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 (AlCl3) 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 AlCl3 (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 AlCl3-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, antienflamaturvar, antiümmoral ve antioksidan özellikleri bilinmektedir. Sunulan çalışmada, önemli terapötik potansiyele sahip olmasa rağmen etki mekanizmasını ortaya koyan çalışmaların azlığı dikkate alınarak in vitro şartlarda insan lenfositlerinde alüminyum genetik mutajenitesi üzerine propolisin etkilerinin değerlendirilmesi amaçlandı. Genetik hasarın değerlendirilmesinde mikroçekirdek (MÇ) testi kullanıldı. Sonuçlar, propolisin (6.25, 12.5, 25, 50 ve 100 mg/L) doza bağlı olarak AlCl3 (25 mg/L) genetik eksitesine 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 AlCl3'nın genotoksisitesi üzerine kırık yıkanıcı etkilerinin olduğunu ortaya koşuldu. flavonoidler serbest radikal giderici olarak rol oynamalarından, propolisin antimutajenik etkileri göstermesinden sorumlulüğü bileşenleri olabilirler.

Anahtar Kelimeler: Alüminyum; Propolis; 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-
cardiotoxicity (Bombi et al., 1990) hepatotoxicity, hematotoxicity (Turgut et al., 2007) and neurotoxicity (Frisardi et al., 2010). Besides, Al caused genetic damage in rat bone marrow cells (Balasubramanyam et al., 2009). This metal also induced MN formations and chromosome aberrations in cultured human peripheral blood lymphocytes (Geyikoglu et al., 2005; Lima et al., 2007). On the other hand, bee products including propolis, royal jelly, and bee pollen are reported to be popular, traditional health foods (Nakajima et al., 2009). The propolis has been used in folk medicine for antioxidant, immune-stimulating, anti-inflammatory and non-toxic natures (Cole et al., 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 al., 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 AlCl₃.

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 al., 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 AlCl₃ (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 AlCl₃ was selected according to the work of Banasik et al. (Banasik et al., 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. AlCl₃ 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 AlCl₃-induced MN formations but low concentrations of propolis (6.25 and 12.5 mg/L) did not completely inhibit MN induction.

![Figure 1](image1.png)

Figure 1. The rates of MN in human lymphocytes treated with Propolis and AlCl₃.

Al= 25 mg/L AlCl₃; 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 AlCl₃ 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 al, 2007) performed the Comet assay and chromosome aberrations analysis to evaluate the DNA-damaging and clastogenic effects of AlCl₃ 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. Al₂(SO₄)₃ significantly caused sister chromatid exchange (SCE) formations in human lymphocytes (Geyikoglu et al, 2005). Kim et al. (Kim et al, 2009) investigated the genotoxicity of Al₂O₃ by using the dye exclusion assay, the comet assay, and the mouse lymphoma
thymidine kinase tk (+/-) gene mutation assay (MLA) and found that Al2O3 could cause primary DNA damage. The genotoxic effects of Al2O3 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 AlCl3 genotoxicity are in line with previously published in vitro data. Likewise Al compounds were reported to exhibit mutagenic activities in vivo (Balasubramanyam et all, 2009; Balasubramanyam et all, 2009).

The present study also demonstrated that the reductions of AlCl3-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 (Benković 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 AlCl3 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 (Maria 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 AlCl3-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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