

## Propolis Ameliorates Human Peripheral Blood Lymphocytes from DNA damage caused by Aflatoxin B<sub>1</sub>

Hasan Türkez<sup>\*1</sup>, Mokhtar I. Yousef<sup>2</sup>

<sup>1</sup> Department of Biology, Faculty of Sciences, Atatürk University, 25240, Erzurum, Turkey,

<sup>2</sup> Department of Home Economic, Faculty of Specific Education, Alexandria University, 21529, Alexandria, Egypt

### 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<sub>1</sub> (AFB<sub>1</sub>) is the most potent pulmonary and hepatic carcinogen. Since the eradication of AFB<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> (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<sub>1</sub> 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<sub>1</sub>-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<sub>1</sub>, 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<sub>1</sub> is a natural contaminant produced by *Aspergillus flavus* and *A. parasiticus* species (Guzman de Pena 2007). AFB<sub>1</sub> 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<sub>1</sub> toxicity (Shon et al. 2004, Lee et al. 2005). Thus, AFB<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub>-induced genotoxicity in human lymphocytes has not so far been studied. Therefore, this study investigated the effect of propolis against AFB<sub>1</sub>-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

\* Corresponding author: hasanturkez@yahoo.com

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  $5 \mu\text{g ml}^{-1}$  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<sub>1</sub> (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<sub>1</sub> and propolis was studied as a control group.

#### **MN test**

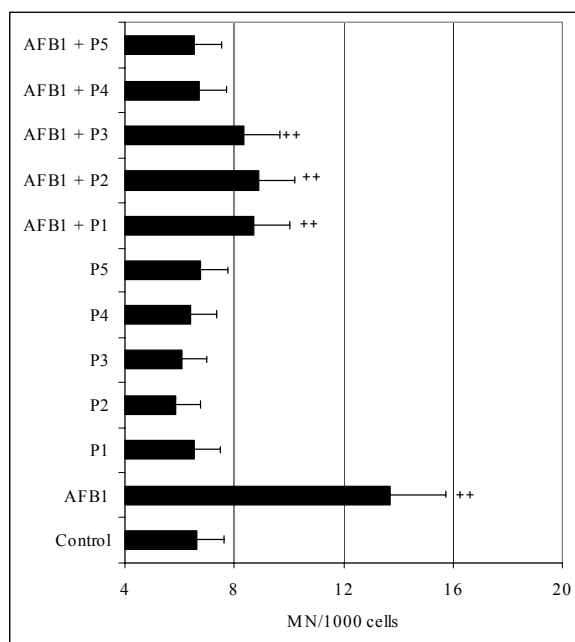
In order to detect the number of micronucleated lymphocytes, cytochalasin B (4.5  $\mu\text{g/ml}$ , Sigma®) were added to cultures at 44th hour. At the end of the 72 h incubation period, the lymphocytes were treated with 0.075 M KCl for 8 minutes at 37°C. After three repetitive fixation with methanol/acetic 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<sub>1</sub> 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 AFB<sub>1</sub> and propolis.



AFB<sub>1</sub> = 3.12 ppm AFB<sub>1</sub>; 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  $\pm$  standard deviation.

**Figure 1.** The rates of MN (‰) in cultured human lymphocytes exposed to AFB<sub>1</sub> 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<sub>1</sub> exposure (Geyikoglu and Turkez 2005, Turkez and Sisman 2007). The genotoxic effects of AFB<sub>1</sub> were also established by using human, mouse and rat liver preparations (Wilson et al. 1997). In an another *in vitro* study, AFB<sub>1</sub> 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<sub>1</sub> in mice using MN and SCE assays. AFB<sub>1</sub> 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<sub>1</sub>-induced p53 and ras mutations (Shen and Ong 1996).

Our findings revealed that treatment with propolis provide anti-genotoxic effects by AFB<sub>1</sub> 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<sub>1</sub>, and the inhibitory effect of phenolic compounds on AFB<sub>1</sub>-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<sub>1</sub> is known to induce mutagenic damage through oxidative stress.

Consequently, the exposure to AFB<sub>1</sub> should be reduced and attention paid to sources of AFB<sub>1</sub> in foods and food related products. Furthermore, using diets rich in propolis could be beneficial in alleviating AFB<sub>1</sub> toxicity.

## ACKNOWLEDGEMENTS

We are grateful to volunteers for the blood samples.

## REFERENCES

- Amici M, Cecarini V, Pettinari A, Bonfili L, Angeletti M, Barocci S, Biagetti M, Fioretti E, and Maria Eleuteri A (2007). Binding of aflatoxins to the 20S proteasome: effects on enzyme functionality and implications for oxidative stress and apoptosis. *Biol. Chem.*, 388: 107-117.
- Belicova A, Krizková L, Nagy M, Krajcovic J, and Ebringer L (2001). Phenolic acids reduce the genotoxicity of acridine orange and ofloxacin in *Salmonella typhimurium*. *Folia Microbiol.*, 46: 511-514.
- Benkovic V, Knezevic AH, Orsolic N, Basic I, Ramic S, Viculin T, Knezevic F, and Kopjar N (2009). Evaluation of radioprotective effects of propolis and its flavonoid constituents: *in vitro* study on human white blood cells. *Phytother. Res.*, 23: 1159-1168.
- Cardador-Martinez A, Albores A, Bah M, Calderón-Salinas V, and Castaño-Tostado E (2006). Relationship among antimutagenic, antioxidant and enzymatic activities of methanolic extract from common beans (*Phaseolus vulgaris* L). *Plant Foods Hum. Nutr.*, 61: 161-168.
- Evans HJ, and O'Riordan ML 1975. Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen tests. *Mutat. Res.*, 31: 135-148.
- Fenech M (1993). The cytokinesis-block micronucleus technique: a detailed description of the method and its application to genotoxicity studies in human populations. *Mutat. Res.*, 285: 35-44.
- Frieauff W, Pötter-Locher F, Cordier A, and Suter W (1998). Automatic analysis of the *in vitro* micronucleus test on V79 cells. *Mutat Res.*, 413: 57-68.

- Geyikoglu F, and Turkez H (2005). Protective effect sodium selenite on genotoxicity to human whole blood cultures induced by aflatoxin B<sub>1</sub>. *Brazil. Arch. Biol. Technol.*, 48: 905-910.
- Gregoris E, and Stevanato R (2010). Correlations between polyphenolic composition and antioxidant activity of Venetian propolis. *Food. Chem. Toxicol.*, 48: 76-82.
- Groopman JD, and Kensler TW (1999). The light at the end of the tunnel for chemical-specific biomarkers: daylight or headlight? *Phytother. Res.*, 15: 307-310.
- Guindon KA, Bedard LL, and Massey TE (2007). Elevation of 8-hydroxydeoxyguanosine in DNA from isolated Mouse lung cells following *in vivo* treatment with aflatoxin B<sub>1</sub>. *Toxicol. Sci.*, 98: 57-62.
- Guzman de Pena D (2007). Exposure to aflatoxin B<sub>1</sub> in experimental animals and its public health significance. *Salud Publica Mex.*, 49: 227-235.
- Kedziora-Kornatowska K, Czuczejko J, Pawluk H, Kornatowski T, and Motyl J (2004). The markers of oxidative stress and activity of the antioxidant system in the blood of elderly patients with essential arterial hypertension. *Cell Mol. Biol. Lett.*, 9: 635-641.
- Koyu A, Ozguner F, Yilmaz H, Uz E, Cesur G, and Ozcelik N (2009). The protective effect of caffeic acid phenethyl ester (CAPE) on oxidative stress in rat liver exposed to the 900 mhz electromagnetic field. *Toxicol. Ind. Health.*, 25: 429-434.
- Lee JK, Choi EH, Lee KG, and Chun HS (2005). Alleviation of aflatoxin B<sub>1</sub>-induced oxidative stress in HepG2 cells by volatile extract from *Allii Fistulosi Bulbus*. *Life Sci.*, 77: 2896-2910.
- Marquez MR, Tejada de Hernandez I, and Madrigal BE (1995). Genotoxicity of aflatoxin B<sub>1</sub> and its ammonium derivatives. *Food Addit Contam.*, 12: 425-429.
- Newairy AS, Salama AF, Hussien HM, and Yousef MI (2009). Propolis alleviates aluminium induced lipid peroxidation and biochemical parameters in male rats. *Food Chem. Toxicol.*, 47: 1093-1098.
- Nieva Moreno MI, Isla MI, Sampietro AR, and Vattuone MA (2000). Comparison of the free radical – scavenging activity of propolis from several regions of Argentina. *J. Ethnopharmacol.*, 71: 109-114.
- Padmavathi R, Senthilnathan P, Chodon D, and Sakthisekaran D (2006). Therapeutic effect of paclitaxel and propolis on lipid peroxidation and antioxidant system in 7, 12 dimethyl benz (a) anthracene-induced breast cancer in female Sprague Dawley rats. *Life Sci.*, 78: 2820-2825.
- Paulino N, Abreu SR, Uto Y, Koyama D, Nagasawa H, Hori H, Dirsch VM, Vollmar AM, Scremin A, and Bretz WA (2008). Anti-inflammatory effects of a bioavailable compound, Artepillin C, in Brazilian propolis. *Eur. J. Pharmacol.*, 587: 296-301.
- Roy M, Sinha D, Mukherjee S, Paul S, and Bhattacharya RK (2008). Protective effect of dietary phytochemicals against arsenite induced genotoxicity in mammalian V79 cells. *Indian J. Exp. Biol.*, 46: 690-697.
- San RH, and Chan RI (1987). Inhibitory effect of phenolic compounds on aflatoxin B<sub>1</sub> metabolism and induced mutagenesis. *Mutat. Res.*, 177: 229-239.
- Shen HM, and Ong CN (1996). Mutations of the p53 tumor suppressor gene and ras oncogenes in aflatoxin hepatocarcinogenesis. *Mutat. Res.*, 366: 23-44.
- Shen HM, Shi CY, Shen Y, and Ong CN (1996). Detection of elevated reactive oxygen species level in cultured rat hepatocytes treated with aflatoxin B<sub>1</sub>. *Free Radic. Biol. Med.*, 21: 139-146.
- Shon MY, Choi SD, Kahng GG, Nam SH, and Sung NJ (2004). Antimutagenic, antioxidant and free radical scavenging activity of ethyl acetate extracts from white, yellow and red onions. *Food Chem. Toxicol.*, 42: 659-666.
- Türkez H, Şişman T (2007). Anti-genotoxic effect of hydrated sodium calcium aluminosilicate on genotoxicity to human lymphocytes induced by aflatoxin B<sub>1</sub>. *Toxicol Ind Health.*, 23: 83-89.
- Valadares BL, Graf U, and Spano MA (2008). Inhibitory effects of water extract of propolis on doxorubicin-induced somatic mutation and recombination in *Drosophila melanogaster*. *Food Chem. Toxicol.*, 46: 1103-1110.
- Wilson AS, Tingle MD, Kelly MD, and Park BK (1995). Evaluation of the generation of genotoxic and cytotoxic metabolites of benzo[a]pyrene, aflatoxin B<sub>1</sub>, naphthalene and tamoxifen using human liver microsomes and human lymphocytes. *Hum Exp Toxicol.*, 14: 507-515.
- Wilson AS, Williams DP, Davis CD, Tingle MD, and Park BK (1997). Bioactivation and inactivation of aflatoxin B<sub>1</sub> by human, mouse and rat liver preparations: effect on SCE in human mononuclear leucocytes. *Mutat Res.*, 373: 257-264.
- Yousef MI, and Salama AF (2009). Propolis protection from reproductive toxicity caused by aluminium chloride in male rats. *Food Chem. Toxicol.*, 47: 1168-1175.
- Yousef MI, El-Demerdash FM, and Al-Salhen KS (2003). Protective role of isoflavones against the toxic effect of cypermethrin on semen quality and testosterone levels of rabbits. *J. Environ. Sci. Health Part B.*, 38: 463-478.
- Yousef MI, Esmail AM, and Baghdadi HH (2004). Effect of isoflavones on reproductive performance, testosterone levels, lipid peroxidation and seminal plasma biochemistry of male rabbits. *J. Environ. Sci. Health Part B.*, 39: 819-833.