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Propolis Ameliorates Human Peripheral Blood Lymphocytes from DNA damage caused by Aflatoxin B_1

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ABSTRACT

Propolis, a natural product derived from plant resins collected by honeybees, has been used for thousands of years in traditional medicine all over the world. Its components are strong antioxidants and free radical scavengers. On the other hand, aflatoxin B_1 (AFB_1) is the most potent pulmonary and hepatic carcinogen. Since the eradication of AFB_1 contamination in agricultural products has been difficult, the use of natural or synthetic free radical scavengers could be a potential chemopreventive strategy. The biological effects of propolis are known, but its interaction with AFB_1 is not known for therapeutic uses. Therefore, this study was designed to examine the protective effects of different concentrations of propolis (6.25, 12.5, 25, 50 and 100 mg/L) against AFB_1 (3.12 ppm) genotoxicity in human lymphocytes *in vitro*. The genotoxic effects were assessed by micronucleus (MN) test in human blood cultures. The results of the present study indicated that AFB_1 significantly ($P<0.05$) increased formations of MNs in peripheral lymphocytes as compared to controls. On the contrary, propolis alone did not show genotoxic effects at the concentrations tested. Furthermore, AFB_1-induced increases in the genotoxicity indices were diminished by the addition of propolis. This anti-mutagenic effect of propolis can be attributed to its powerful scavenger ability.

Key Words: Aflatoxin B_1, antimutagenicity, *in vitro*, lymphocytes, micronucleus assay, propolis.

INTRODUCTION

Propolis, also known as bee glue, is a resinous hive product collected by honey bees from plant exudates and contains more than one hundred components (Newairy et al. 2009). Propolis has been used in folk medicine since ancient times and is known for its antimicrobial, antiparasitic, antiviral, anti-inflammatory, antitumoral and antioxidant properties (Nieva Moreno et al. 2000, Yousef et al. 2003, Yousef et al. 2004, Padmavathi et al. 2006, Paulino et al. 2008). Flavonoids are thought to be responsible for many of its biological and pharmacological activities (Newairy et al. 2009). On the other hand, AFB_1 is a natural contaminant produced by *Aspergillus flavus* and *A. parasiticus* species (Guzman de Pena 2007). AFB_1 is known to cause hepatotoxicity, teratogenicity, immunotoxicity and even death in animals and humans (Guindon et al. 2007). Furthermore, this mycotoxin has been classified as a carcinogenic agent for humans by the International Agency for Research on Cancer (IARC) (Guzman de Pena 2007). Reactive oxygen species (ROS) and lipid peroxidation (LPO) have been reported to be major mechanisms in AFB_1 toxicity (Shon et al. 2004, Lee et al. 2005). Thus, AFB_1 causes MN, Sister chromatid exchanges (SCE), unscheduled DNA synthesis, and chromosomal strand breaks as well as forms adducts in rodent and human cells (Groopman and Kensler 1999).

It has been pointed out that oxidative damage after AFB_1 exposure, together with hepatotoxicity or hepatocarcinogenesis could be inhibited by intake of antioxidants and/or free radical scavengers (Lee et al. 2005). At this context, propolis found to improve health and prevent serious disorders including heart disease, diabetes and cancer (Padmavathi et al. 2006, Paulino et al. 2008, Newairy et al. 2009). To our best knowledge, the role of propolis against AFB_1-induced genotoxicity in human lymphocytes has not so far been studied. Therefore, this study investigated the effect of propolis against AFB_1-induced DNA damages for improving the therapeutic gain of the propolis. So here we focused on alterations in MN formations in lymphocytes as genotoxic endpoint since MN induction in cells has been shown repeatedly to be a sensitive and specific parameter to assess both clastogenic and aneugenic potential of a test compound (Frieauff et al. 1998).

MATERIAL AND METHODS

Experimental design

The heparinized blood samples obtained from two healthy non-smoking donors with no history of exposure to any genotoxic agent. Questionnaires were given to each blood donor to evaluate exposure history; and informed consent forms were signed by each of them. Human peripheral blood lymphocyte cultures were set

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up according to a slight modification of the protocol described by Evans and O'Riordan (1975). The heparinized blood (0.5 ml) was cultured in 6 ml of culture medium (Chromosome Medium B, Biochrom, Leonorenstr. 2-6.D-12247, Berlin) with 5 µg ml^{-1} of phytohemagglutinin (Biochrom). The propolis samples collected from hive bee's located in the province of Erzurum, Turkey. About 10 g of propolis was dissolved in an appropriate amount of ethanol (Merck®). The extract was evaporated and filtrated aseptically under flow cabinet. The sticky extract yielded, was used to prepare determined concentrations for applications. AFB_1 (Sigma Chemical Co., St Louis, MO. USA) (3.12 ppm) and propolis (6.25, 12.5, 25, 50 and 100 ppm) were added to the cultures just before incubation, separately and together. Each individual lymphocyte culture without AFB_1 and propolis was studied as a control group.

MN test

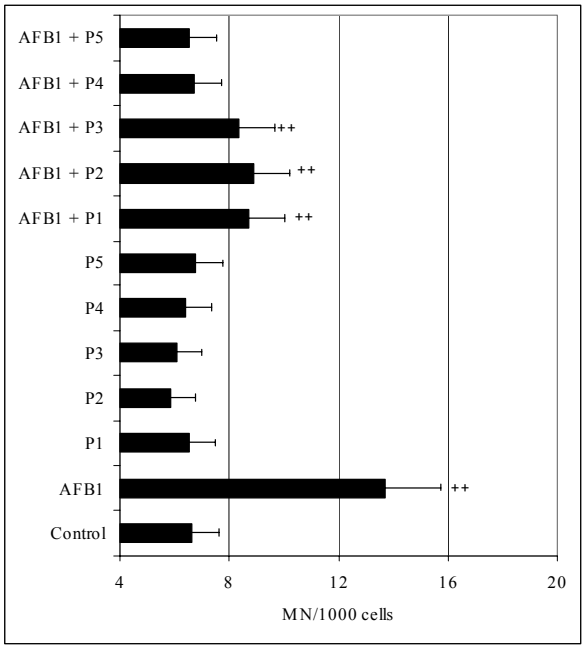
In order to detect the number of micronucleated lymphocytes, cytochalasin B (4.5 µg/ml, Sigma®) were added to cultures at 44th hour. At the end of the 72 h incubation period, the lymphocytes were treated with 0.075 M KCl for 8 minutes at 37°C. After three repetitive fixation with methanol/acetic acid (3:1, v/v), cell suspension was dropped onto cold slides. The slides were air-dried at room temperature and then stained with 5% Giemsa for 15 minutes. All slides were coded before scoring. The criteria for scoring micronuclei were as described by Fenech (1993). At least 2000 binucleated lymphocytes were examined per concentration (two cultures per concentration) for the presence of one, two or more micronuclei.

Statistics

The statistical analysis of experimental values in the MN test was performed by Student's *t*-test and using the S.P.S.S. 12.0 software. Statistical decisions were made with a significance level of 0.05.

RESULTS

The ability of AFB_1 to induce MN in cytokinesis blocked cells, as well as a decrease in the MN frequency in cultures treated with propolis is reflected in Figure 1. Our results showed that propolis (at all concentrations) did not alter MN frequencies in human lymphocyte cell. Moreover, the positive effect of propolis in dose depending decreasing the incidence of MN in comparison with an unprotected level was attained when cultures were treated simultaneously with AFB1 and propolis.



AFB1 = 3.12 ppm AFB1; P1 = 6.25 ppm propolis; P2 = 12.5 ppm propolis; P3 = 25 ppm propolis; P4 = 50 ppm propolis; P5 = 100 ppm propolis; ++ represents statistically significant differences from control group ($P < 0.05$). Values are means±standard deviation.

Figure 1. The rates of MN (‰) in cultured human lymphocytes exposed to AFB1 and propolis.

DISCUSSION

The results obtained by us indicate a significant increase in the ratios of MN in lymphocytes, which is in accordance with the previous reports. In similar to our finding, it was reported that frequencies of SCEs in human lymphocytes were significantly increased by AFB_1 exposure (Geyikoglu and Turkez 2005, Turkez and Sisman 2007). The genotoxic effects of AFB_1 were also established by using human, mouse and rat liver preparations (Wilson et al. 1997). In an another *in vitro* study, AFB_1 was found to produce genotoxic effects in human liver microsomes and human lymphocytes (Wilson et al. 1995). In addition to these *in vitro* investigations, Marquez et al. (1995) betrayed the mutagenicity of AFB_1 in mice using MN and SCE assays. AFB_1 toxicity was thought to be related with LPO and oxidation of DNA *in vivo* and *in vitro* (Shen et al. 1996). Thereby, this xenobiotic could contribute to the formation of the genome leading to carcinogenesis (Amici et al. 2007). Recent studies have also provided additional evidence that ROS and oxidative DNA damage may be involved in AFB_1-induced p53 and ras mutations (Shen and Ong 1996).

Our findings revealed that treatment with propolis provide anti-genotoxic effects by AFB_1 at different degree. There is considerable evidence that the propolis presents positive effects with increasing concentrations without leading to any genetic damage on human blood cells. It was established that propolis alone were non-genotoxic. The biologically fundamental macromolecules such as nucleic acids and proteins in mammalian cells defense themselves with antioxidants (Kedziora-Kornatowska et al. 2004). And it was suggested that polyphenolic components, caffeic acid (CA) derivatives and flavonoids in particular, were matter of interest for its antioxidant property (Gregoris and Stevanato 2010). It was established that the phenolic compounds did not react covalently with AFB_1, and the inhibitory effect of phenolic compounds on AFB1-induced mutagenesis could be due to the inhibition of the activation enzymes (San and Chan 1997, Cardador-Martinez et al. 2006). CA was exhibited antimutagenic properties and this positive effect of CA was assumed to be a result of its ability to scavenge ROS (Belicova et al., 2001, Benkovic et al. 2009). Likewise, Roy et al. (2008) revealed natural phytochemicals including flavonoids might have the efficacy in reducing genotoxic effects, in scavenging ROS and in enhancing the process of DNA repair. As a matter of fact, recent studies indicated that propolis could strengthen the tissue antioxidant defense system by reducing reactive ROS and increasing main antioxidant enzyme activities such as superoxide dismutase, catalase and glutathione peroxidase (Koyu et al. 2009, Newairy et al. 2009, Yousef et al. 2009). Again, propolis significantly decreased genotoxic effects of some agents such as doxorubicin and irinotecan due to its strong antioxidant nature (Valadares et al. 2008, Benkovic et al. 2009). So the results of this study may be attributed to antioxidant activity of propolis, as AFB_1 is known to induce mutagenic damage through oxidative stress.

Consequently, the exposure to AFB_1 should be reduced and attention paid to sources of AFB_1 in foods and food related products. Furthermore, using diets rich in propolis could be beneficial in alleviating AFB_1 toxicity.

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