The Effect of Propolis on Aluminum-Induced Genotoxicity in Human Lymphocytes

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Abstract

Propolis has been used in folk medicine since ancient times and is known for its antimicrobial, antiparasitic, antiviral, anti-inflammatory, antitumoral and antioxidant properties. In view of the great therapeutic interest in propolis and the small number of studies regarding its mechanism of action, the aim of the present study was to evaluate the antimutagenic effects of propolis against the genotoxicity of aluminum chloride ($AlCl_3$) using human lymphocytes *in vitro*. The micronucleus (MN) test was performed to assess genetic damage. The results showed that different concentrations of propolis (6.25, 12.5, 25, 50 and 100 mg/L) were found to exert an antimutagenic effect against the genotoxicity of $AlCl_3$ (25 mg/L) in a dose dependent manner. The obtained results also showed that the effective antimutagenic concentrations of propolis had no clastogenic or aneugenic effects in lymphocytes. From the obtained results, propolis at tested concentrations displays chemopreventive effects on $AlCl_3$-induced genotoxicity. Flavonoids may be the components of propolis responsible for its antimutagenic effects, once these compounds may act as free radicals scavenger.

Key Words: Aluminum; Propolis; Lymphocytes; Micronucleus assay; *In vitro*.

İnsan Lenfositlerinde Alüminyum ile Uyarılmış Genotoksisite Üzerine Propolisin Etkisi

Özet

Çok eski zamanlardan beri halk hekimliğinde kullanılmakta olan propolisin antimikrobiyal, antiparazitik, antiviral, antienflamatuvar, antitümoral ve antioksidan özellikleri bilinmektedir. Sunulan çalışmada, önemli terapötik potansiyele sahip olmasına rağmen etki mekanizmasını ortaya koyan çalışmaların azlığı dikkate alınarak *in vitro* şartlarda insan lenfositlerinde alüminyum klorit mutajenitesi üzerine propolisin etkilerinin değerlendirilmesi amaçlandı. Genetik hasarın değerlendirilmesinde mikroçekirdek (MÇ) testi kullanıldı. Sonuçlar, propolisin (6.25, 12.5, 25, 50 and 100 mg/L) doza bağlı olarak $AlCl_3$ (25 mg/L) genotoksisitesine karşı antimutajenik etkili olduğunu gösterdi. Elde edilen sonuçlar aynı zamanda lenfosit kültürlerinde propolisin antimutajenik etkili olduğu konsantrasyonlarda klastojenik veya anöjenik olmadığını da gösterdi. Bulgular ışığında, propolisin test edilen konsantrasyonlarda $AlCl_3$'ün genotoksisitesi üzerine kimyasal koruyucu etkilerinin olduğu ortaya konuldu. Flavonoidler serbest radikal giderici olarak rol oynadıklarından, propolisin antimutajenik etkiler göstermesinden sorumlu bileşenleri olabilirler.

Anahtar Kelimeler: Alüminyum; Propolis; Lenfosit; Mikroçekirdek testi; *İn vitro*

1. Giriş

Aluminium (Al) is present in several manufactured foods and medicines and is also its toxic effects (Suay et all, 2002). Several *in vitro* and *in vivo* studies indicate that Al has pro-added to drinking water during purification purposes (Newairy et all, 2009). The extensive use of Al was cautionable due to oxidative effects (Exley, 2004). Therefore, Al provoked nephrotoxicity (Kutlubay et all, 2007),

cardiotoxicity (Bombi et all, 1990) hepatotoxicity, hematotoxicity (Turgut et all, 2007) and neurotoxicity (Frisardi et all, 2010). Besides, Al caused genetic damage in rat bone marrow cells (Balasubramanyam et all, 2009). This metal also induced MN formations and chromosome aberrations in cultured human peripheral blood lymphocytes (Geyikoğlu et all, 2005; Lima et all, 2007). On the other hand, bee products including propolis, royal jelly, and bee pollen are reported to be popular, traditional health foods (Nakajima et all, 2009). The propolis has been used in folk medicine for antioxidant, immune-stimulating, anti-inflammatory and non-toxic natures (Cole et all, 2010). The recent investigations found that propolis could effect antibody production, macrophage activation and lymphocyte proliferation (Sforcin, 2007).

Al have been implicated in serious pathological disorders such as Alzheimer and Parkinson diseases (Lima et all, 2007; Carpenter, 2001). Hence, in recent years, to examine useful antidotes against the toxic effects of Al is especially important. The supplementation with free radical scavengers (e.g. propolis) may protect the organisms from the harmful effect of Al. The role of propolis in human blood against aluminum-induced DNA damage has not so far been studied. In this study, our goal was to investigate the efficacy of propolis in the lymphocytes of aluminum-applied cultures. Thus, here we focused on alterations in MN formations in lymphocytes as genotoxic endpoint. With this aim, the present study was designed to determine rate of MN formations in cultures after exposure to 6.25, 12.5, 25, 50 and 100 mg/L concentrations of propolis and 25 mg/L $AlCl_3$.

2. Materials and methods

2.1. Cell culture and experimental design

The whole blood samples were collected from two healthy non-smoker donors with no history of exposure to any genotoxic agent. The cultures were set up according to the protocol described by Evans and O'Riordan (Evans et all, 1975) with a slight modification. The peripheral blood lymphocytes (0.5 ml) were cultured in 6 ml of culture medium (Chromosome Medium B, Biochrom®) with phytohemagglutinin. 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. Then, propolis (6.25, 12.5, 25, 50 and 100 mg/L) and $AlCl_3$ (25 mg/L) (Sigma®) were added alone or together to the cultures except control group. The tested agents were applied to the cultures after incubation for 24 h. The application dose of $AlCl_3$ was selected according to the work of Banasik et al. (Banasik et all, 2005). And the doses of propolis were selected according to our pre-study on dose–response relations by using an automatic blood analyzer (COULTER GEN-S, Miami, USA).

2.2. 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 (Fenech, 1993). At least 2000 binucleated lymphocytes were examined for the presence of one, two or more micronuclei per concentration.

2.3. Statistical analysis

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.

3. Results

The results of present study clearly showed that propolis (at all concentrations) did not alter MN frequencies in human lymphocyte cell. $AlCl_3$ at 25 mg/L concentration significantly increased MN formations in lymphocytes as compared with controls (Fig. 1). Nevertheless, propolis (25, 50 and 100 mg/L) reduced the number of $AlCl_3$-induced MN formations but low concentrations of propolis (6.25 and 12.5 mg/L) did not completely inhibit MN induction.

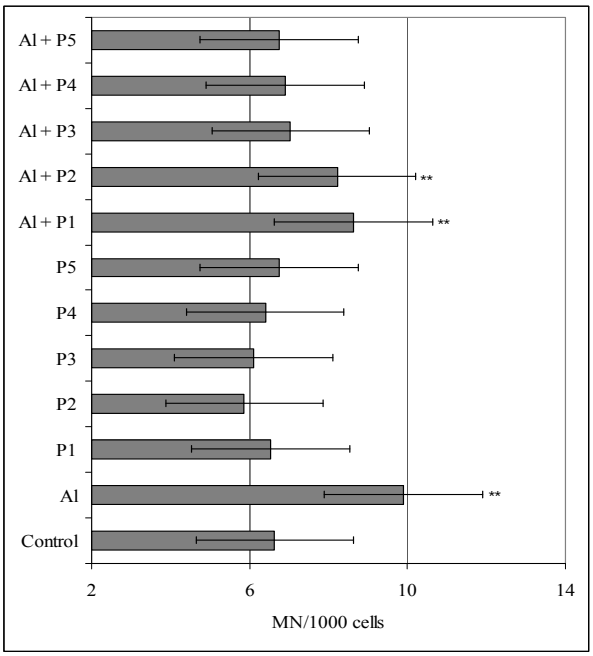

Figure 1. The rates of MN in human lymphocytes treated with Propolis and $AlCl_3$.

Al= 25 mg/L $AlCl_3$; P1 = 6.25 mg/L propolis; P2 = 12.5 mg/L propolis; P3 = 25 mg/L propolis; P4 = 50 mg/L propolis; P5 = 100 mg/L propolis; ** represents statistically significant differences from control group ($P < 0.05$). Values are means±standard deviation.

4.Discussion

Our results clearly indicated the $AlCl_3$ induced genotoxic damage in human lymphocytes. In similar to this finding, there are a few reports on *in vitro* Al genotoxicity. Lima et al. (Lima et all, 2007) performed the Comet assay and chromosome aberrations analysis to evaluate the DNA-damaging and clastogenic effects of $AlCl_3$ in different phases of the cell cycle of cultured human lymphocytes. They showed that this compound was clastogenic and indirectly affected the construction of mitotic fuse in all tested concentrations. $Al_2(SO4)_3$ significantly caused sister chromatid exchange (SCE) formations in human lymphocytes (Geyikoğlu et all, 2005). Kim et al. (Kim et all,2009) investigated the genotoxicity of Al_2O_3 by using the dye exclusion assay, the comet assay, and the mouse lymphoma

thymidine kinase tk (+/-) gene mutation assay (MLA) and found that Al_2O_3 could cause primary DNA damage. The genotoxic effects of Al_2O_3 were established on Chinese hamster ovary (CHO-K1) cells using SCE and MN formations (Di Virgilio et all, 2009). The mutagenic activity of waste material originating from an Al products factory was also determined by the Salmonella/microsome assay, using the bacterial strain TA98 (Varella et all, 2004). Our findings on $AlCl_3$ genotoxicity are in line with previously published *in vitro* data. Likewise Al compounds were reported to exhibite mutagenic activities *in vivo* (Balasubramanyam et all, 2009; Balasubramanyam et all, 2009).

The present study also demonstrated that the reductions of $AlCl_3$-induced MN formations were caused by the protective effect of propolis. The previous studies showed that propolis did not lead to DNA damages in human white blood cells (Benkovic et all, 2009). Moreover, this substance significantly decreased genotoxic effects of some agents such as doxorubicin (Valadares et all, 2008) and irinotecan (Orsolic et all, 2009). This protective mechanism is not very clear but it can be related with strengthening the tissue antioxidant defense system by reducing reactive oxygen species (ROS) and increasing main antioxidant enzyme activities such as super oxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST) and glutathione peroxidase (GSH-Px)(Newairy et all, 2009; Koyu et all, 2009; Yousef et all, 2009). Otherwise, the results of the study by Abubakar et al. (Abubakar et all, 2003) suggested that Al toxicity might be mediated by free radical generation. Similarly, Garcia-Medina et al. (García-Medina et all, 2009) reported that Al modified the activity of antioxidant enzymes and elicited higher levels of lipid peroxidation and oxidized proteins. Thus, propolis could modulate the $AlCl_3$ induced genetic damage by preventing free radical generation or stimulating the components of antioxidant defense system. As a matter of fact, a significant positive correlation was found between the antioxidant capacity and flavonoid content of propolis (María et all,2009) although the composition of propolis could change due to vegetation of the area from where it was collected (Shiva et all, 2009). In the several propolis samples from Crotia, the level of flavones and flavonols varied from 2.2 to 2.3% (average 2.2%), the content of flavanones varied between 10.3 and 20.7% (average 16.2%) (Kosalec et all, 2004). And, the total flavonoid contents of propolis samples varied from 1.22 ± 0.33 to 7.79 ± 0.39 g/100 g crude extract for Iranian propolis samples (Shiva et all, 2009). Likewise, the characteristic flavonoid of Tunisian propolis, myricentin 3,7,4‘,5‘-tetramethyl ether, was determined in the most of analyzed samples (Martos et all, 1997). In addition, the flavonoid contents were investigated in Portuguese propolis extracts, and detected dihydroflavonols, flavones, flavanones, and flavonols as in free or methylated/esterified forms such as apigenin, pinobanksin, pinocembrin and chrysin (Falcao et all, 2010).

The findings of this investigation clearly indicated that propolis modulated $AlCl_3$-induced genetic damage in human blood cultures due to its antioxidant and detoxifying nature. So the propolis can be a novel resource of therapeutics as recognized in this study against genetic and oxidative damages.

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References

Newairy, A.S., Salama, A.F., Hussien, H.M., ve Yousef, M.I., 2009. Propolis Alleviates Aluminium Induced Lipid Peroxidation and Biochemical Parameters in Male Rats, Food Chem. Toxicol., 47, 1093-1098.

Suay, L.L. ve Ballester, D.F., 2002. Review of Studies on Exposure to Aluminum and Alzheimer's Disease, Rev. Esp. Salud Publica., 76, 645-658.

Exley, C., 2004. The Pro-oxidant Activity of Aluminum, Free Radic. Biol. Med., 36, 380-387.

Kutlubay, R., Oğuz, E.O., Güven, C., Can, B., Sinik, Z. ve Tuncay, O.L., 2007. Histological and Ultrastructural Evidence for Protective Effects on Aluminium-Induced Kidney Damage by Intraperitoneal Administration of Alpha-tocopherol, Int. J. Toxicol., 26, 95-101.

Bombi, G.G., Corain, B., Favarato, M., Giordano, R., Nicolini, M., Perazzolo, M., Tapparo, A. ve Zatta, P., 1990. Experimental Aluminum Pathology in Rabbits: Effects of Hydrophilic and Lipophilic Compounds, Environ. Health. Perspect., 89, 217-223.

Turgut, S., Bor-Kucukatay, M., Emmungil, G., Atsak, P. ve Turgut, G., 2007. The Effects of Low Dose Aluminum on Hemorheological and Hematological Parameters in Rats, Arch. Toxicol., 81, 11-17.

Frisardi, V., Solfrizzi, V., Capurso, C., Kehoe, P.G., Imbimbo, B.P., Santamato, A., Dellegrazie, F., Seripa, D., Pilotto, A., Capurso, A. ve Panza, F., 2010. Aluminum in the Diet and Alzheimer's Disease: From Current Epidemiology to Possible Disease-Modifying Treatment, J Alzheimers Dis., in press.

Balasubramanyam, A., Sailaja, N., Mahboob, M., Rahman, M.F., Misra, S., Hussain, S.M. ve Grover, P., 2009a. Evaluation of Genotoxic Effects of Oral Exposure to Aluminum Oxide Nanomaterials in Rat Bone Marrow, Mutat. Res., 31, 41-47.

Geyikoğlu, F., Türkez, H. ve Keles, M.S., 2005. The Role of Fruit Juices in The Prevention of Aluminum Sulphate Toxicity in Human Blood *In Vitro*. Fres. Environ. Bull., 14, 878-883.

Lima, P.D., Leite, D.S., Vasconcellos, M.C., Cavalcanti, B.C., Santos, R.A., Costa-Lotufo, L.V., Pessoa, C., Moraes, M.O. ve Burbano, R.R., 2007. Genotoxic Effects of Aluminum Chloride in Cultured Human Lymphocytes Treated in Different Phases of Cell Cycle, Food Chem. Toxicol., 45, 1154-1159.

Nakajima, Y., Tsuruma, K., Shimazawa, M., Mishima, S. ve Hara, H., 2009. Comparison of Bee Products Based on Assays of Antioxidant Capacities, BMC Complement. Altern. Med., 9, 4.

Cole, N., Sou, P.W., Ngo, A., Tsang, K.H., Severino, J.A., Arun, S.J., Duke, C.C. ve Reeve, V.E., 2010. Topical 'Sydney' Propolis Protects against UV-Radiation-Induced Inflammation, Lipid Peroxidation and Immune Suppression in Mouse Skin, Int. Arch. Allergy. Immunol., 152, 87-97.

Sforcin, J.M., 2007. Propolis and the Immune System: A Review, J. Ethnopharmacol., 113, 1-14.

Carpenter, D.O., 2001. Effects of Metals on the Nervous System of Humans and Animals, Int. J. Occup. Med. Environ. Health., 14, 209-218.

Evans, H.J. ve O'Riordan, M.L., 1975. Human Peripheral Blood Lymphocytes for the Analysis of Chromosome Aberrations in Mutagen Tests, Mutat. Res., 31, 135-148.

Banasik, A., Lankoff, A., Piskulak, A., Adamowska, K., Lisowska, H. ve Wojcik, A., 2005. Aluminum-induced micronuclei and apoptosis in human peripheral-blood lymphocytes treated during different phases of the cell cycle, Environ. Toxicol. 20, 402-406.

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.

Kim, Y.J., Choi, H.S. ve Song, M.K., 2009. Genotoxicity of Aluminum Oxide (Al1O3) Nanoparticle in Mammalian Cell Lines, Mol. Cell. Toxicol., 5, 172-178.

Di Virgilio, A.L., Reigosa, M., Arnal, P.M. ve Fernández Lorenzo de Mele, M., 2009. Comparative Study of the Cytotoxic and Genotoxic Effects of Titanium Oxide and Aluminium Oxide Nanoparticles in Chinese Hamster Ovary (CHO-K1) Cells, J. Hazard. Mater., in press.

Varella, S.D., Pozetti, G.L. ve Vilegas, W., 2004. Mutagenic Activity in Waste From an Aluminum Roducts Factory in Salmonella/Microsome Assay, Toxicol. *In Vitro*, 18, 895-900.

Balasubramanyam, A., Sailaja, N., Mahboob, M., Rahman, M.F., Hussain, S.M. ve Grover, P., 2009b. *In Vivo* Genotoxicity Assessment of Aluminium Oxide Nanomaterials in Rat Peripheral Blood Cells Using the Comet Assay and Micronucleus Test, Mutagenesis, 24, 245-251.

Benkovic, V., Knezevic, A.H., Orsolic, N., Basic, I., Ramic, S., Viculin, T., Knezevic, F. ve 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.

Valadares, B.L, Graf, U. ve Spano, M.A., 2008. Inhibitory Effects of Water Extract of Propolis on Doxorubicin-Induced Somatic Mutation and Recombination in *Drosophila melanogaster,* Food Chem. Toxicol., 46, 1103-1110.

Orsolic, N., Benkovic, V., Lisicic, D., Dikic, D., Erhardt, J. ve Horvat Knezevic, A., 2009. Protective Effects of Propolis and Related Polyphenolic/Flavonoid Compounds Against Toxicity Induced by Irinotecan, Med. Oncol., in press.

Koyu, A., Ozguner, F., Yilmaz, H., Uz, E., Cesur, G. ve 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.

Yousef, M.I. ve Salama, A.F., 2009. Propolis Protection From Reproductive Toxicity Caused by Aluminium Chloride in Male Rats, Food Chem. Toxicol., 47, 1168-1175.

Abubakar, M.G., Taylor, A. ve Ferns, G.A., 2003. Aluminium Administration Is Associated With Enhanced Hepatic Oxidant Stress That May Be Offset By Dietary Vitamin E in the Rat, Int. J. Exp. Pathol., 84, 49-54.

García-Medina, S., Razo-Estrada, A., Gómez-Oliván, L., Amaya-Chávez, A., Madrigal-Bujaidar, E. ve Galar-Martínez, M., 2009. Aluminum-Induced Oxidative Stress in Lymphocytes of Common Carp (*Cyprinus carpio*) Fish Physiol. Biochem., DOI: 10.1007/s10695-009-9363-1.

María I. Isla, Iris C. Zampini, Roxana M. Ordóñez, Soledad Cuello, Belén Carrasco Juárez, Jorge Esteban Sayago, María I. Nieva Moreno, María Rosa Alberto, Nancy R. Vera, Enrique Bedascarrasbure, Alejandro Alvarez, Fabián Cioccini, Luis M. Maldonado. Journal of Medicinal Food. December 2009, 12(6): 1334-1342Effect of Seasonal Variations and Collection Form on Antioxidant Activity of Propolis from San Juan, Argentina

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Kosalec, I., Bakmaz, M., Pepeljnjak, S. ve Vladımır-Knezevıc, S., 2004. Quantitative

analysis of the flavonoids in raw propolis from northern Croatia, Acta Pharm. 54, 65-72.

Martos, I., Cossentini, M., Ferreres, F., Francisco, A. ve Tomás-Barberán, F.A., 1997. Flavonoid Composition of Tunisian Honeys and Propolis, J. Agric. Food Chem. 45, 2824-2829.

Falcao, S.I., Vilas-Boas, M., Estevinho, L.M., Barros, C., Domingues, M.R.M. ve Cardoso, S.M., 2010. Phenolic characterization of Northeast Portuguese propolis: usual and unusual compounds, Anal. Bioanal. Chem. 396, 887-897.