in human lymphocyte culture by considering SCE as a biomarker of genotoxicity. SCE is a process, during DNA replication, in which two sister chromatids break and rejoin with one another, physically exchanging regions of the parental strands in the duplicated chromosomes.[3]

Nutraceuticals (often referred to as phytochemicals or functional foods) are natural, bioactive, chemical compounds that have health-promoting, disease-preventing, or medicinal properties. Curcumin (diferuloylmethane), a polyphenol, is the active ingredient of the dietary spice turmeric (*Curcuma longa)* and has been consumed for medicinal purposes for thousands of years; it has persuasive anticancer properties, as demonstrated in a plethora of human cancer cell lines/animal carcinogenesis models.[4] It acts as a free radical scavenger and an antioxidant, inhibitinglipid peroxidation and oxidative DNA damage.[5,6] Carvacrol is a terpene constituent of various essential oils extracted from the family Labiatae, including *Origanum* species. It has been shown to exhibit a range of biological activities such as antibacterial, antifungal, insecticidal, analgesic, and antioxidant activities.[7–11] Carvacrol has also been found to have inhibitory effects in various types of tumorigenesis. It was found to inhibit 7, 12 dimethyl benz (a) anthracene (DMBA)-induced tumorigenesis in rats and the growth of melanomas *in vitro*.[12] Ameliorative effects of curcumin and carvacrol have been studied in mice and human lymphocytes using various mutagens.[13,14] It is now well established through molecular epidemiological studies that both genetic and environmental factors are responsible for individual susceptibility to mutagen. Hereditary differences in the effectiveness of detoxification/activation of carcinogens play a vital role in host susceptibility. Glutathione S-transferases (GST) are one of the most frequently studied polymorphisms concerning metabolism of xenobiotics. As GST function widely in metabolic detoxification of xenobiotics, their genetic polymorphism can play an important role in determining individual sensitivity to various reactive chemicals. *GSTM1* and *GSTT1* are members of the GST multigene family, and are mostly concerned with the detoxification of a broad range of environmental carcinogens, endogenously produced reactive oxygen species, and lipid peroxidation products, yielding excretable hydrophilic metabolites.[15] The null *GSTM1* and *GSTT1* increase genotoxicity risk.[16] There are very few *in vitro* studies showing the effect of genetic polymorphism of *GSTM1* and *GSTT1* on the genotoxicity of malathion. In our study, we investigated the effect of *GSTM1* and *GSTT1* polymorphism and its relation with the genotoxicity of malathion/antigenotoxicity of curcumin and carvacrol, as measured by SCE frequency.

Materials and methods

Sample collection

Venous blood (5 mL) was taken from healthy individuals in two separate vacutainer tubes containing sodium heparin and dipotassium EDTA for lymphocyte culture set-up and DNA extraction, respectively. All participants signed a consent form and also filled a questionnaire about their health status history. All the individuals participated in this study were men of age group 18–30 years and were *bona fide* resident of Haryana state, North India (Aryan race). The protocol was duly approved by the Human Ethical Committee of Kurukshetra University, Haryana, India.

Human lymphocyte culture

Short-term peripheral blood lymphocyte cultures were set up using the earlier studied technique of Moorhead et al[17] with minor modifications. Culture was set up in duplicate by adding whole heparinized blood (0.4 mL) into 5 mL of RPMI 1640 culture medium (Himedia, Mumbai, India) containing L-glutamine (1%), fetal calf serum (20%) (Himedia), penicillin (100 UI/mL), streptomycin (100 μg/mL) solution (Himedia), and phytohemagglutinin (2%) (Bangalore Genei, Bangalore, India). The cultures were incubated at 37°C and 5% CO_2 for 72 hours.

Sister chromatid exchange

For SCE analysis, after 24 hours of incubation, 5-bromo-2-deoxyuridine (Sigma-Aldrich, Sigma-Aldrich Co, St. Louis, MO, USA) was added to the culture at a final concentration of 10 μg/mL. Malathion (Sigma-Aldrich) was added at the beginning of culture in concentrations ranging from 10 μg/mL to 50 μg/mL. Out of these concentrations, the maximum genotoxic dose of malathion, i.e., 30 μg/mL, was chosen to check the antigenotoxic effect of curcumin and carvacrol (Sigma-Aldrich). To check the antigenotoxic potential of curcumin and carvacrol against malathion, separate cultures with various combinations of malathion and curcumin/carvacrol were set up. In one set-up, heparinized fresh blood was treated with 30 μg/mL of malathion along with 25 μg/mL and 50 μg/mL of curcumin, while in another set-up, 2.5 μg/mL and 5.0 μg/mL of carvacrol were added against 30 μg/mL of malathion. Combined effect of both curcumin and carvacrol was also observed against malathion. Blood was also treated with curcumin and carvacrol alone to check their genotoxic effects if any. Blood without any mutagen/curcumin and carvacrol acted as a control, while blood having dimethylsulfoxide was taken as a negative control. The cultures were then incubated for 72